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Polymeric Gels

Characterization, Properties and Biomedical Applications

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Introduction to polymeric gels

1

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1.1 Introduction

The term "gel" is originated from the word- "gelatin." Both the words, "gel" and "jelly" are thought to be originated from the Latin word—"*gelu*," means frost, freeze, or congeal (Venugopal, 2011). Thus, the origin of the term, "gel" indicates the idea of liquid setting to the solid-like or semisolid materials, which do not flow (Rathod and Mehta, 2015). However, these retain some characteristics of the liquid materials due to the elastic or semielastic nature. During the late 18th century, the term- "gel" was firstly used as the chemists, chemical engineers, chemical scientists, and chemical researchers attempted to categorized semisolid materials on the basis of their phenomenological properties rather than their compositions in the molecular level (Rathod and Mehta, 2015).

In the year of 1993, Almdal et al. proposed a phenomenological definition of "gels." According to them, "gel" is a soft and/or solid-like substances consisting at least two components, one of which should be a liquid in abundance (Almdal et al., 1993). In the "Encyclopedia of Polymer Science and Engineering," it was mentioned that "a gel is a crosslinked polymer network swollen in a liquid medium. Its properties depend strongly on the interaction of these two components" (Tanaka, 1987). In the "Webster's New Twentieth Century Dictionary," the definition of "gel" is found as "Gel, a jelly-like substance formed by a colloidal solution in its solid phase: opposed to sol" (McKechnie, 1966). In general, gels are described as semisolid systems made of at least two constituents: one of these (polymers) structures is a three-dimensional (3-D) network by virtue of covalent or noncovalent bondings in the medium of other constituents (liquid), wherein the smallest quantity of the liquid is adequate to ensure the elastic characters of the gel, even though it may exceed 10−100 times the polymer quantity (Rathod and Mehta, 2015). This chapter deals with the introductory discussion of polymeric gels. In this chapter, various important properties of polymeric gels and different kinds of polymeric gels, including physical gels, hydrogels (smart hydrogels, superporous hydrogels, interpenetrating polymer networking [IPN] hydrogels, etc.), macrogels, microgels, nanogels, organogels, emulgels, aerogels, xerogels, cryogels, etc., are discussed in brief.

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1.2 Polymeric gels and their properties

1.2.1 Polymeric gels

Polymeric gels are defined as soft and/or solid-like systems made of one polymer or more than one polymer (Hägerström, 2003). In general, polymeric gels are polymer/solvent systems (mostly binary systems), in which there subsists a 3-D network made of macromolecules and/or their aggregates enabling to retain a larger volume of solvent(s) (Rogovina et al., 2008). In some cases, these polymeric gel systems comprise elastic cross-linked networks and also fluidfilling into the interstitial gaps of the network and capable of experiencing larger deformation(s) (Osada and Gong, 1998). During the past few decades, these are recognized as a fascinating category of materials containing polymer(s). At the present time, the area of polymeric gels has gained an attractive position in the wide-reaching researches around the world by numerous materials scientists, polymer engineers, chemical engineers, chemists, biomedical scientists, pharmaceutical scientists, etc. Numerous research endeavors have been carried out on the preparations, characterizations, evaluations, and exploitations of various polymeric gels comprising widely different structures and characteristics (Hägerström, 2003; Rogovina et al., 2008).

1.2.2 Properties of polymeric gels

In general, polymeric gels possess at least one of these following properties: (1) infinite molecular weight, (2) insoluble and infusible, and (3) ability to reversibly swell or shrink (up to even 1000 times in volume) (Osada et al., 2001). Depending on several factors such as constituent polymers, polymeric matrix types, nature of cross-linking, size, etc., polymeric gels can have very diversified and a distinctive set of properties. Important properties of polymeric gels are swelling, pseudoplastic (non-Newtonian) rheological behavior, syneresis, aging, electrostatic potential distribution, electrical oscillation, electrical contraction, mechanoelectric effect, interaction with oppositely charged surfactants, etc. Mostly, these gels exhibit stimuli responsive behavior by means of applying external stimuli such as pH, pressure, temperature, electric field, etc. (Jagur-Grodzinski, 2010). Thus, polymeric gel systems usually are able to produce force or accomplish their performances on the external milieu (Osada and Gong, 1998).

1.2.2.1 Swelling

Polymeric gels are capable to swell through absorbing an increased volume of liquid (solvent). Usually, solvents penetrate the gel matrix so that the gel−gel interactions are restored via the gel−solvent interactions (Bohidar et al., 2003; Wack and Ulbricht, 2007). Inadequate swelling behavior is generally the result of some extent of cross-linking in the gel matrices that averts the matrix dissolutions. Such gel systems swell noticeably when the solvent mixtures possess a solubility parameter as compared to that of the gellant. Based on the swelling parameter, several highly swellable

polymeric gels are being prepared using polyacrylates (Song et al., 2017). These gels are called as "superabsorbing polymers (SAPs)." On the contact with the aqueous medium, SAPs swell and form hydrogel structure. Moreover, these can occupy up to 1000 times wetness of their dry weight, while storing the absorbed aqueous medium even under pressure (Buchholz and Graham, 1998).

1.2.2.2 Rheological behavior

Solutions containing gelling materials and dispersions of flocculated materials are generally pseudoplastic in nature (i.e., demonstrating a non-Newtonian flow performance, characterized by declining in the viscosity with an increment in the shear-rate values) (Osada et al., 2001). Weak configuration of the inorganic particles dispersed in the aqueous medium is disrupted by the shear stress applied owing to collapsing of interparticulate association, demonstrating a better trend to flow. Likewise, the applied shear stress supports the molecules toward the stress and decreasing the flow resistance for the macromolecules (Rathod and Mehta, 2015).

1.2.2.3 Syneresis

Numerous gel systems usually experience contraction on their standing. The interstitial fluid is expressed, accumulating at the gel surface. This course of action is referred to as syneresis (Bhasha et al., 2013). The occurrence of syneresis is not only restricted to the organic hydrogels but has also been noticed in the inorganic hydrogels and organogels. As the concentrations/amounts of polymer(s) constituents decrease, syneresis of the polymeric gels becomes generally more pronounced. This occurrence has been associated to the relaxation of elastic stresses produced for the duration of the setting of gel systems. When the stresses are reduced, the interstitial gap accessible for the solvent(s) is decreased, compelling the expression of liquid(s) (Neeraj et al., 2017).

1.2.2.4 Aging

In general, colloidal systems demonstrate a slower spontaneous aggregation and it is known to as aging of gel system (Sowmya et al., 2015). In the gel systems, aging causes the gradual development of comparatively denser networking(s) of the gelling agent (Neeraj et al., 2017). This aging process of gels carries on even after the initial gelation because the liquid concentrations/amounts are reduced from the newly produced gel systems.

1.2.2.5 Structure

The rigidness of gel systems comes up from the occurrence of the networking(s) developed by means of the interlinking of the gelling component(s). The character of the gelling component(s) and the force type are thought as accountable for the relationships determining the networking structure and the gel properties (Neeraj et al., 2017).

1.2.2.6 Electrostatic potential distribution

Numerous polymeric gels possess electrostatic charges in their polymeric networking structures. The polyelectrolyte polymeric gels are capable to swell in the aqueous medium and absorb a significant fraction (approximately 2000 folds of the polymer mass) of the aqueous medium within the gel structure (Sowmya et al., 2015). However, these will not degrade in the aqueous medium. Unlike the linear structured polyelectrolyte solutions, the polyelectrolyte gels display various unique characters such as phase transition, occurrence of unfrozen liquid, chemomechanical performances, specific adsorption equilibrium, etc. The unique characters and performance detected in the polymeric gels are well illustrated by the character of charged polymeric networking together with the counterions. The charge density of counterions declines very harshly with an increment in the distance from the polymeric chains. The mechanism of electrically conduction in the polymeric gels is fundamentally unchanged as that found in the linear polymeric solutions (i.e., the transfer of freely bound counterions plays a leading role in the electrical conduction) (Neeraj et al., 2017).

1.2.2.7 Electrical oscillation

Electrical oscillation is also another important property of the polymeric gels. For examples, a metal/polymeric gel/metal structure composed of aqueous-swollen polymeric gels sandwiched in between two electrodes made of platinum wires possesses the capability of switching "on" and "off" reversibly in-between two stable states (Neeraj et al., 2017). One of the two stable states is due to the higher impedance corresponding to the "switch off," and the another state is also due to the lower impedance corresponding to the "switch on" (Sowmya et al., 2015). The character of the electrical oscillation of the polymeric gels is connected with the temperature and extent of cross-linking of the system; the increment of these parameters augments the amplitude and the rate of electrical oscillation. This is believed that electrical oscillation of the polyelectrolyte gels is linked with the transferring procedure of macroions and microions accompanying the corresponding dynamic segmentational action of the charged networking in the aqueous solvent(s) (Rathod and Mehta, 2015).

1.2.2.8 Electrical contraction

If the aqueous-swollen cross-linked polyelectrolyte gels are introduced in between a pair of planar electrodes and a direct current voltage is employed, these undergo anisotropic contraction of the polymeric gel. The electrically stimulated contraction of the polymeric gels is originated via the transfer of hydrated ions and aqueous medium in the networking made of polymer(s), and the observed contractile performance is basically an electrochemical incident (Sowmya et al., 2015). When an external electrical field is employed across the polymeric gels, both the macroions and microions receive the electric forces in the reverse trend (Rathod and Mehta, 2015; Neeraj et al., 2017).

1.2.2.9 Mechanoelectric effect

The polyelectrolyte polymeric gels are usually able to deform or contract under an electric stimulus and thus, these gels are capable of converting electrical energy into the mechanical effects. Also, the reverse phenomena can be observed in these gels (Sowmya et al., 2015). This indicates that the mechanical deformation is capable of generating an electrical potential for these gels. The pHs of polymeric gels change reversibly while a small amount of weak polyelectrolyte polymeric gels is pressed. The pH alteration is related with an improved extent of ionizations of the −COOH groups under the deformation: being compressed in one trend, the polymeric gels expand laterally and also stimulate a one-dimensional dilatation of the polymeric networking in this trend (Neeraj et al., 2017). This happening produces an amplified chemical free energy (i.e., reduction in the entropy) of the polymeric chains that should be balanced via a simultaneous increment in the degree of ionizations (Rathod and Mehta, 2015).

1.2.2.10 Interaction with oppositely charged surfactants

In view of the fact that the polyelectrolyte gels have profound electrostatic potential valleys alongside the polymeric chains at the cross-linked sites (Neeraj et al., 2017). This can attract the oppositely charged surfactants and also form the complexes due to interaction with oppositely charged surfactants (Sowmya et al., 2015). The surfactant bindings to the solvated and cross-linked polyelectrolytes possessing charges on the side chains and the chain backbones have also been widely investigated by using diverse types of surfactants possessing opposite charges. In general, three types of surfactant bindings have been proposed: (1) cooperative and stoichiometric, (2) cooperative and nonstoichiometric, and (3) noncooperative and stoichiometric (Osada and Gong, 1998).

1.3 Classification of polymeric gels

On the basis of the cross-linking character, polymeric gels can be classified into three main categories: (1) physical gels, (2) covalently cross-linked gels, and (3) entanglement network gels (Bohidar et al., 2003).

1.3.1 Physical gels

Generally, various kinds of physical gels are being prepared using synthetic, semisynthetic, and natural polymers (Kavanagh and Ross-Murphy, 1998). Some examples of constituent polymers in the formula of various kinds of physical gels are carbomers, gelatin, marine polysaccharides, plant polysaccharides, etc. Various kinds of physical gels can be prepared by using different synthetic polymers such as ionomers in solvents of low dielectric constant, isotactic polymers in solvents, and numerous A-B-A type block copolymers (Osada et al., 2001). In the polymeric gel systems, the aggregation of heterostructural chains of polymeric structure is often a general

attribute. Because the cross-linking of these gels is temporary in nature, a number of physical gels are thermoreversible as these can be liquefied and/or dissolved. These physical gels will also reform again without any occurrence of the real hysteresis. However, thermoreversibility and irreversibility of different kinds of physical gels depend on the material types and characters. This is very attention grabbing to note that in the physical gels, the length of disordered chain in between the junction zones is usually shorter and less flexible in comparison with that of the sections of "random coil" in the chemical gels (Kavanagh and Ross-Murphy, 1998). On the basis of further differences of the physical gels, these can be categorized as follows: (1) "strong" physical gels and (2) "weak" physical gels (Rogovina et al., 2008). Both the physical gels (i.e., "strong" physical gels and "weak" physical gels) respond solid-like at the smaller deformations. The strong physical gels (e.g., swollen elastomers) are also solid in nature at the larger deformations, whereas the weak physical gels are actually structured fluids, so that these flow just about as the liquids at the larger deformations (Kavanagh and Ross-Murphy, 1998). The thermodynamic profiles of the formation of physical gels may also be different: one-phase systems or two-phase systems with the lower or upper critical solution temperatures and the systems with deficient phase separation. The general and most important properties of different kinds of physical gels are that the networking junctions appear and disappear reversibly with the alteration of temperatures. In general, these physical gels produced on the cooling of solutions and melting on applying heat. However, the polymeric phase encompasses the basis for networking junctions in the polymeric solutions containing lower critical solution temperature and is released with an increment in the temperature correspondingly (Rogovina et al., 2008). Recent years, numerous physical gels are employed as vehicle for the delivery of various kinds of drugs (Malakar et al., 2012; Jana et al., 2014a,b; Das et al., 2013, 2017).

1.3.2 Covalently cross-linked gels

A variety of covalently cross-linked polymeric gels (also known as chemically cross-linked polymeric gels) are prepared through different methods, including addition polymerization of oligomeric multi-functional precursors, vulcanization of high-molecular weight linear polymeric chains, end linking of the reactive chains with branching units, etc. (Kavanagh and Ross-Murphy, 1998; Bohidar et al., 2003). During the past few decades, different covalently cross-linked polymeric gels have widely been explored. Both the smaller and larger deformation mechanical characteristics of these gels have been characterized in various studies (Bohidar et al., 2003). Because the networking junctions in the covalently cross-linked gels are formed via covalent bonding in between the macromolecules and the cross-linkers, such gels can be damaged only via the bond rupturing or by the thermal degradations of constituent polymer(s) (Osada et al., 2001). Covalently cross-linked gels are usually characterized by means of an equilibrium elasticity modulus (Kavanagh and Ross-Murphy, 1998). The issues accountable for the elasticity of covalently cross-linked polymeric gels are the larger volume of solvent(s) and the flexibility of polymeric chains. On the basis of the volume of the solvent(s), these can be fragile or even able to show certain degree

of elasticity. Simultaneously, these gel systems are characterized by rubber-like elasticity. In addition, it is notable here that the polymeric hydrogels are a unique group of covalently cross-linked polymeric gels, which are produced by cross-linking of 3-D hydrophilic polymeric networking swollen in the aqueous medium (Hennink and van Nostrum, 2002).

1.3.3 Entanglement network gels

The entanglement network gels of polymers are formed/prepared via the topological interactions of polymeric chains either in the melt or in the solutions, when the concentration and molecular weight of the formed/prepared entanglement network gels becomes greater than the critical molecular mass for the entanglements (Kavanagh and Ross-Murphy, 1998). This kind of entanglement networking is usually seen in the polymeric gels containing polymer(s) of higher-molecular weight(s), particularly in the elastomers. These act like the "pseudogels" at the frequencies greater than the lifetime of the topological entanglements (Osada and Khokhlov, 2001). These entanglement network polymeric gels are usually dissolved in a suitable solvent to produce more dilute polymeric solutions. In contrast, the covalently cross-linked gels generally swell but not dissolve. However, the entanglement networkings can be recognized from the covalently cross-linked gels through the using of simple rheological procedures such as dynamic mechanical analyses (Kavanagh and Ross-Murphy, 1998).

1.4 Hydrogels

1.4.1 Hydrogels: basics and properties

Hydrogels are mostly made of hydrophilic polymer(s) (Mishra and Mishra, 2016). These are of 3-D structure with the hydrophilic networking able to absorb a larger amount of liquids or the biological fluids devoid of losing their physical structures as these do not dissolve speedily (Peng et al., 2011; Nayak and Pal, 2016). The capability of hydrogels for the absorbing fluids arises because of the hydrophilic functional groups of the constituent polymeric backbone. The penetration of the liquids inside the hydrogel structure permits free diffusion of the solute molecules, whereas the constituent polymer(s) serves up as the hydrophilic matrices to hold the liquid (Anisha et al., 2010).

Hydrogels contained a single polymer are not capable of meeting divergent requirements in terms of properties and performances (Hua et al., 2010; Mishra and Mishra, 2016). These are produced through the adding of the cross-linked polymer(s) to the aqueous medium or the biological fluids, and afterward, permitting to swell (Nilimanka, 2013). Generally, hydrogels can be produced from the polymer(s) containing different hydrophilic groups such as hydroxyl groups, carboxyl groups, sulphonic acid groups, amide groups, and imide groups, either embedded in or grafted to their polymeric structures. The essential characters of hydrogels are hydrophilicity, higher swelling capability, liquid capturing ability, softness, elasticity, flexibility, absorbent, etc. (Hoffman, 2002). For the chemical and physical hydrogels, various

kinds of macromolecular structural features can be constructed, which comprises linear copolymers, entangled cross-linked networking of linear polymers and their copolymers, complexes of polyion/polyion, polyion/multivalent ions or hydrogen bonding, IPNs and semiinterpenetrating polymer networkings (semi-IPNs), hydrophobic domain−stabilized hydrophilic networkings, physical blending of polymers, etc. (Ullah et al., 2015).

For the syntheses of physical cross-linked hydrogels and chemically cross-linked hydrogels, a variety of synthesis methodologies are used. The important methods for the syntheses of physical cross-linked hydrogels are hydrogen-bonding interaction, hydrophobic interaction, ionotropic interaction, stereo-complexation, inclusion complexation (i.e., supramolecular chemistry), etc. Chemically cross-linked hydrogels are synthesized by polymerization, radiation, small molecule cross-linking, polymer/polymer cross-linking (i.e., condensation reaction) etc. (Ebara et al., 2014). Recently, click chemistry has been employed to synthesize click hydrogels of different patterns and dimensions (Jiang et al., 2014). Because of the higher yielding, higher degree of reactivity, excellent specificity, milder reaction states, bioorthogonality, etc., click chemistry approaches have emerged as the most potential strategy to synthesize click hydrogels. These click hydrogels are also being currently developed for the use in different biomedical applications such as tissue engineering, wound dressings, drug delivery, cell and enzyme encapsulations, etc. (Crescenzi et al., 2007) (Fig. 1.1).

1.4.2 Classification of hydrogels

Hydrogels are classified according to their polymeric composition, configuration, type of cross-linking, and network electrical charge.

1.4.2.1 Classification of hydrogels according to the polymeric composition

According to the polymeric composition, hydrogels are classified into these three categories: (1) homopolymeric hydrogels, (2) copolymeric hydrogels, and (3) IPN hydrogels (Ahmed, 2015).

Homopolymeric hydrogels are referred to the polymeric networking hydrogels originated from a particular class of monomer, which is an essential structural component encompassing of any polymeric networking. Even, these hydrogels may comprise cross-linked skeletal structure depending on the character of monomer and polymerization methods (Ahmed, 2015).

Copolymeric hydrogels generally contain two or more dissimilar monomeric species with at least a single hydrophilic monomeric constituent, assembled in a random, block, and/or alternating structural configuration along the polymeric networking chains.

IPN hydrogels are the multipolymeric structures. These are recognized as a special category of polymeric hydrogels made of two independent cross-linked polymers contained in a networking form (Nayak and Pal, 2015; Pal and Nayak, 2015). When

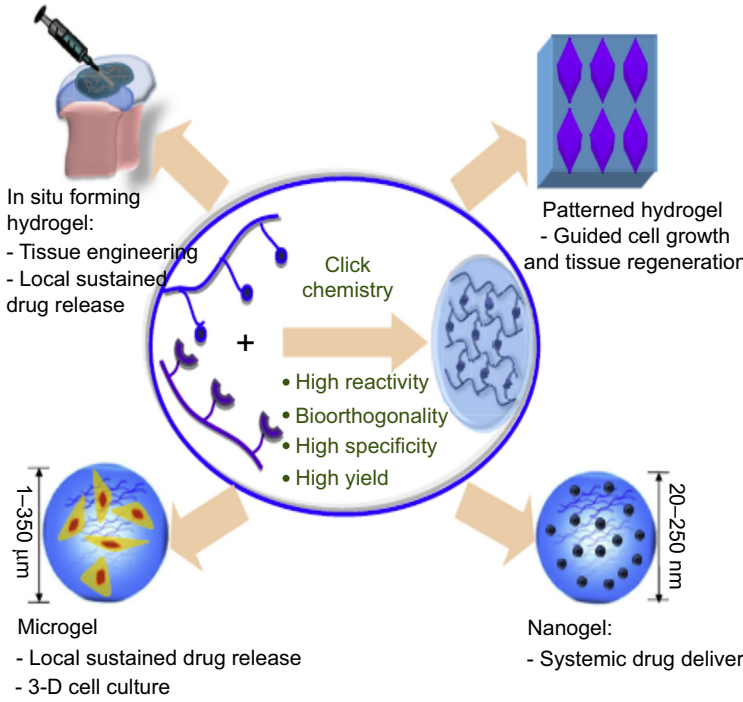

Figure 1.1 Preparation and potential biomedical applications of click hydrogels, microgels, and nanogels.
From: Jiang, Y., Chen, J., Deng, C., Suuronen, E.J., Zhong, Z., 2014. Click hydrogels, microgels and nanogels: Emerging platforms for drug delivery and tissue engineering. Biomaterials 35, 4969−4985; Copyright © 2014 Elsevier Ltd.

a second polymeric hydrogel networking is polymerized within a prepolymerized hydrogel structure, an IPN structure is formed (Jana et al., 2013). This process is usually carried out via dipping a prepolymerized hydrogel matrix into a solution containing monomers along with an initiator for the polymerization. The constituent polymers in the IPNs may be synthetic, semisynthetic, and/or natural polymers. The major benefits of IPN-based hydrogels are that comparatively denser hydrogel matrices can be fabricated, which feature the improved mechanical characteristics (e.g., stiffer, tougher, etc.), more extensively controllable physical characteristics in comparison with the conventionally structured hydrogels (Ignat and Stanciu, 2003). In the semi-IPN hydrogel, one of the constituent polymer(s) should be a cross-linked polymer and the other constituent polymer is non−cross-linked polymer (Nayak and Pal, 2015). Semi-IPN hydrogels are able to maintain a fast kinetic response rates to the temperature or pH more efficiently, while still as long as most of the advantages of IPNs in the drug delivery applications (e.g., higher drug encapsulation efficiencies, controlled size distributions, modifying swelling behavior, slower drug releasing, etc.) (Ignat and Stanciu, 2003).

1.4.2.2 Classification of hydrogels according to the configuration

Depending on the physical structures and chemical compositions, hydrogels can be classified into these three categories: (1) amorphous (noncrystalline), (2) crystalline, and (3) semicrystalline (i.e., complex mixture of crystalline as well as amorphous phases).

1.4.2.3 Classification of hydrogels according to the type of cross-linking

On the basis of cross-linking types, i.e., physical or chemical characters of the cross-linking junctions, hydrogels can be classified into two different categories: (1) physically cross-linked networking hydrogels and (2) chemically cross-linked networking hydrogels (Maitra and Shukla, 2014). The physically cross-linked networking hydrogels comprise transient junctions of cross-linking, which arise from either the polymeric-chain entanglements or the physical interactions, such as hydrophobic interactions, ionotropic interactions, hydrogen bonding, etc. In contrast, chemically cross-linked networking hydrogels comprise the permanent junctions of cross-linking. The mechanisms of formations of physically and chemically cross-linked networking hydrogels are presented in Fig. 1.2.

1.4.2.4 Classification of hydrogels according to the network electrical charge

Based on the absence and presence of the electrical charges located on the cross-linked polymeric chains in the hydrogel structure, hydrogels can be classified into four main

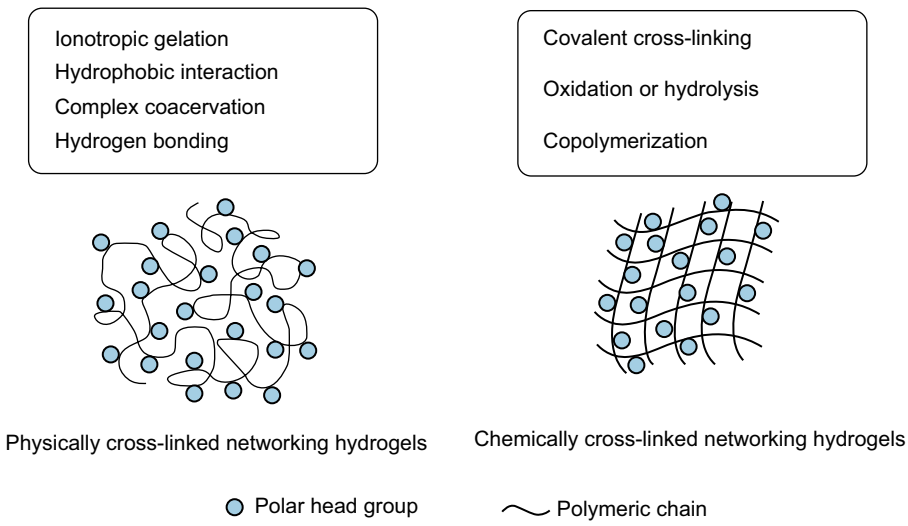

Figure 1.2 Formation mechanisms of physically and chemically cross-linked networking hydrogel formations.

classes: (1) ionic (cationic and anionic) hydrogels, (2) nonionic (i.e., neutral), hydrogels, (3) ampholytic hydrogels (possessing both acidic and basic groups), and (4) Zwitterionic (i.e., polybetaines; containing both cationic and anionic groups in each repeating units) (Ahmed, 2015).

1.4.3 Polymers used in the preparation of hydrogels

The constituent polymers in the in the preparation of hydrogels may be synthetic, semisynthetic, and/or natural polymers (Maitra and Shukla, 2014; Ahmed, 2015). Some important examples of the synthetic polymers employed in the preparation of hydrogels are carbomer (Carbopol polymers), poloxamers, polyvinyl alcohol, poly *N*-vinyl pyrrolidone, polyethylene oxide, polyacrylic acid, polymethacrylic acid, polystyrene, polyethylene glycol diacrylate/dimethacrylate, polyethylene glycol acrylate/methacrylate, etc. The semisynthetic polymers extensively utilized in the preparation of hydrogels are methylcellulose, carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, etc. Due to the improved biocompatibility, water absorption ability, controlled swelling capability, and economical reason, numerous natural polymers are being employed in the preparation of hydrogels and the natural polymers employed for this purpose are chitosan, alginates, hyaluronic acid, pectins, gellan gum, dextran, xanthan gum, guar gum, psyllium polysaccharide, tamarind polysaccharide, locust bean gum, sterculia gum, gum tragacanth, etc.

1.4.4 Smart hydrogels

Hydrogels possessing sensor characteristics (environmental stimuli) can experience reversible volume phase transitions or gel−sol phase transitions on small alterations of the environmental stimuli (Chaterji et al., 2007). These kinds of stimuli responsive hydrogels are also described as "smart" hydrogels (Fig. 1.3). Smart hydrogels generally react to the different environmental stimuli, such as temperature, pressure, pH, light, sound, ultrasound, electric field, magnetic field, mechanical stress, solvent composition, chemicals, or ionic strength, or a combination thereof (Ebara et al., 2014). Reaction to the environment stimuli is an essential event in the living systems. Mimicking this sensing character of the living systems presents a ready solution to the numerous medical problems (Chaterji et al., 2007). These are capable of responding to the minute alterations in the ambient stimuli and also show remarkable changes of hydrogel characters. Stimuli responsive smart hydrogels can be either physical- or chemical-stimuli responsive in character. Different physical stimuli (such as temperature, pressure, light, sound, electric field, magnetic field, mechanical stress, etc.) can influence the level of energy sources and modify the interactions in the molecular level at the critical onset. Chemical stimuli (such as pH, solvent composition, chemicals, ionic strength, etc.) can modify the molecular interactions in between the polymeric chains and the solvent(s). In recent times, different biochemical stimuli have also been recognized as a special class of stimuli, which entails the responses to enzymes, ligands, antigens, antibodies, etc. (Ebara et al., 2014).

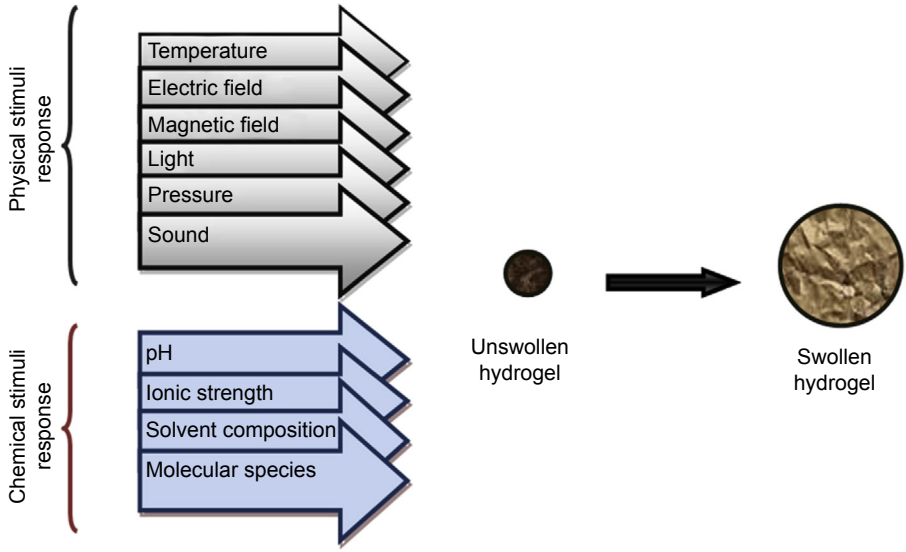

Figure 1.3 Smart (stimuli responsive) hydrogel.
From: Ahmed, E.M., 2015. Hydrogel: preparation, characterization, and applications: a review. Journal of Advanced Research 6, 105−121; Copyright © 2013 Production and hosting by Elsevier B.V. on behalf of Cairo University.

Significantly, smart polymeric hydrogels have been achieving the momentum in the biomaterial and biomedical researches. These smart polymeric hydrogels represent a newer invention of biomaterials, which are currently being synthesized at a prolific tempo for the use in biomedical uses, including drug delivery applications, scaffold systems for the tissue engineering prostheses, biosensors, actuators, templates for the nanoscale biomedical devices (biomedical nanoparticles) (Chaterji et al., 2007).

1.4.5 Superporous hydrogels

Superporous hydrogels are another special class of hydrogels having faster swelling capability with the pore sizes much bigger than the characteristic mesh sizes of conventional hydrogels (Hossein et al., 2007). The swelling rate of the superporous hydrogels is greatly faster in comparison with that of the conventional polymeric hydrogels and this dissimilarity can be understood in the light of the differentiation in the morphological characteristics of the two categories of hydrogels. As the mesh sizes of the conventional polymeric hydrogels are smaller, the swelling in such comparatively closed systems such as superporous hydrogels is restricted by the diffusion of water through the glassy-polymeric matrices. In contrast, these hydrogels comprise larger interconnected pores ensuing in the capillary-intake of liquids. These superporous hydrogels are prepared for the use as novel gastroretentive devices to increase the gastric residence time of various drugs in the gastrointestinal tract (Chen et al., 2000; Nayak et al., 2010; Malakar et al., 2014).

1.4.6 Biomedical applications of hydrogels

In general, hydrogels bear resemblance to the biological tissues (in the swollen condition) exhibiting remarkable mechanical capability because of these soft, flexible, and biocompatible characters (Mohite and Adhav, 2017). For this reason, hydrogels are being employed as biomaterials in medical and pharmaceutical applications (Peppas, 1986; Rokhade et al., 2007; Das et al., 2012). These are also widely used in some other industrial applications such as agriculture, water treatment, food, cosmetics, etc. (Maziad, 2004; Naziha et al., 2015). Hydrogels possess different kinds of physical forms viz. solid molding (utilized in the soft contact lenses), coatings (utilized as coating layer on inner capillary walls in the capillary electrophoresis and in catheters, implants, pills, tablets, etc.), polymeric membranes or polymeric sheets (utilized in gels of 2-D electrophoresis and as drug reservoirs in the transdermal patches), pressed powered matrices (utilized in capsules and pills for the oral administration), microparticles and beads (utilized as drug reservoirs in controlled drug releasing), encapsulated solid materials (utilized in osmotic pumps), encapsulated liquids (in the formation of gels on cooling or heating), adhesive agents (utilized in the preparation of bioadhesive carriers and wound dressings), etc. (Peppas, 1986; Rosiak and Yoshii, 1999; Kashyap et al., 2005; Das et al., 2012; Singh and Pal, 2011; Nayak et al., 2014; Jana et al., 2015; Ahmed, 2015). Some examples of hydrogels for biomedical applications are listed in Table 1.1.

1.5 Macrogels, microgels, and nanogels

On the basis of particle sizes (average diameters), polymeric hydrogels can be further classified into three classes: macrogels, microgels, and nanogels (Jiang et al., 2014). The individual gel particles possessing particle sizes of less than 1 μm are generally known as macrogels, whereas gels of submicron particle sizes (from 100 nm to 1 μm) are known as microgels (Musial et al., 2015). On the other hand, gels of particle sizes less than 100 nm are called as nanogels. Likewise, gels possessing particle-size range a little greater than 100 nm (in the vicinity of the nanometric range) are generally known as quasinanogels.

Microgels are often referred to the cross-linked latex particles, which swell in the aqueous environment and release liquids because of alterations in the thermodynamic conditions such as presence of various additional solvents, the alteration in the environmental temperatures, ionic strengths of the solutions, pHs, etc. (Vinogradov, 2006; Musial et al., 2015). Actually, the swelling pattern directs to the considerable hydration of the microgel particles. On the basis of the dispersion medium type, microgels exhibit extensive swelling pattern because of the presence of specific functional groups in the structure of constituent polymer(s) (Musial et al., 2015). As compared with the other carrier systems, microgels present some important benefits such as excellent control over the particle sizes and particle shapes, greater extent of colloidal stability, desired functionality, improved responsive behavior, etc. (Vinogradov, 2006). These abilities make microgels as an

Table 1.1 Some examples of hydrogels for biomedical applications

Biomedical applications	Hydrogels	References
Drug delivery	pH-sensitive sodium alginate/polyvinyl alcohol hydrogel beads for controlled release of diclofenac sodium	Hua et al. (2010)
	Alginate−methyl cellulose mucoadhesive microcapsules	Pal and Nayak (2011)
	Potato starch−blended alginate beads for prolonged release of tolbutamide	Malakar et al. (2013a)
	Fenugreek mucilage−gellan mucoadhesive beads for controlled release of metformin HCl	Nayak and Pal (2014)
	Modified starch (cationized)−alginate beads containing aceclofenac	Malakar et al. (2013b)
	Jackfruit seed starch−blended gellan gum mucoadhesive beads of metformin HCl	Nayak et al. (2014)
	Ispaghula mucilage−alginate mucoadhesive beads	Nayak et al. (2013)
	Jackfruit seed starch−alginate mucoadhesive beads of metformin HCl	Nayak and Pal (2013)
	Calcium alginate/gum Arabic beads containing glibenclamide	Nayak et al. (2012)
	Tamarind seed polysaccharide−alginate mucoadhesive microspheres for oral gliclazide delivery	Pal and Nayak (2012)
	Tamarind seed polysaccharide−alginate composite beads for controlled diclofenac sodium delivery	Nayak and Pal (2011)
	Tamarind seed polysaccharide−alginate mucoadhesive microspheres for oral metformin HCl delivery	Nayak et al. (2016)
	Okra gum−alginate mucoadhesive beads for controlled glibenclamide release	Sinha et al. (2015)
	Chitosan−tamarind seed polysaccharide IPN microparticles	Jana et al. (2013)
	Chitosan−poly(ethylene oxide-g-acrylamide) IPN hydrogel microspheres for the controlled release of capecitabine	Agnihotri and Aminabhavi (2006)

	Zinc−pectinate−sterculia gum IPN beads encapsulating ziprasidone HCl	Bera et al. (2015)
	Novel alginate hydrogel core−shell systems for combination delivery of ranitidine HCl and aceclofenac	Jana et al. (2015)
	pH-sensitive chitosan-*N*, *N*′-dimethyl acrylamide semi-IPN microspheres	Babu et al. (2008)
	Semi-IPN microspheres of acrylamide grafted dextran and chitosan for controlled release of acyclovir	Rokhade et al. (2007)
Protein delivery	γ-irradiated chitosan−polyvinyl pyrrolidone hydrogels as pH-sensitive protein delivery system	Dergunov and Mun (2009)
	Alginate/hydroxypropyl methylcellulose hydrogel beads for in vitro release of bovine serum albumin	Argyrios et al. (2008)
Tissue engineering	Ionically cross-linked alginate hydrogels as scaffolds for tissue engineering	Kuo and Ma (2001)
	In situ cross-linkable hyaluronan hydrogels for tissue engineering	Shu et al. (2004)
Wound dressings	Sterculia gum−polyvinyl alcohol−polyvinyl pyrrolidone for making hydrogel wound dressings	Singh and Pal (2011)
	Radiation synthesis poly (*N*-vinyl-2-pyrrolidone)-κ-carrageenan hydrogels for wound dressing applications	Sen and Avci (2005)
	Polyvinyl alcohol and polyvinyl pyrrolidone−blended hydrogel for wound dressing	Razzak et al. (2001)
Enzyme immobilization	Alginate/bacterial cellulose nanocomposite beads	Kim et al. (2017)

improved class of hydrogel matrices with some important properties such as controlling drug-binding and drug-releasing kinetics, long-standing stability and better shelf life, biodegradation, biodistribution, bioaccumulation, biotargeting, and functionality in perspective of the uses in drug delivery (Vinogradov, 2006; Smeets and Hoare, 2013).

The term "nanogel" was first described to define cross-linked bifunctional networking of a polyion and a nonionic polymer candidate for the delivery of polynucleotides made of cross-linked polyethylene imine and polyethylene glycol (Sultana et al., 2013). Nanogels are defined as the nanosized particles synthesized via chemically or physically cross-linked polymeric networking formations, which are capable of improved swelling in the aqueous milieu (Kuroda et al., 2002). With the continuous progress in the nanotechnological field, the developments of numerous nanogels being demonstrated the prospective of the nanogels for delivering a variety of drugs in sustained and controlled way. One of the important advantageous aspects of nanogels is the faster swelling−deswelling properties. These nanogels exhibit excellent permeation abilities because of the very smaller size and are also capable of crossing the blood−brain barrier. In addition, nanogels are capable of improving the solubilization of numerous hydrophobic drug candidates and diagnostic substances (Soni et al., 2016). Nanogels form various complexes with different drugs, proteins, peptides, and DNA materials. These are also employed to coat the surfaces of particles, solid surfaces including cells, liposomes, etc. (Nishikawa et al., 1996). Nanogels are macromolecular gel systems and these are particularly designed to attain longer range of circulation half lives of their cargo in vivo, with their capability to transport this cargo at the desired site(s) (Fig. 1.4).

Recently, besides hydrogel preparations, click and pseudo-click chemistry strategies are employed to prepare various microgels and nanogels. These click microgels and nanogels are also found as effective biomimetic scaffolds, systemic drug delivery carriers, local drug delivery carriers, etc., in various biomedical and pharmaceutical applications (Jiang et al., 2014) (Fig. 1.1).

1.6 Organogels

Organogels are actually semirigid systems having an immobilized externally apolar phase, and the apolar phases get immobilized within the spaces of 3-D networking structured because of the physically interactions between various kinds of self-assembled structural compounds, which are known as gelators (Sahoo et al., 2011). Thus, the organogels are considered as bicontinuous systems comprising gelators and apolar solvent(s) (i.e., apolar phases) that may or may not restrain aqueous molecules trapped within the different self-assembled structures of gelator(s) (Suzuki and Hanabusa, 2010). The gelators in the formula of organogels when incorporated in the concentration less than 15% (approximately) may experience chemically and physically interactions to form self-assembled fibrous structural systems that get entrapped with each other ensuing in the development of 3-D networking structures of various organogels. Some common examples of gelators utilized in the preparation

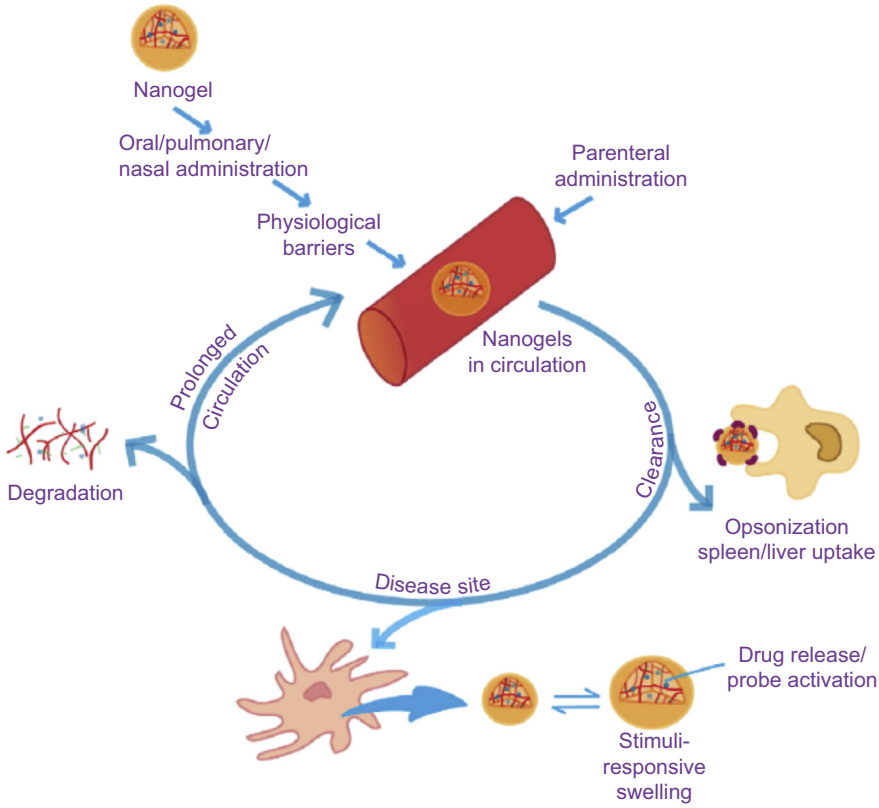

Figure 1.4 In vivo behavior of nanogels.
From: Soni, K.S., Desale, S.S., Bronich, T.K., 2016. Nanogels: an overview of properties, biomedical applications and obstacles to clinical translation. Journal of Controlled Release 240, 109−126; Copyright © 2015 Elsevier B.V.

of organogels include lecithin, cholesteryl anthraquinone derivates, sterol, sorbitan monostearate, etc. The developed 3-D networking structures prevent the flow of externally apolar phases. Some important physic-chemical characteristics of organogels comprise viscoelasticity, thermostability, thermoreversibility, nonbirefringence, chirality effects, optical clarity, etc. Generally, organogels are the thermodynamically stable systems (Sahoo et al., 2011). The thermoreversible characteristics of various organogels have achieved a great deal of interests for the prospective uses of these as system in drug delivery applications. Some important kinds of organogel-based systems investigated as drug delivery carriers or vehicles are lecithin organogels, pluronic lecithin organogels, premium lecithin organogels, polyethylene organogels, sorbitan organogels, microemulsion-based organogels, etc. (Sahoo et al., 2011). Even, organogels have already been investigated as matrices for the delivery of various drugs and bioactive materials (Kumar and Katare, 2005; Vintiloiu and Leroux, 2008).

1.7 Bigels

In general, bigels contain two immiscible liquid phases that are independently stabilized by means of the independent gelators (Cates and Clegg, 2008). The immobilization of the immiscible liquid phases causes a noticeable decrease in the interfacial free energy. Because of the microarchitectural structure, bigels are also known as biphasic gels.

Bigels have shown a number of uses in the biomedical and pharmaceutical industrial applications. Simple and economical preparation procedure of bigels averts the high expenditure incurred during the preparation of nanoliposomes, nanoparticles, etc. One of the significant causes for selecting bigels in various applications is to facilitate synergistic actions of both oleogels and hydrogels within a single gel system. Because of the combination of oleogels and hydrogels, bigels are being utilized as carriers or vehicles for the simultaneous delivery of both lipophilic and hydrophilic drug candidates (Rehman et al., 2014). These are able to show moisturizing action if applied onto the skin surface. Bigels are spreadable in nature and also offer the improved hydration of the skin surface (*stratum corneum*). The easy and higher extent of water washability characteristics of the bigels makes these as a suitable vehicle/carrier for the topical delivery of several kinds of bioactive molecules (Cates and Clegg, 2008).

1.8 Emulgels

Emulgels are mainly emulsion-based gels. There are two classes on the basis of the dispersed and continuous phases (Asija, 2013). These are either of the oil/water or water/oil types, which are gelled by incorporating a gelling agent. The development of emulgels is controlled by characteristics of the nonionic surfactants, which are also reliant on the temperature. These nonionic surfactants produce micelles at the lower temperature and at the higher temperature; there is a conversion to reverse micelles. At the transition temperature, the surfactant-phase coexists both with aqueous and oily phases. These result the shape of soft emulgels. On the basis of the forces acting in between the droplets of dispersed phase, emulgels are also categorized into two different classes: adhesive emulgels and nonadhesive emulgels. The adhesive emulgels are the emulsion-based gels, which do not relax and control a higher degree of packing because of the occurrence of attractive forces in between the oil droplets. In contrast, the nonadhesive emulgels usually relax in a fashion that it dilates to the condition of nondeformed droplets of emulsion(s) (Panwar et al., 2011).

Recent years, a considerable research effort has been directed toward the use of emulgel systems for the topical drug delivery. The topically drug releasing from the emulgel systems is actually controlled by the drug diffusion mechanism. The internal phase of the emulgel systems operates as a drug reservoir for different drugs and these release the drugs slowly into the external phase of the emulsion. The drugs loaded within the emulgels permeate through the *stratum corneum* by both intracellular and intercellular penetrations. Emulgels possess a number of positive dermatological characteristics. These are thixotropic in nature, and the spreadability, extrudability, removability, greaselessness, emollient action, longer shelf life, and an agreeable appearance make the emulgels preferable over the conventional gel systems (Panwar et al., 2011).

1.9 Aerogels

Aerogels are generally sol−gel materials that the liquid constituent of the gel systems have been restored with the gas to disappear the integral solid nanostructures devoid of the collapsing of pores in which, by volume, 90%−99% is air (García-González et al., 2011). The density of aerogels range 1−1000 kg/m^3 and this stimulates the remarkable alterations in the gel characteristics. Because of the higher porosity, dual structural features of both macroscopic (i.e., condensed state matter) and microscopic (i.e., nanoscale skeleton), aerogels show multipurpose and unique characters such as highly specific surface area, refractive index, dielectric constant, sonic velocity, ultralow thermal conductivity, ultrawide adjustable density, etc. (Du et al., 2013).

In general, the preparation methodology of aerogels includes these following steps:

1. Sol−gel transition (i.e., gelation): Nanoscale sol particles are cross-linked and hierarchically assembled into a wet−gel spontaneously or catalyzed by means of catalysts via hydrolysis or condensation reactions.
2. Network perfection (i.e., aging): Mechanically reinforce the weak solid structures produced during the sol−gel procedure.
3. Gel−aerogel transition (i.e., drying): The solvent within the wet−gel is restored by air without severe damage of microstructure.

All these above three steps decide the aerogel microstructure. These also influence the characteristics and uses of aerogels. There are different kinds of polymeric aerogels such as cellulosic aerogels, polysaccharide-based aerogels, resin-based aerogels, polyamide-based aerogels, polyvinyl alcohol−based aerogels, etc. All these aerogels exhibit different kinds of structures and characteristics. On the basis of polymer types and preparation conditions, the structures of the polymeric aerogels can be changed from the colloidal-like nanoparticles to the nanofibrillar or microfibrillar networkings, even to the sheetlike structures (Du et al., 2013).

1.10 Xerogels

Xerogels are a type of solid-formed gels, which are being prepared through drying slowly at the room temperature with an unconstrained shrinkage (Czarnobaj, 2008). Xerogels generally possess the properties of higher porosity and larger surface area together with very smaller pore sizes. These are prepared by the sol−gel methodology. In the sol−gel method for the preparation of xerogels, various metal alkoxide precursors, water and ethyl alcohol, are required. The hydrolysis as well as condensation procedure of the liquid alkoxide directs to the preparation of the solid oxide networking (i.e., sol−gel transition process). The sol−gel transition process generated silica xerogels are safe materials in nature and also found biocompatible in vivo. These xerogel-based materials do not produce any kinds of adverse tissue reactions and also degrade to silicic acid within the body. The generated silicic acid is then eliminated through the kidneys (Czarnobaj, 2008).

1.11 Cryogels

Cryogels are generally supermacroporous gel networking developed by the cryogelation of apposite monomers or the polymeric precursors at the subzero temperature (Lozinsky, 2002). The advantageous aspect of the cryogel system is an exceptional combination of highly porous characteristics with sufficient osmotic stability and mechanical strength because of which the gel system is being predicted as prospective scaffold materials for a variety of biomedical uses (Lozinsky et al., 2003; Henderson et al., 2013). The most essential feature of cryogels is the simplest approach through which cryogels are synthesized and the application of aqueous solvent(s) for the cryogel syntheses making these fit for the diverse biological and biomedical applications (Henderson et al., 2013). Different modifications of these cryogel systems have been sought, which entails coupling of a variety of ligands to its surfaces, grafting of the polymeric chains to the surface of cryogels or IPN of two or more polymers to develop a cryogel for diverse applications. The procedure of cryogelation is ideally thought to occur by means of these following steps: phase separation with the ice crystal formations, cross-linking, and polymerization followed by thawing of the ice crystals forming an interconnected porous cryogel network (Kumar et al., 2010).

1.12 Conclusion

Gels are generally semisolid systems. The polymeric gels are made of polymers, which structure 3-D networking by virtue of covalent or noncovalent bondings in the liquid medium ensuring the elastic characters of the gel. During the past few years, numerous physical gels, covalently cross-linked gels, and entanglement network gels were investigated, developed, characterized, and utilized in a variety of industrial applications, including biomedical and pharmaceutical applications. Nowadays, numerous polymeric gels have gained an attractive position in the wide-reaching researches around the world by numerous materials scientists, polymer engineers, chemical engineers, chemists, biomedical scientists, pharmaceutical scientists, etc. The important kinds of these polymeric gels, which find their industrial applications are hydrogels (including smart hydrogels, superporous hydrogels, IPN hydrogels, etc.), macrogels, microgels, nanogels, organogels, emulgels, aerogels, xerogels, cryogels, etc.

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Protein-based gels: preparation, characterizations, applications in drug delivery, and tissue engineering

2

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2.1 Introduction

Gels are defined as cross-linked three-dimensional (3-D) structures consisting of two components, namely, structuring materials, and solvent phase. These structuring materials can be either organic or inorganic molecules and may either be cross-linked by physical or chemical interactions (Sahiner et al., 2006). The interactions among these structuring materials are responsible for the formation of the gel network. Interest in the protein biopolymers for fabricating gels has increased over the recent years. These biopolymers have favorable properties, such as water solubility, biocompatibility, nontoxicity, and sustainability making them hold immense potential for applications in drug delivery and tissue engineering (Jiang et al., 2014). These structuring agents are usually derived from natural sources such as plant and animal origins. Because the protein and the peptides are readily available, they are inexpensive and often preferred over synthetic polymers. This chapter provides an in-depth review on the different protein-based gels including hydrogels, microgels, and nanogels. This entry also provides insight into the applications of the proteins such as collagen, gelatin, elastin, and silk proteins in drug delivery and tissue engineering.

2.2 Types of protein-based gels

As discussed earlier, the gels are defined as 3-D network of structuring agents, which immobilizes the solvent phase. The designing of the 3-D network can be achieved in the various length scales, i.e., macro- to nanoscale. Depending on the length scale of the gels, they are often categorized as hydrogels, microgels, and nanogels. The hydrogels and the organogels are usually categorized as macrogels. If the dimension of the gels is in the range of 1−1000 μm, then the gels are regarded as microgels. On the other hand, a size range of 1 and 100 nm suggest the formation of nanogels.

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This section details about the different types of protein-based gel systems and their methods of preparation.

2.2.1 Hydrogels

The word hydrogel is made up of two words, namely, hydro (meaning water) and gel (meaning a jellylike substance) i.e., hydrogels usually consist of an aqueous solvent as the liquid phase. The structuring agents are usually hydrophilic polymers, which form 3-D polymeric networks. Although inorganic structuring agents for the formation of hydrogels have also been proposed, in this study, we will restrict ourselves to the polymeric gels. The formation of the 3-D network of the polymeric chains is either via physical or chemical cross-linking. Depending on cross-linking methodologies, the hydrogels can be categorized either as chemical or physical gels. Chemical gels or permanent gels are formed when the polymers are cross-linked via the formation of a covalent bond. Such gels possess low processibility as the covalent bonds are formed during the formation of the gels and cannot be easily modified or altered. Physical (reversible) gels can allow easy processibility and hence eliminate the need for the chemical cross-linking agents, which might be toxic sometimes.

Furthermore, the hydrogels have been further classified as conventional and stimuli-responsive. Conventional hydrogels absorb water when introduced into an aqueous medium and exhibits constant equilibrium swelling. The swelling process of such hydrogels remains unaffected by the changes in the surrounding environmental conditions, namely, temperature, pH, and magnetic and electric field. Drug release from these hydrogels takes place by diffusion mechanism and can be regulated by tailoring the movement of the drug molecules through the polymer matrix and/or inducing erosion of the polymer matrix of the hydrogels. On the other hand, stimuli-responsive hydrogels exhibit alteration in the swelling properties in response to the changes in the surrounding environment. Drug release from these hydrogels is usually initiated by the change in the surrounding environment of the hydrogels. Some of the environmental changes include chemical changes, temperature, pH, or enzymatic activity. Stimuli responsive hydrogels are found useful for targeted drug delivery to localized areas making them popular for biomedical applications. Such hydrogels have also found applications in tissue engineering.

2.2.2 Microgels

If the gellike features are at the microscopic scale, the gelled structures are categorized as “microgels.” Microgels represent a 3-D network, which comprises cross-linked polymer molecules in the micron size range (0.5−5 μm). Such structures usually possess both polymeric and particle-like characteristics. The structure of the microgels owes its stability to the presence of covalent bonds and strong noncovalent interactions (Dickinson, 2015; Farjami and Madadlou, 2017). Microgels are increasingly being considered as important colloidal units and stabilizing agents. This can be accounted to their ability to undergo deformation under stress, elicit surface activity depending on their composition and show environment-responsive characteristics due to the change in the temperature and pH (Dickinson, 2015; Farjami and Madadlou, 2017).

2.2.3 Nanogels

The advancement of nanotechnology in the field of medicine has enabled sustained delivery of essential therapeutic biomolecules at the nanoscale. Recent years have seen an emergence of nanogels, liposomes, lipid nanoparticles, and polymeric nanoparticles as nanocarriers for drug delivery. Nanogels are hydrogel networks at a nanoscale range. They are typically of the size 1−100 nm. Nanogels exhibit the capacity of high drug loading.

Because the diameter of the nanogels is very small, they have lesser surface-to-volume ratio. This facilitates their movement through the cellular membrane, increases cellular uptake of the drug and enables the targeting of the therapeutic agents to the specific organs or tissues. Novel nanoscaled platforms are being created for applications in therapeutics and diagnostics (Loo et al., 2005; Tréhin et al., 2006; Cyrus et al., 2006; Zharov et al., 2006).

Nanogels are classified into two major classes: firstly on the basis of their environment-responsive nature, and secondly on the type of cross-linking technology applied. On the basis of responsive nature, nanogels can be categorized as stimuli responsive or nonresponsive gels (Soni et al., 2016). Stimuli responsive gels swell up due to environmental changes, viz., electric and magnetic fields, pH, and temperature. Nonresponsive gels just swell up when placed in an aqueous environment. Based on the second classification type, nanogels are of two types: chemical cross-linked gels or physical cross-linked gels.

2.3 Preparation of the protein-based gels

2.3.1 Methods of preparation of hydrogel

Natural structural building blocks for hydrogel fabrication are numerous and are derived from variety of nature-based components such as proteins (e.g., collagen, silk, elastin, gelatin, etc.). These materials have immense potential to form hydrogels as they exhibit chemical, mechanical, and structural resemblance to the extracellular matrix, thus offering superior biological compatibility, further substantiate precise cellular responses. Besides, proteolytic enzymes in the body result in degradation of the hydrogel matrices thereby rendering them one of the most potential candidates for biomedical applications (Varaprasad et al., 2017; Dang et al., 2017).

Jayaramudu et al. (2013) explained the use of simple free-radical polymerization method for the fabrication of protein-based hydrogels in which protein molecules contribute to the 3-D interpenetrating hydrogel network. Pourjavadi et al. (2006) highlighted the preparation of collagen hydrogels in which of vinyl monomers underwent graft polymerization using cross-linkers within different initiator systems. Incorporating vinyl monomers enhances the hydrophilicity of protein molecule thus increasing water absorption ability of the hydrogels. In the process of cross-linking graft copolymerization of acrylic acid onto the protein backbone of hydrolyzed collagen in a homogeneous medium, *N*,*N*-methylene bisacrylamide acted as a cross-linker and potassium persulfate was an initiator (Varaprasad et al., 2017;

Pourjavadi et al., 2006). Dang et al. (2017) explained the preparation of thermosensitive hydrogels using acid soluble collagen. Akhtar et al. (2016) had vividly discussed both physical and chemical cross-linking processes involved in formation of hydrogels. The methods involve melt polycondensation of butanediol, polyethylene glycol (PEG), and dimethyl terephthalate, which were used to synthesize biocompatible polymers, formation of hydrogen bond, cross-linking via ionic interactions (cross-linking done at room temperature, physiological pH) (Akhtar et al., 2016). Our group had also reported the physical methods of hydrogel involving electrostatic, hydrophobic, or hydrogen-bonding interactions among the polymer chains. The electrostatic method of cross-linking involves ionic cross-linking and polyelectrolyte complexes. These methods use the ability of the anionic groups to form ionic bonds with that of the cationic chitosan. The various anionic molecules/metals that have been used to ionically cross-link CS include sulfates, citrates, phosphates, Pt (II), Pd (II), and Mo (VI), whereas the various polyelectrolytes include heparin, gelatin, DNA, albumin, xanthan, fibroin, alginate, carboxymethyl cellulose, dextran sulfate, chondroitin sulfate, hyaluronic acid, pectin, keratin, collagen, and polyacrylic acid. Details of the process can be seen in the report (Pal et al., 2013).

Protein-based hydrogels usually suffer from poor mechanical behavior. Due to this reason, scientists have proposed the cross-linking of the protein structure using cross-linking agents. The numerous reactive groups, acting as sites, in proteins, can be subjected to chemical modification and cross-linking for the fabrication of polymeric structures. The chemical method of formation of hydrogels includes the formation of a covalent bond. This is generally carried out using a cross-linker. A cross-linker may be defined as a molecule having at least two reactive groups and engages into a chemical reaction, which forms a bridged structure among the polymer chains (Pal et al., 2013). The cross-linking agents may either be of synthetic (e.g., glutaraldehyde) or natural (e.g., genipin) origin. Certain hydrophilic groups in the polymer, such as NH_2, COOH, OH, could be exploited for the development of the hydrogels. Covalent linkages between polymer chains may be identified based on the reactions of amine-carboxylic acid or an isocyanate-OH/NH_2 or formation of Schiff base. Hydrophilic polymers having −OH groups may be cross-linked through aldehyde (glutaraldehyde). However, tight conditions (such as high temperature, low pH, added methanol acting as quencher) are applied to establish cross-linking. Polymers having amine groups in polymer can be cross-linked using same cross-linker under milder conditions resulting in formation of Schiff bases and this is specially designed for the cross-linked protein synthesis, for example, gelatine (Akhtar et al., 2016). Furthermore, polyamides and polyesters may also be synthesized through condensation reactions among the −OH groups or−NH2 with −COOH or derivatives, respectively, for the synthesis of the hydrogel. Hydrophilic polymers containing amide groups can be efficiently cross-linked by *N*,*N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide (EDC). Kuijpers et al. (2000) used EDC to prepare gelatin hydrogels. Vulpe et al. (2016) showed the formation of collagen hydrogel using carbodiimide. Ahn et al. (2013) carried out a comparative analysis of cross-linking kinetics versus collagen hydrogel properties by using *N*-cyclohexyl-*N*′-(2-morpholinoethyl) carbodiimidemetho-p-toluenesulfonate (CMC), with that of EDC and the results

indicated that the CMC cross-linked hydrogels could be fabricated at ambient temperatures, whereas cross-linking with the EDC reaction had to be carried out at low temperatures as it was too rapid to control. Our group had showed the fabrication of gelatin hydrogel using genipin as the cross-linker. Gelatin remains in solution state above 35°C and gels below that temperature. The composition of gelatin is made up of a large variety of side chains. To make chemical modifications many methods have been proposed out of which one such technique is to introduce cross-linkable groups. It is carried out when warm gelatin solution is cast into a mold and cooled to room temperature or refrigerated and eventually cross-linked. Cross-linking is usually facilitated below a certain sol−gel transition temperature, which paves the way for physical cross-linking of triple helix regions to get stabilized with chemical cross-links such as genipin and glutaraldehyde. Temperature and concentration of genipin/glutaraldehyde have immense impact on the stability of the hydrogel. The use of cross-linking agents in suitable concentrations and higher cross-linking temperature can considerably enhance the mechanical behavior of the gels (Thakur et al., 2012a,b). Kapoor and Kundu (2016) nicely explained the fabrication of silk protein−based hydrogels in their review. Hydrogels are prepared mainly using gelation process induced by the treatments like change in pH, temperature, chemical cross-linking, etc. A schematic diagram of the process of fabrication is shown in Fig. 2.1.

Apart from the protein-based gels mentioned above, Maltais et al. (2010) reported the fabrication of soy protein hydrogels through the cold gelation process (as heat treatment could have a deteriorating effect for the sensitive active compounds in food). This method is carried out by the denaturation of soy protein solution by heating followed by cooling at room temperature and subsequent addition of a Maillard type cross-linker, i.e., glutaraldehyde or glycerol in the presence or absence of a salt (Caillard et al., 2010). Besides, hydrogels can also be prepared by making the polymer mixtures photosensitive, and thus, allowing photoinitiated chemical reaction. Irradiation with rays of suitable wavelength leads to the formation of a 3-D cross-linked matrix (Pal et al., 2013). Carvalho reported the synthesis of hybrid hydrogel using functionalized chitosan with gelatin. A mixture of both the solutions was homogenized. In the sequence, the photoinitiator (Irgacure 2959, 0.2%w/v) was added to the solution and kept on a magnetic stirrer. UV radiation (4.78 mW/cm^2, 6 W, at $\lambda = 254$ nm) was used to initiate photocrosslinking for 10 min in a dark chamber. After the exposure, the reaction was stopped by adding 1.0 mL of ascorbic acid solution (1.0%w/v) per sample, which acts as a biocompatible antioxidant (free radical scavenger) and eliminates free radicals in the medium. UV−vis spectroscopy was used to monitor the photocrosslinking reaction (Carvalho and Mansur, 2017).

2.3.2 Methods of preparation of microgel

Microgels are commonly synthesized by emulsification method. Herein, a multiphase mixture, usually consisting of a polymeric aqueous phase and an organic phase, is homogenized to form an emulsion of tiny aqueous droplets of hydrogel precursors within an organic phase. Factors such as the process of homogenization (ultrasonication

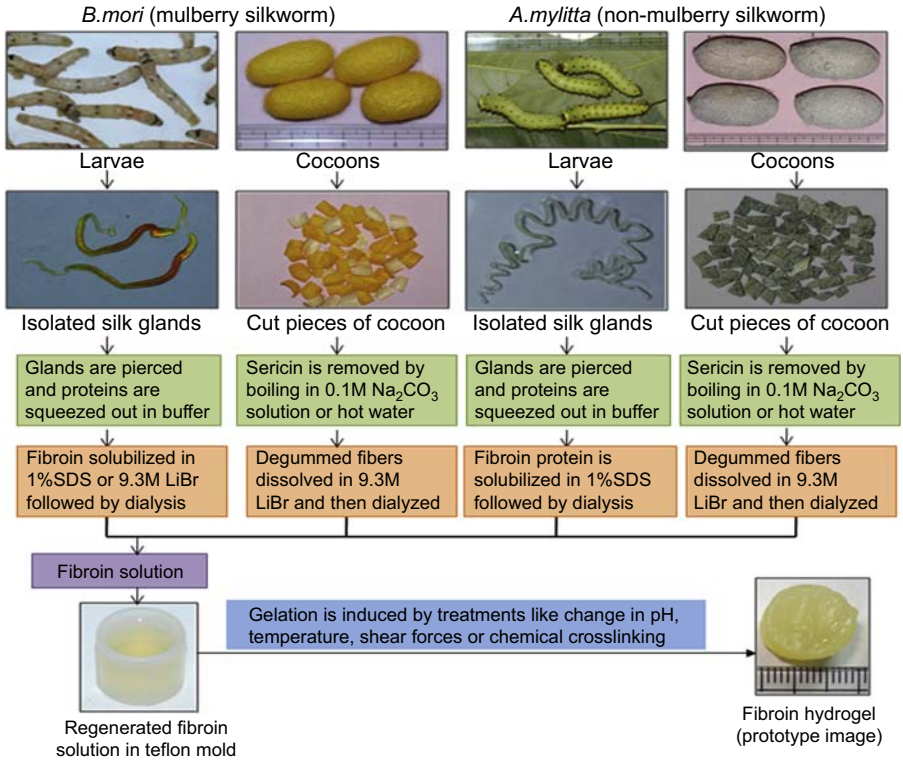

Figure 2.1 Schematic representation of silk fibroin protein-based hydrogel formation. Reproduced from Kapoor, S., Kundu, S.C., 2016. Silk protein-based hydrogels: promising advanced materials for biomedical applications. Acta Biomaterialia 31, 17−32, 2016/02/01/, with permission from Elsevier.

or mechanical agitation), the each phase's viscosity, and the presence/absence of emulsifiers have been found to alter the droplet size of the emulsified internal polymer phase. The internal droplets of the aqueous polymeric phase are then solidified either by chemical or physical cross-linking methods. The solidification of the polymeric phase results in the formation of spherical hydrogels. If the formed spherical hydrogels are micron-sized, then the hydrogels are regarded as microgels (Farjami and Madadlou, 2017; Oh et al., 2008). Besides, these hydrogels are regarded as matrix-type particulate hydrogels. During the preparation of such particulate hydrogels, the addition of an immiscible phase within the dispersed aqueous polymeric phase results in the formation spherical hydrogels, wherein a layer of the polymer matrix entraps the immiscible phase. Such particulate structures are regarded as core−shell particle hydrogels. Many biopolymeric protein−based (e.g., collagen) particulate hydrogels have been prepared and exploited for biomedical applications (tissue engineering, wound healing, and drug delivery) using this method. The major advantage of the preparation of the particulate hydrogels by this methodology is its easy processing techniques. Spizzirri et al. (2013) studied the fabrication of spherical hybrid hydrogels, which were

made up of gelatin and multiwalled carbon nanotubes. It was formed by emulsion polymerization in the presence of sodium methacrylate and *N*,*N*′-ethylenebisacrylamide (Spizzirri et al., 2013). Wu and McClements (2015) synthesized hydrocolloid microgels by electrostatic complexation technique utilizing aqueous solutions of gelatin and pectin electrostatic complexes. The assembly of the hydrogel particles was initiated due to positively charged proteins and negatively charged polysaccharides by electrostatic complexation. The association of the two biopolymers to form a water-in-water emulsion with a polymer-rich dispersed phase and a solvent-rich continuous phase was carried out under optimized solution conditions (e.g., pH, ionic strength, temperature, and shearing). Gelation of the polymer-rich dispersed phase further results in the formation of hydrogel particles. Glutaraldehyde was used as the cross-linker after adjusting pH at 5 (Wu and McClements, 2015).

Natural biopolymer-based microgels can also be fabricated through different physicochemical and mechanical approaches. The structure, electric charge, and physicochemical properties of the microgels depend on the preparation method. The synthesis conditions, for instance, pH value, temperature, ionic strength, shear stress, type of solvent, biopolymer concentration, and the nature of cross-linking agent are significant factors that govern the properties of the microgels. Altering the cross-linking density of the microgels has been found to tailor the texture of the microgels (Farjami and Madadlou, 2017). Samal et al. (2013) showed the fabrication of silk fibroin protein (SFP) microgel by proteolytic enzyme activity. The preparation of lower concentration SFP solutions was carried out by diluting the original SFP solution in water. Stock solutions of α-chymotrypsin were added to 5 mL of 2% SFP followed by incubation at 37°C for 12 h. After the incubation period, the enzyme-treated SFP solution turned milky and resulted in the separation of two phases. The amorphous region of the protein is selectively cleaved by α-chymotrypsin and is followed by the self-assembly of the crystalline region into silk microgels at the physiological temperature.

The various techniques used to fabricate microgels have been summarized in Table 2.1.

2.3.3 Nanogel

Current conventional approaches used for the preparation of nanogels are physical entrapment, ionotropic gelation, covalent conjugation, and desolvation (Pal et al., 2013).

Koul et al. (2011) also demonstrated the synthesis of interpenetrating polymer network (IPN) nanogels utilizing gelatin and poly(acrylic acid) by inverse miniemulsion technique. The process as described involves three steps. First, a mixture of acrylic acid, gelatine, and NaOH was sonicated in the presence of ammonium persulfate (used as an initiator for radical generation) to obtain stable droplets. Then accelerator tetramethyl ethylene diamine and the cross-linker *N*—*N*-methylenebisacrylamide have been used to polymerize and cross-link acrylic acid. The semi-IPN thus formed is further used to make full IPN by the incorporation of glutaraldehyde as cross-linker for gelatine (Koul et al., 2011). Sarika et al. (2015) also studied the synthesis of nanogels by

Table 2.1 Fabrication methods of microgels

Method		Features
Molecular association		Performed through assembling a single type of biopolymer, i.e., self-association or alternatively mixed biopolymer types, i.e., associative complexation.
Self-association	Thermal denaturation of globular protein	Formed by simply heating solution of globular proteins above their thermal denaturation temperature.
	Cross-linking random coil proteins	Formed by cross-linking gelling biopolymers at a concentration below that is needed for the formation of macroscopic gel.
	Desolvation and simple coacervation	Both are phase separation techniques that depend on biopolymer solvation. Lowering solvency leads to molecular self-assembly and particle formation. In coacervation, phase separation of one polymer is induced by adding salts having higher affinity toward water than the polymer or by changing pH/temperature that promotes aggregation of polymer.
Mechanical methods		Microgel formulations are generated by specific mechanical devices and ultimately gelled via physicochemical methods.
	Extrusion method	A biopolymer solution is injected into another aqueous solution containing a gelling agent (gelatin injected into a cold liquid) (Fig. 2.2). Droplets gelation is induced in the hardening solution as a result of cross-linking agents (e.g., mineral ions, glutaraldehyde, or enzymes). Alternatively, a temperature change (heating or cooling) may be applied for gelation induction of thermally setting biopolymers (such as gelatin). Microgel particle size is determined by needle diameter, feed solution viscosity, and the flow rate.

	Atomization method	Here, mechanical devices break a bulk liquid into a large number of uniformly sized droplets, which are subsequently dried to hard particles, and then rehydrated in a controlled fashion to obtain the microgel.
	Shearing methods	Here, biopolymer gels are broken up to form gellike particles (broken gel) by the application of shear to the biopolymer system. Gelation is induced either chemically or thermally.
	Emulsion-based processes	The process involves the use of water in oil emulsion as template to produce biopolymer particles with specific dimensions, and then biopolymers within the droplets are cross-linked via various processes (enzymatic treatment, thermal denaturation, addition of ions, UV light) depending on the gelation mechanism.
	Micromolding methods	Here, first the mold is fabricated using PDMS/silicon/rubber. The biopolymer solution is placed onto the mold and gelation is induced by changing temperature or by incorporating gelling agents.

Courtesy: Farjami, T., Madadlou, A., 2017. Fabrication methods of biopolymeric microgels and microgel-based hydrogels. Food Hydrocolloids 62, 262−272, 2017/01/01/; Oh, J.K., et al., 2008. The development of microgels/nanogels for drug delivery applications. Progress in Polymer Science 33, 448−477, 2008/04/01/.

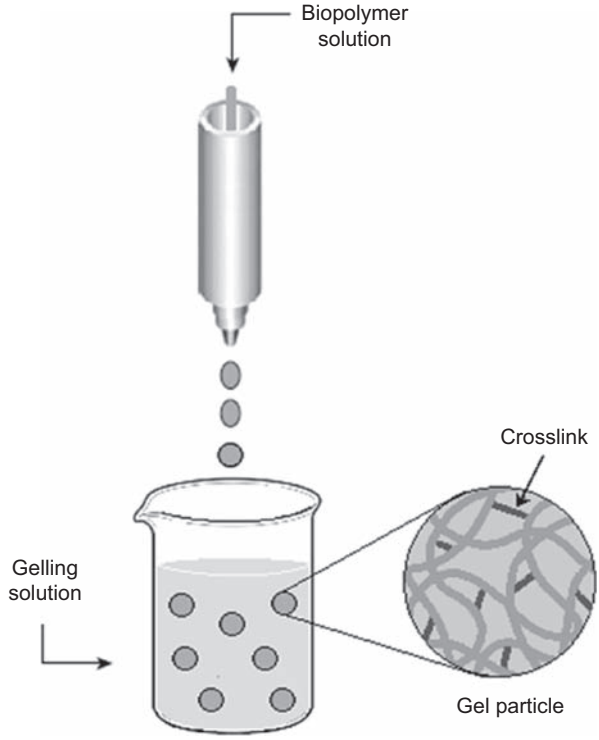

Figure 2.2 Schematic representation of microgel formation by extrusion method. Reproduced from Farjami, T., Madadlou, A., 2017. Fabrication methods of biopolymeric microgels and microgel-based hydrogels. Food Hydrocolloids 62, 262−272, 2017/01/01/, with permission from Elsevier.

the same method, i.e., inverse miniemulsion method. These nanogels were developed from gelatin and alginic aldehyde. The preparation of stable inverse miniemulsions was carried out by sonicating the noncontinuous aqueous phase (gelatin and alginic aldehyde mixture) in a continuous organic phase (Span 20 dissolved in cyclohexane). The cross-linking during the formation of inverse miniemulsion was initiated in the presence of borax by Schiff's base reaction between alginic aldehyde (AA) and gelatin (gel) (Sarika et al., 2015).

Ahsan and Rao (2017) showed the development of thermoresponsive gelatin nanoparticulate formation by double-desolvation method. Firstly, gelatin solution was prepared followed by the first desolvation state in which acetone was rapidly added to the solution. Sedimentation of precipitate containing gelatin was allowed and the supernatant comprising of soluble low-molecular weight gelatin was discarded. The sediment was again dissolved in water (50 mL) by gently heating the solution at 50°C. The redissolved gelatin, now containing the high-molecular weight fraction, was freeze dried in liquid nitrogen and lyophilized. 0.1 g of this freeze-dried high-molecular weight gelatin was dissolved in 10 mL water under gentle heating (50°C). It was filtered with a 0.22 m filter and its pH was adjusted to the expected value

using 100 mM HCl or 100 mM NaOH. A temperature of 25°C and pH 7 was required to synthesize nanoparticles with native gelatine. A 50% concentrated acetone solution was used, which was followed by cross-linking with 5 L glutaraldehyde diluted in 1 mL of acetone. To synthesize nanoparticles with denatured gelatin, the temperature was maintained at 50°C and the pH was adjusted to 3.5. The concentration of acetone used was above 75% and cross-linking was done with 50 L glutaraldehyde diluted in 1 mL of acetone (Ahsan and Rao, 2017). Kanoujia et al. (2016) also demonstrated the preparation of silk protein (sericin)—based nanogels by desolvation method using genipin as cross-linker. Here, ethanol was used as desolvating agent. Briefly, 2 mL (2%w/v) of Seri solution was taken in a container, and ethanol (12 mL) was added. The nanoparticles were precipitated and cross-linked by using different concentrations of genipin. The resulting cross-linked nanoparticles suspensions were centrifuged for 15 min to remove ethanol and unreacted cross-linkers. The collected pellet was washed and redispersed in deionized water by sonication using probe sonicator at 50% amplitude for 10 min to get nanoparticle suspension in deionized water. The obtained nanoparticles were filtered through a 0.45 μm syringe filter and lyophilized without any cryoprotectant (Kanoujia et al., 2016). Chen et al. (2014) highlighted the development of pH-sensitive soy protein nanogels prepared by self-assembly of denatured soy protein. First, soy protein dispersion was prepared and then dispersion was adjusted to the pH (5.9) for making nanogel. The dispersion indicated lowest polydispersity index and was poured into 100 mL glass vials, followed by heating in a water bath without stirring at 95°C for 30 min. Once the heating was complete, the samples were immediately cooled at 4°C (Chen et al., 2014).

2.4 Application of protein-based gels

This section has been dedicated on the applications of the protein matrices for drug delivery and tissue engineering.

Collagen: Collagen, a popular natural polymer is derived from the mammalian tissues (skin, tendons, cartilage, and bone). It is a principal component of extracellular matrices (ECMs) of the mammalian tissues. A collagen molecule is made up of three polypeptide chains, which fit in to form a triple helical structure. The helical conformation is attributed to the presence of repetitive amino acid sequence (Gly-X-Y; X and Y are mostly proline and hydroxyproline). At the end of the helix, telopeptides help in forming cross-striated fibrils. Cross-links occur between molecules to improve the stability of the fibrils. Soluble collagen, starting material for collagen research, can be extracted by cleaving telopeptide region by adding proteolytic enzyme (such as pepsin) (Antoine et al., 2014). Collagen gels, which are physically formed, are thermoreversible in nature but present poor physical and mechanical properties as compared with covalently cross-linked (glutaraldehyde or diphenylphosphoryl azide) collagen gels. Gelation parameters have substantial impact on the gel ultrastructure. Factors such as temperature and pH could be altered to tweak the characteristics of the fibrous polymeric network of the collagen gels. Collagen gels formed at lower temperature exhibit larger pore sizes with enhanced cellular

response (Yang et al., 2010). On the contrary, collagen gels formed at higher temperatures (37°C) and higher pH accelerate fibrillogenesis but result in the formation of the gels with smaller diameter fiber and pore sizes (Antoine et al., 2014). Collagen satisfies the biological design as it is composed of particular amino acid sequence combinations, which are easily familiar to the cells and the enzymes secreted by these cells (e.g., collagenase). Due to its compatibility with different cell types and easy degradation, it can be implemented either in tissue culture scaffolds or artificial skins. Its ability to promote cell attachment can be tailored either by chemical modification or by adding fibronectin, chondroitin sulfate, and hyaluronic acid into the collagen matrix. Collagen gels can be molded into different shapes by filling molds with a collagen solution followed by inducing gelation. In the recent years, 3-D printing of collagen using layer-by-layer gelation technique by application of heat has been used. The applications of collagen gels have branched out to reconstructing organs such as skin, small intestines, liver, etc. (Lee and Mooney, 2001).

Drug delivery: Collagen matrix applications in sustained drug delivery are depicted by the delivery of ciprofloxacin, an antimicrobial agent, from a succinylated type-1 collagen matrix in wound management (Sripriya et al., 2007). Collagen has also been explored in combination with other materials as a possible tool for wound management. Another area of application is cancer management, where a collagen matrix can be an excellent vehicle for chemotherapeutic agents, as indicated by the in vitro release profile of the bioactive agents such as 5-fluorouracil, bleomycin A2, and mitomycin C, which were entrapped in a collagen-poly(HEMA) hydrogel matrix (Jeyanthi and Rao, 1990). One of the characteristic stages of cancer is metastasis, which is known to have a degradative effect on the collagen containing extracellular matrix. To address this property, Kojima et al. (2013) have developed a polymer prodrug embedded within the collagen gel, used as a drug delivery matrix, for controlling metastasis. The results indicate that this hybrid gel had a combinational effect of suppression of tumor growth and attenuating the in vivo metastasis activity (Kojima et al., 2013). Collagen has also been used to deliver bioactive cytokines using a dense collagen gel vehicle for craniosynostosis (Premaraj et al., 2006). Nanda et al. (2014) fabricated collagen microgels for the release of dexamethasone from hybrid scaffolds (PLLA−collagen) for human mesenchymal stem cells. The results demonstrated that by controlling the pore structure and the interconnected pores formed by the collagen fibers lead to the sustained release of dexamethasone. They reported the successful application of the developed matrix in the regeneration of functional bone tissue (Nanda et al., 2014). Bettini et al. (2015) designed collagen-based hydrogel scaffolds, which were porous in nature and contained iron oxide nanoparticles. The presence of nanoparticles enabled the controlled drug release of fluorescein when a weak static magnetic field was applied (Bettini et al., 2015). An overview of the different bioactive agents released from collagen-based matrices is tabulated in Table 2.3.

Tissue engineering: An ideal scaffold for tissue engineering mimics the natural ECM. Carbodiimide cross-linked type-II collagen−CH−based hydrogels have been shown to exhibit chondrocyte proliferation (Pieper et al., 2002). Similarly, Zhang et al. (2011) have designed genipin−cross-linked hydrogels with collagen−chondroitin sulfate−hyaluronic acid hybrid biomimetic scaffolds and investigated

cell compatibility in vitro for chondrocyte transplantation in cartilage tissue engineering. Li et al. (2012) also incorporated Icariin (Chinese-based medicine), which demonstrated an excellent chondrocyte growth-promoting response for cartilage tissue engineering application. Porous collagen scaffolds are often used in a combination with hydroxyapatite for its bone tissue regeneration application (Wahl et al., 2007). These porous scaffolds are typically prepared using freeze-drying techniques or stereolithography methods. Kim et al. (2009) have explored the area of fabricating 3-D collagen matrices using a cryogenic direct-plotting system. Advancements have led to the development of scaffolds with various geometries including cylindrical tubes, which are explored for blood vessel regeneration. Additionally, cyclic AMP contributes to engineered human microvessels using type-I collagen gels (Wong et al., 2010). The use of a collagen−glycosaminoglycan (GAG) coculture model has been further extended for applications in heart valve tissue engineering (Flanagan et al., 2006). A diverse range of such applications is shown in Table 2.2.

Gelatin: Gelatin is a biopolymer of animal origin (i.e., the skin and bones of bovine, porcine, and fish sources) that is derived from the heat-induced hydrolytic degradation of collagen. Depending on the method of extraction of collagen, different types of gelatin—either acidic or basic, can be derived, with each having a different isoelectric point (pI) (Thakur et al., 2013). Alkaline-processed gelatin (which is usually from bovine sources) has a larger amount of carboxyl groups, making them highly negatively charged and having a lower pI as compared with an acidic-processed gelatin (usually porcine in origin), which possesses a pI similar to that of collagen. Furthermore, depending on its type, gelatin changes its polyion complexation to either of the two charged (positive or negative) bioactive agents. Acidic gelatin (pI ∼ 5.0) can assist in the controlled delivery and release of basic bioactive agents, whereas basic gelatin (pI ∼ 9.0) is best adapted for acidic compounds (Thakur et al., 2013). The gelatin formed gels that are thermoreversible in nature, and its gel-to-sol transition takes place at ∼30−35°C (Thakur et al., 2011). Owing to its sol−gel transition temperature, gelatin must be cross-linked with chemical fixatives (e.g., glutaraldehyde, diisocyanates, carbodiimides, genipin) to improve its mechanical and thermal stability (Thakur et al., 2011; Nickerson et al., 2006). If cross-linking occurs below its sol−gel transition temperature, physical cross-linking is stabilized by chemical fixatives. Gelatin can also be 3-D printed into porous matrices that are subsequently cross-linked. Given its biodegradable nature and its excellent biocompatibility, gelatin alone or its combination with other biopolymers have been explored for potential pharmaceutical and tissue engineering applications.

Drug delivery: Gelatin carrier−mediated drug delivery has shown a wide scope of applications (Shown in Table 2.3). Prosthetic valve endocarditis, a severe complexity of cardiac valve replacement, is an infection when the bacteria make a way to the site of surgery and adhere to the cardiac valve. To overcome this condition, Kuijpers et al. (2000) studied the capacity of both the in vivo and in vitro release of lysozyme from in situ gelatin hydrogels to prevent valve endocarditis Applications related to other antibiotic delivery that have proven beneficial include the release of tetracycline and bisphosphonate, which are incorporated in gelled foam pellets for the reduction of periodontal bone loss in rat models (Yaffe et al., 2003), as well as the release of ciprofloxacin hydrochloride for the management of ocular infection

Table 2.2 Protein gels and their tissue engineering applications

Gel	Material/combination	Application (TE)
Collagen	Polyvinyl alcohol/collagen/hydroxyapatite (HA), nano-HA/collagen/Poly L Lactic Acid (PLLA),	Bone
	poly(lactic-co-glycolic acid) (PLGA)/collagen, collagen/HA/chondroitin sulfate	Cartilage
	Collagen/silk	Ligament
	Collagen−chitosan nanofiber, PLGA microsphere/collagen,	Blood vessel
	Cross-linked collagen/chondroitin sulfate/hyaluronic acid,	Skin
	Collagen/chitosan/heparin	Liver
	Collagen/hyaluronic acid, collagen/heparan sulfate	Nerve
	Dendrimer−cross-linked collagen,	Cornea
Gelatin	Hydroxyapatite chitosan/gelatin, cross-linked gelatin	Bone
	Gelatin/chitosan/hyaluronan	Cartilage
	Gelatin/silk fibroin (SF)	Ligament
	Gelatin/Polytetrafluoroethylene (PTFE), gelatin/poly(ε-caprolactone, vascular endothelial growth factor immobilized gelatin,	Blood vessel
	Cross-linked gelatin	Skin
	Cross-linked sodium alginate/gelatin, chitosan/gelatin	Liver
	Photocrosslinkable gelatin, gelatin/hydroxyphenylpropionic acid	Nerve
Elastin	Bone morphogenic protein (BMP)-containing elastin	Bone
	Alginate/elastin/polyethylene glycol,	Blood vessel
	Collagen/elastin	Skin
	Elastin-like proteins,	Nerve
Silk protein	SF/chitosan/PLLA, chitosan/fibroin−hydroxyapatite,	Bone
	SF modified porous poly(e-caprolactone),	Cartilage
	Gelatin/SF	Ligament
	Collagen/fibroin, polylactide/SF−gelatin,	Blood vessel
	PLLA/fibroin, chitosan/SF, chitosan/SF/heparin	Liver

Data from Van Vlierberghe, S., et al., 2011. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. Biomacromolecules 12, 1387−1408, 2011/05/09.

(Jain et al., 2011). When it comes to the release behavior of chemotherapeutic agents, it has been observed that these drugs have a relatively low therapeutic index and require an effective dosing paradigm, which has to be administered through a carefully designed vehicle. Muvaffak et al. (2004) have highlighted that in vitro colchicine (antimitotic drug) release has an initial toxic effect on breast cancer cell lines (MCF-7). Ohta et al. (2009) in their in vivo study on rabbits against VX2 liver tumors, demonstrated anticancerous efficacy by the prolonged release of cisplatin from gelatin carriers. Wang et al. (2012) prepared multiresponsive gelatin microgels loaded with Doxorubicin and Rhodamine B (model drugs). Its release was triggered by exploiting its sensitivity to salt concentrations and pH. The results indicated that these microgels demonstrate excellent biocompatibility and biodegradability. Similarly, chitosan−gelatin microgels were cross-linked with succinimide-end PEG for thermoresponsive sustained drug delivery of hydrophobic active molecules (Wang et al., 2012, 2016). Furthermore, Fan and Wang (2016) designed gelatin−dopamine nanogels for the intracellular delivery of Doxorubicin. The novel gelatin−based nanogels demonstrated efficient cell membrane penetration making it an excellent drug delivery matrix (Fan and Wang, 2016).

Tissue engineering: The utilization of gelatin for the fabrication of scaffolds or constructs is a common practice; however, its mechanical instability limits its application. To overcome this constraint, gelatin-based matrices are typically cross-linked with chemical fixatives. Apart from that, fabricating porous gelatin−based scaffolds by freeze-drying and phase separation techniques is adopted in tissue engineering (Van Vlierberghe et al., 2009). Interestingly, the preparation of gelatin-based cell carriers was done by cryogenic treatment, which was subsequently followed by lyophilization (Van Vlierberghe et al., 2007, 2009). The cryogels developed through this process favored the adherence and the proliferation of various human cells (e.g., fibroblasts, endothelial cells, glial cells, osteoblasts, and epithelial cells) (Dubruel et al., 2007). Alternative techniques to process gelatin are also adopted, including electrospinning (Li et al., 2008) and stereolithography (Xu et al., 2007). In addition, collagen-derived gelatin is used in a combination with GAGs (Vanderhooft et al., 2009), calcium phosphates, or both when it is a tissue-specific regeneration (Lien et al., 2009). Li et al. (2016) demonstrated the successful incorporation of gelatin microgels to improve adhesiveness and the bioactivity of PEG-based adhesive. Researchers such as Kim et al. (2016) have studied the suitability of gelatin methacryloyl nanogels for transdermal delivery of macromolecules and established the potency of the nanogels as a transdermal delivery carrier for hydrophilic macromolecules.

Elastin: Elastin, an important structural protein, which is obtained from tropoelastin, is the most linearly elastic biosolid. It is a 70 kDa protein consisting of alternating hydrophilic and cross-linking regions. Elastin comprises of repetitive amino acid sequences such as VPGVG, APGVGV, and VPGG. Elastin is widely used to reconstruct mechanically active tissues such as tendons, blood vessels, and elastic cartilage (Krishna and Kiick, 2010). However, elastin exhibits a tendency to

calcify, is difficult to purify, and has poor mechanical stability which necessitates cross-linking to enhance its stability (Daamen et al., 2008). A recombinant elastin developed by Barbosa et al. (2009) has provided a promising alternative to natural elastin. These repetitive polypeptides are made up of VPGXG pentapeptide sequences, where X could be any natural amino acid excluding proline (Bessa et al., 2010). Recombinant elastin exhibits thermoresponsive behavior, which indicates polymer solubility below the transition temperature. However, above this critical temperature, an ordered structure is obtained due to the self-assembly of the hydrophobic chains. For example, a recombinant elastin—poly(VPAVG) that indicates maximum resemblance to the native elastin—forms a micellar assembly above its transition temperature. This property could be further explored for drug delivery applications (Rincón et al., 2006).

Drug delivery: As discussed earlier, recombinant elastin—based carriers show promise for drug delivery applications. Table 2.3 provides an overview of the bioactive agents that can be delivered using elastin-based carrier devices.

Tissue Engineering: Table 2.2 depicts an outline of the applications of elastin-based constructs in tissue engineering. Elastin scaffolds can be fabricated through the use of electrospinning techniques, but insoluble elastin can also form gels (Ratner and Bryant, 2004). The processing of elastin can either be done alone or in combination with gelatin or collagen through electrospinning method (Buttafoco et al., 2006), and the resultant scaffolds are also cross-linked using EDC/N-hydroxysuccinimide (NHS). Electrospun elastin has been reported to moderate proliferation, migration, and differentiation of smooth muscle cells (Daamen et al., 2008). Adding more to its advantages, elastin-like polymers indicate excellent biocompatibility because of their strong resemblance to the natural elastin and their property to break down to native amino acids (Bessa et al., 2010). Also, to enhance its applications in cardiac tissue engineering, elastin can also be used as combinations with peptide sequences and growth factors, which improve its cell-interactive properties (Debelle et al., 1998).

Silk protein: The usage of silk fibrin for biomedical applications has been carried out for centuries. Silk is naturally harvested from the silk worms *Bombyx mori* or *Antheraea mylitta*. Natural silk is composed of two main components: fibroin, a filamentous component that maintains mechanical stability, and a gummy protein sericin, which is responsible for holding the fibers together (Cao and Wang, 2009). Both these proteins comprise 18 amino acids, such as serine, alanine, and glycine, but in different compositions (Altman et al., 2003). The fibroin molecule's composition is such that the two-third consists of the crystalline portion, whereas the remaining one-third has an amorphous region. Repetitive amino acid sequences (-Gly-Ala-Gly-Ala-Gly-Ser-) are present along the crystalline portion, forming an antiparallel β-sheet structure, this enhances the stability of the fiber and improves its mechanical properties (Altman et al., 2003; Acharya et al., 2009). The properties of silk are such that it possesses very high chemical, thermal, and mechanical stability when compared with the other protein-based materials. In aqueous solution, silk protein aggregates as random coils and the presence of calcium usually facilitates gelation. As an increase in concentration or temperature is facilitated, more interaction leading to faster gelation is observed. Besides, decreasing pH and incorporation of

Table 2.3 Protein-based gels and its applications in bioactive agent transportation

Hydrogel	Carrier material	Bioactive agents	Application	References
Collagen (C)	Dendrimer−C hybrid, C-poly(HEMA), C-CS, EDA cross-linked C, C, type-I C vitrigel,	Doxorubicin, 5-fluorouracil, bleomycin A2, mitomycin C, thymosin β4, FITC, human growth hormone, vancomycin, deoxyuridine, FITC−dextran, ^{125}I-calmodulin, vascular endothelial growth factor (VEGF), dexamethasone	Metastasis-associated delivery, drug delivery,	Jeyanthi and Rao (1990), Kojima et al. (2013), De Paoli Lacerda et al. (2005), Takezawa et al. (2007) and Chiu and Radisic, 2011
Gelatin (G)	G, G-β-cyclodextrin, cross-linked G, G-PVA, HA-G, CS-G, CS-honey-G, G-g-poly(N-isopropylacrylamide),	Bovine serum albumin, indomethacin, tolbutamide, insulin-like growth factor I, lysozyme, erythropoietin, BMP-2, cisplatin, adriamycin, guanidinoethyl disulfide, simvastatin, Fibroblast Growth Factors (FGF), pilocarpine	Drug release, growth factor release, antitumor delivery,	Thakur et al. (2012a,b), Konishi et al. (2005), Li et al. (2009), Tanigo et al. (2010) and Lai and Hsieh (2012)
Elastin (E)	E,	BMP, cell,	Drug release, cellular delivery,	Bessa et al. (2010) and Bandiera (2011))
Silk protein	Silk fibroin (SF)−PVA, SF/polyacrylamide, silk−elastin-like protein polymer, silk elastin like polymers (SELP) -815K, and poloxamer 407, sonication induced silk, silk−elastin-like protein polymer,	FITC−dextrans, trypan blue dye, BMP-2, viral gene, VEGF, ciprofloxacin, dexamethasone	Drug delivery, gene delivery	Kapoor and Kundu (2016), Kundu et al. (2012), Mandal et al. (2009), Zhang et al. (2011), Diab et al. (2012), Price et al. (2012) and Yu et al., 2016

hydrophilic polymer reduces protein−protein interaction and renders faster gelation. Higher protein concentration or higher temperature accounts for gel with smaller pore size and enhanced mechanical properties.

Drug delivery: Silk fibroin (SF)−based carrier matrices, which incorporate bioactive compounds, are successfully employed in medicinal therapy. It has mainly found its application in controlled drug delivery and cancer treatment (Kon'kov et al., 2010). The advantages of SF are its biocompatibility, slow biodegradability, and its endowment with excellent mechanical properties. The protein can also be processed into different forms of matrices (e.g., films, nanofibers, scaffolds, and gels), which are most appropriate for a large variety of drug delivery applications (Yucel et al., 2010). The main application of SF matrices was to deliver protein drugs and maintain its potency. To modify the release kinetics of SF matrices, various alterations are made to the delivery system design, SF crystallinity, molecular weight, concentration, and the structure of the embedded agents (Wenk et al., 2011). Guziewicz et al. (2011) have tested the effectiveness of SF-based hydrogels for controlled, localized delivery of therapeutic monoclonal antibodies. A successful attempt was also made by Mandal et al. (2009) to design 3-D scaffolds by incorporating drug-loaded calcium alginate beads in an SF protein. The scaffolds were investigated for in vitro controlled dual drug release of bovine serum albumin (66 kDa) and FITC−inulin (3.9 kDa) (Mandal and Kundu, 2009). SF nanoparticles were also developed by Zhao et al. (2014) using electrospraying technique. The nanoparticles were loaded with *Cis*-dichlorodiamminoplatinum (CDDP) and indicated a sustained inhibitory effect on A549 lung cancer cells (Zhao et al., 2014). Table 2.3 provides an overview of bioactive agents released from silk-based carriers.

Tissue Engineering: SF, the main structural protein of silkworm silk, can be explored to design artificial cartilages, bone tissue fragments, and blood vessels and to regenerate nervous tissue due to its properties such as its mechanical strength (Kon'kov et al., 2010). However, the incorporation of growth factors in SF scaffolds has also been considered for bone and cartilage tissue engineering and also for vascular and nerve regeneration devices. Several research groups have worked with lactose surface modifications to fine-tune the cell adhesion behavior of silk-based materials (Gotoh et al., 2004). Fini et al. (2005) have developed injectable SF hydrogels to study the role of implanted hydrogels in the management of critical size defects in rabbit distal femurs using osteoblast cultures. An improvement in terms of an increase in the levels of TGF-β1 was observed, which led to accelerated bone healing and the recovery of critical size defects in rabbit femurs (Fini et al., 2005). This research indicated that the scaffolds, capable of releasing growth factors, provide a biologically active template for tissue regeneration. Uebersax et al. (2008) have demonstrated the feasibility of a controlled release of insulin-like growth factor I from SF scaffolds, which induces chondrogenic differentiation of human mesenchymal stem cells for cartilage repair. Research carried out by Jones et al. (2009) highlighted the importance of creating a coculture of osteoblasts and osteoclasts when developing SF scaffolds for bone tissue engineering. For its application in cartilage tissue engineering, SF−chitosan-blended 3-D scaffolds were fabricated, where SF fibroin was the substrate for cell adherence and growth, whereas chitosan that had a similar structure to GAG showed promise for cartilage repair (Bhardwaj et al., 2011). Additionally, the

applications of SF−chitosan blend in cartilage tissue engineering can be extended to tracheal tissue reconstruction (Zang et al., 2011). Harkin et al. (2011) have researched the application of SF as a compatible biomaterial for the reconstruction of tissues in the human eye, such as the cellular reconstruction of corneal stroma and endothelium, corneoscleral limbus, and outer blood−retinal barrier (Ruysch's complex). Furthermore, SF microgels embedded PEG hydrogels were developed by dual method and were proved to be potent candidates for 3-D cell culture, which would include temporal control of the hydrogel stiffness (Ryu et al., 2016). Table 2.2 briefly highlights the applications of silk protein in tissue engineering.

2.5 Conclusion

Protein-based gels usually comprise isolated proteins from natural extracellular matrix. Their feasibility for modification allows these gels to be applied in multiple fields, such as drug delivery, tissue engineering, regenerative medicine, and others. This chapter elaborated the properties of different proteins as well as the hydrogel fabrication and modification approaches. Finally, the recent advancements in the application of protein-based gels in drug delivery and tissue engineering have been discussed.

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Synthetic polymeric gel

3

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3.1 Introduction

Synthetic gels are defined as three-dimensional (3D) networks composed of elastic polymer molecules and fluid (water for hydrogels) filling the interstitial spaces conferring capability of undergoing substantial deformations to 3D network. In forming a gel, the solid polymer chains swell in the liquid matrix but do not dissolve in it, thus enabling the network to exhibit viscoelastic properties. In the past several decades, synthetic gels have commanded attraction for biomedical applications. In fact, excluding some hard tissues such as bones, teeth, and nails, human body is also gellike structure. Gel structure confers several advantages to the organism, most importantly, ability to transport nutrients and gases. Gels are formed from precursors when polymer chains are cross-linked. Depending on the mechanism of cross-linking, gels are classified into permanent/irreversible gels or reversible gels. Irreversible gels are predominantly formed through covalent cross-linking (Hennink and van Nostrum, 2002). Such bonds can be formed by chemical reactions in presence of cross-linkers or under photoirradiation. These gels reach the equilibrium swelling state depending on the interaction between water and polymer network and degree of cross-linking density (Rosiak and Yoshii, 1999). In reversible gels, the polymer networks are physically entangled or held by secondary forces such as ionic bond, hydrogen bond, crystallite formation, or hydrophobic interactions (Hennink and van Nostrum, 2002).

Synthetic polymer gels have wide range of applications in the field of tissue engineering, sustained and pulsatile drug delivery, wound dressing, and medical devices such as contact lens, biosensors, and actuators. Furthermore, certain synthetic gels can respond to environmental stimuli in reversible manner, a property which can be harnessed for generating mechanical energy from changing chemical potentials. In comparison to natural polymer gels, synthetic gels provide greater possibility of tailoring the molecular weight or mechanical properties, greater batch-to-batch reproducibility of physical and chemical properties, lesser immunological reactivity, and higher mechanical strength. However, among the class of synthetic gels, a wide number of polymers with different properties are available. In selecting a polymer gel, the most important gel parameters are the biocompatibility, gelation temperature, and time, the conditions required for gelation, and if they are physiologically amenable, swelling kinetics, biodegradation properties, mechanical properties, and the ease/cost of commercial availability. Among them, gelation kinetics and

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swelling behavior are important parameters and hence is discussed briefly before presenting various synthetic gels used in biomedical applications.

3.2 Cross-linking, water uptake, and gel rheology

Appropriate characterization of polymer gels is important prior to their specific application in biomedical engineering. Different characteristics include the cross-linking, swelling, and gel rheology, which directly influence the stability of the gel and its interaction with surrounding environment. Parameters used to understand swelling characteristics are the swelling ratio (mass or volume swelling ratio), number of average molecular weight, and the network mesh size (Zhu and Marchant, 2011). Fig. 3.1 represents different cross-linking schemes of polymer matrix. The stiffness properties of gels are becoming an important characteristic in tissue engineering application because the rigidity of scaffold materials defines cellular microenvironment and influences the cellular morphology, adhesion, as well as genetic and epigenetic characteristics. On the other hand, flowable nature of gel is important

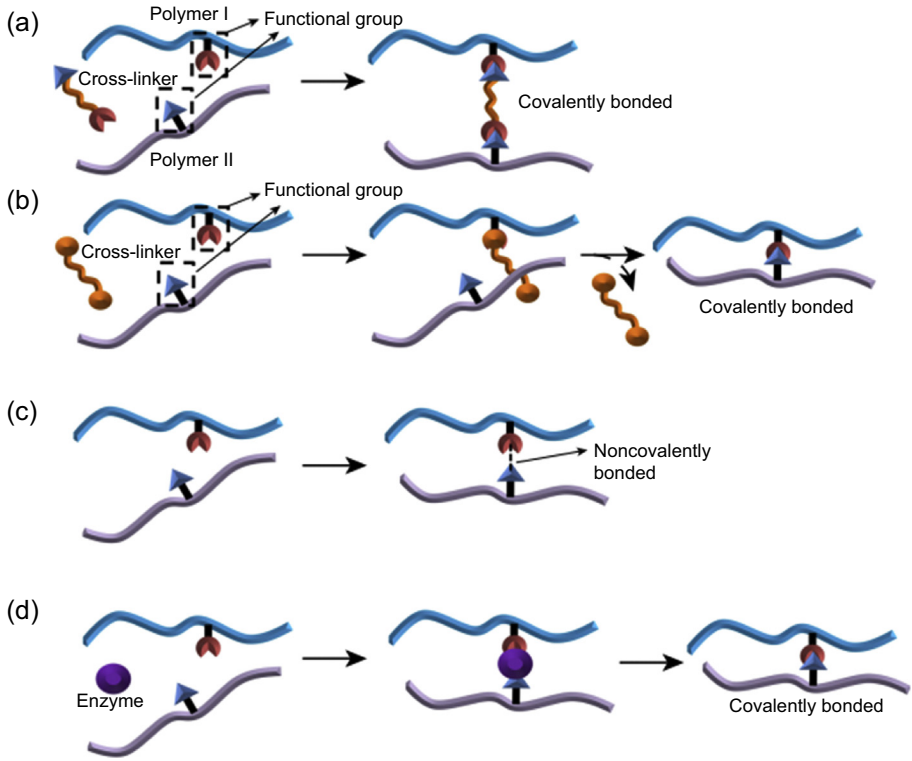

Figure 3.1 Represents different modes of cross-linking of polymers, (a) Chemical cross-linker covalently bonded (b) Chemical cross-linker non-covalently bonded; (c) Physical cross-linking; and(d) Enzymatic cross-linking (Reddy et al., 2015).

to judge their suitability as vehicles in injectable therapeutic delivery (Huebsch and Mooney, 2009). Various factors affect the rheology of the hydrogel including molecular weight and its distribution, monomer, associated hydrophobic and hydrophilic functional groups, etc. (Mendoza, 1998). The sol−gel synthesis route has been represented in Fig. 3.2. Rheological parameters such as complex viscosity (n*), elastic (G″) and viscous (G′) modulus, and phase angle (α) define the viscoelastic properties of polymer materials. The overall internal structure of gel is characterized by the critical stress (σc), which describes the stress at which the internal structure of polymers starts to disperse (Gulrez et al., 2011). The 3D cross-linked gel exhibit lattice structure. Mobility of polymer gel is controlled by degree of cross-linking. However, under physiological condition the polymer network is gradually solvated by trapped solvent. The distribution of cross-linking units is different in heterogeneous and homogenous gel. In former structure cross-linking units mostly concentrated in local unit, whereas in homogeneous gel they are evenly distributed. Spatial size of gel network can be measured by water and solute permeation in the medium. Properties of polymer gel mostly depend on structure as well as distribution of polymer network. Gel−solvent interaction is another crucial factor for deciding the fate of network structure. Response of gel to external environmental factors, such as heat, pH, electrical field, etc., exhibits variations with altered characteristics such as electrical conductivity, stereoregularity, and functions. The property of synthetic hydrogel can be altered by altering the chemical composition and method of preparation. Due to their tunable properties such as porosity, mechanical strength, water retention, and biodegradability, such hydrogels are commonly used for different biomedical applications.

3.3 Synthetic gels

3.3.1 Polyethylene glycol

It is one of the most familiar synthetic polymers for tissue engineering as well as targeted drug delivery. At low molecular weight (1000 Da) this polymer behaves like viscous liquid, whereas in higher molecular weight it appears as waxy white solid. For biomedical or tissue engineering applications, approximately 20,000 Da molecular weight is used. Interestingly, the solubility pattern of (polyethylene glycol) PEG is amphiphilic. Besides water, it can be soluble in a number of organic solvents such as methylene chloride, ethanol, acetone, etc. PEG is insoluble in aliphatic hydrocarbon such as hexane, etc., and such solubility pattern helps in synthesis and isolation of product from reaction mixture. For instance, the synthesis reaction of PEG is usually carried out in toluene or other organic solvents in which PEG is soluble, whereas isolation process is usually carried out by addition of nonsolvent phase such as hexane, ethyl ether, etc (Sieber et al., 1999). In biological system, PEG exhibits partitioning behavior between aqueous medium and cell membrane. The cell fusion behavior of PEG is greatly utilized in production of hybridoma and monoclonal antibodies (Bettencourt and Almeida, 2012). By adding the hydrophobic polymer components in PEG structure, the solubility and partitioning pattern of PEG can be altered, which

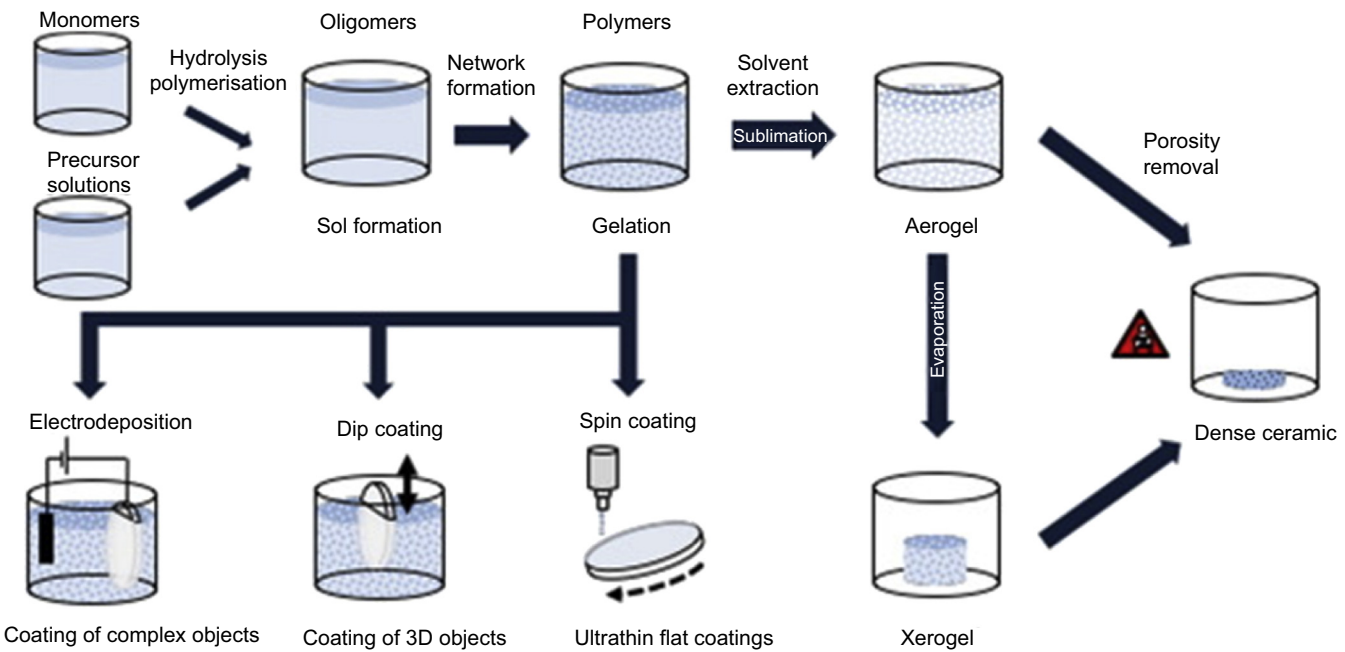

Figure 3.2 Represents mechanisms for sol-gel transformation (Owens et al., 2016).

also helps in phase separation of the polymer (Bailey and Koleske, 1976). Another interesting property of PEG is lower consolute temperature (LCT), or cloud point which is approximately 100°C in water. This indicates if the temperature of this polymer in aqueous solution is increased beyond 100°C, PEG becomes insoluble and formation of two phases takes place. This inverse solubility−temperature relationship has wide spectrum of applications in biomedical science (Bailey and Koleske, 1976). It is important to note that, the LCT of PEG is not a fixed phenomenon. Several parameters such as molecular weight, pH, and salt concentration greatly influence the LCT of PEG. The partition property of PEG makes it an eligible candidate for tissue culture media and for organ preservation.

In biological environment, interaction of protein molecules with PEG spheres causes temporary deformation of PEG structures, and degree of deformation depends on molecular weight of polymer. Attachment of PEG with any molecules actually improves the solubility of that molecule in organic solvent, and such property helps in developing PEG−enzyme system, which is stable even in the dry organic solvent. The solubility pattern of PEG also helps in designing the structure that can cross the cellular membrane, which may help in drug delivery or other cell-specific targeted therapy (Veronese and Mero, 2008). The toxicity of PEG molecules also depends on molecular weight. The PEG with molecular weight less than 400 D exhibits mild toxicity, otherwise PEG has excellent biocompatibility and insignificant immunogenicity, which makes it a perfect material for various pharmaceutical applications (Abuchowski and Davis, 1981). In a study, Richter and Akerblom have tested the immunogenic reactivity of PEG in contact with biological solution. Their study indicated that PEG is poorly immunogenic; however, PEG−protein complex elicits feeble anti-PEG response, which gradually decreased with exposure time (Richter & Akerblom, 1983). Besides pharmaceutical applications, PEG−protein conjugates also have other biotechnological applications such as synthesizing artificial enzymes using PEG linkers, which are flexible and hydrophilic in nature. Yomo, Urabe, and Okada have applied the properties of PEG linkers for preparing semisynthetic oxidases (Harris, n.d.). In another study, Yoshinaga et al. have integrated the PEG−BSA (Bovine serum albumin) complex with catalytically active metal porphyrin for formation of hybrid catalysts, which are active in organic media (Yoshinga and Harris, 1989).

The amphiphilic nature of the PEG helps in interacting with the cellular membrane. However, the mechanism of their interaction is still debatable. In biological system the membrane uptake of PEG molecules takes place through membrane fusion method (Yamazakit and Ito, 1990). In a study Beckman et al. (1988) suggested that endothelial cells can uptake superoxide dismutase and catalage conjugated with PEG. Their observation also suggested three possible mechanisms about cellular uptake—(1) direct penetration through cellular membrane, (2) binding of PEG−enzyme on the membrane surface, and (3) endocytosis. Among them the first mechanism is controversial because the solubility pattern of PEG does not allow the PEG bound substrate to be soluble in phospholipid membrane with aliphatic core (Yamazakit and Ito, 1990). Contradict with this fact another study by Veronese et al. (1992), reported that when PEG conjugate superoxide dismutase is added

in the whole blood, unlike free enzyme, the conjugates could not be completely recovered from blood plasma. They have suggested that PEG−enzyme made bound to the cell membrane by making another conjugation like PEG−protein. Such association reflects that PEG−protein conjugation is coupled with cells and is not freely available in plasma. Bittner et al. (1986) in their study revealed the possibility of application of PEG to fuse the nerve cells in human body. In their study they exhibited that PEG is able to fuse invertebrate nerves through interaction of PEG with the cell membrane. In another similar work, Geron and Meiri (1985) reported the PEG assisted fuse of synaptic vessel with cellular membrane of nerve cells, whereas Clifton et al. (1989) showed PEG conjugated Vitamin E-succinate is even able to penetrate the blood−brain barrier. However, another research group reported the contradictory observations. In two independent studies by different research group have revealed that PEG is impermeable to cerebral capillaries of hangfish. Spigelman et al. (1984) showed that solvent containing PEG 300 was permeable through blood−brain barrier.

PEG is a material of choice for hydrogel formation due to its hydrophilic nature. PEG exhibits "stealth" properties, which help the control release of formulation conjugated with PEG under biological ambience. PEGylation is important for association of PEG with drug/protein/other active agents, which improve the pharmacokinetic and pharmacodynamic properties of delivered drugs. For transplantation of living cells like islets of Langerhans at target site, cells were encapsulated with PEG or its derivatives like photopolymerized poly(ethylene glycol) diacrylate and the microspheres delivered at the target site (Gulrez et al., 2011). However, this method shows limited permeability of nutrition media through microspheres but sometime affects cellular viability. To enhance the viability of encapsulated cells, various modification of PEG hydrogel has been performed. For example, addition of RGD sequence on PEG scaffolds enhances the cellular viability (Park et al., 2008; Nuttelman et al., 2005).

3.3.2 Polyvinyl alcohol

Polyvinyl alcohol (PVA) is most commonly used water-soluble synthetic polymer for biomedical applications. Unlike PEG, PVA is insoluble in organic solvent and only sparsely soluble in ethanol. Because of its solubility pattern and easy degradability, PVA is also known as "green polymer." PVA shows compatibility with number of polymers and it can be easily mixed up with various natural materials, which extend the range of its applicability. Different studies exhibited that the mechanical property of PVA can be improved without compromising the degradability through inclusion of natural fiber and fillers (Goodship and Ogur, 2009). PVA hydrogel can be formed either through physical cross-linking by repeated freezing−thawing cycles or through chemical cross-linking by glutaraldehyde, etc. (Zhu and Marchant, 2011). The solubility of PVA in organic solvent can be modulated through addition of acrylate co-polymers, which makes the PVA ampholytic that contains both cationic and anionic comonomers. Such polyampholytic copolymers are used for purification from target protein from crude samples (Harris, n.d.). The physicochemical property of PVA is determined by the degree of hydrolysis during synthesis procedure. Because PVA is

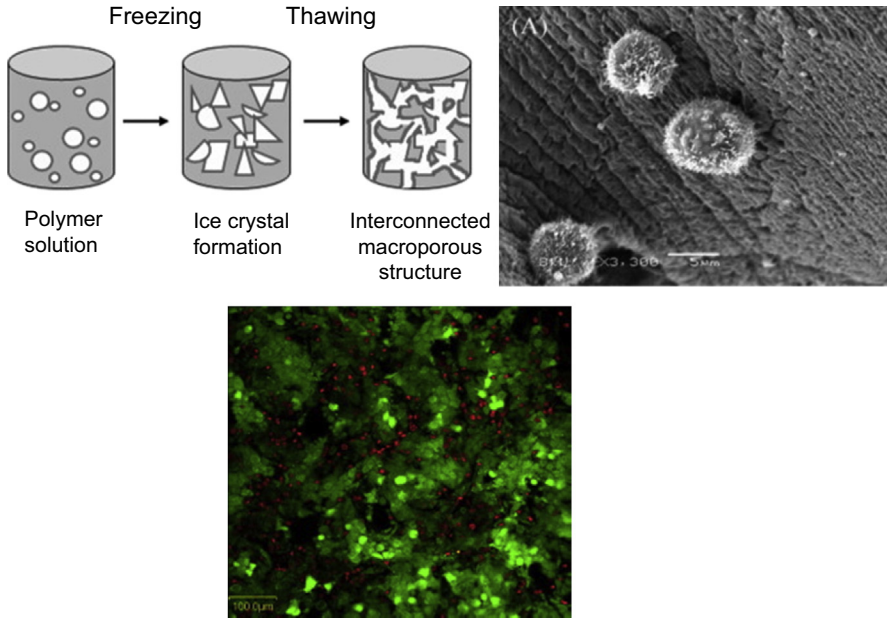

Figure 3.3 Representing (a) fabrication process of cryogel, (b) SEM micrograph showing attachment of CD34 human acute myeloid leukemia KG-1 cells attached on cryogel surface, and (c) live/dead cell assay of attached cells on cryogel surface (Hixon et al., n.d.).

water-soluble polymer hence prior to any biological application, cross-linking of polymers is important to maintain the integrity. In this respect degree of cross-linking plays an important role in deciding the stability in biological environment, fluid uptake, degradation property, etc. For biomedical application the physical cross-linking is more useful as it does not leave any residual toxic cross-linking agents (Harris, n.d.). Moreover, the physical cross-linking method provides more tunable mechanical property of this polymer in comparison with other cross-linking methods. In this process PVA hydrogel is formed through repeated free−thaw cycle and finally the product obtained through this method is known as PVA cryogel (PVA-C). In recent date, the PVA-C is an important cryogel for biomedical application. Molecular weight of PVA has direct impact on the nature of cryogel formed. Fig. 3.3 depicts the schematic representation of cryogel formation and attachment of cells on PVA-C surface. The higher molecular weight of polymer increases the size of crystalline structure. However, after a certain point this effect is not observed possibly due to reduction in available space and mobility of the polymer chain (Harris, n.d.). Wan et al. (Harris, n.d.) reported that high molecular weight PVA showed better degree of crystallinity and uniformity of the structure. Lozinsky et al. (Harris, n.d.) in their study revealed that the hydrogel of PVA with higher molecular weight is more rigid in comparison with the higher molecular weight polymer. The biocompatibility of PVA hydrogel or implant was assessed by various research groups. Tadavarthy et al. (Harris, n.d.) in 1975 demonstrated the compatibility of this polymer for Ivalon embolic material. PVA hydrogel cross-linked through irradiation has application in

ophthalmology as vitreous substitute. Covey et al. (Harris, n.d.) in a clinical study demonstrated the biocompatibility of PVA particles in vein embolization. However, in this regard only one controversial report is available. A study by Nakamura et al. (2001) demonstrated tumor formation in mice after insertion of PVA hydrogel. DeMerlis and Schoneker (2003) have investigated the oral toxicity of PVA and their study concluded that the LD50 of PVA indicates low acute toxicity, which is considered as orally safe product. Wang et al. (DeMerlis and Schoneker, 2003) tested the biocompatibility of PVA composite containing hydroxyapatite (HA) and gelatin for cartilage tissue engineering in in vivo model. Another group has examined the application of hydrogel containing PVA−carboxymethylated cellulose blend for adhesion barrier (Taghizadeh and Sabouri, 2013). PVA/chitosan blend cross-linked with genipin also exhibited biocompatibility in in vitro assay studied by various research groups (Muzzarelli et al., 2015). In drug delivery, a combination of PVA/carboxymethyl chitosan has been implemented in rat and exhibits no cytotoxic or hemolytic effect (Wan et al., 2014).

3.3.3 Polymethyl methacrylate

Polymethyl methacrylate (PMMA) was first discovered by British chemists in the year of 1930 (Henri, 2007). It is optically transparent thermoplastic, which was initially used as a substitute of glass due to light weight, shatter resistance, and high impact strength (Demir et al., 2006). In PMMA the methyl group in the polymer side chain prevents its compact crystalline packing. The polymer is first used during the World War II as aircraft window and in gun turret. Other application of this polymer includes optical, pneumatic actuator/sensor, drug delivery, using electro diffusion, etc. (Kost and Langer, 2001; Shi et al., 2011; Beruto et al., 2002). Application of PMMA in nanotechnology is reported by Wang et al. (2005a) in which they prepared carbon nanotube−polymer composite. PMMA shows high resistance to UV radiation and it possesses good optical properties and cellular compatibility (Charles and Edward, 2003). Unique mechanical property of this polymer makes it the hardest thermoplastic with resistance against scratch or other chemical assaults. However, its resistivity is relatively low against few chemical groups such as chlorinated and aromatic hydrocarbons, esters, or ketones (Van Krevelen and Nijenhuis, 2000). Tacticity of this polymer can be altered depending on the adjacent polymer groups (Mark, 2007). Being an organic polymer the solubility of PMMA is expected to be "like-dissolve-like" that means the solute dissolves in the solution that has similar chemical structure. However, PMMA exhibits more complex as it swells in the solvent and subsequently forms soft layer of its surface, which is finally dissolved by diffusion of solvent through the structure (Ali et al., 2015). Similar to solubility, this polymer exhibits complex thermal decomposition behavior. In 2013, Rani et al. showed that the PMMA grafted sodium alginate synthesis under initiator such as ceric ammonium nitrate. Using the similar process Routray et al. (2013) developed PMMA grafted on cellulose acetate in presence of dimethyl sulfoxide. In another study, Harish Prashanth et al. (2005) grafted PMMA on chitosan in presence of potassium persulfate for formation of chitosan−PMMA film. PMMA−chitosan blend is widely studied by different research groups for application in drug delivery and other biomedical applications.

Zuber et al. (Amer and Ahmed, 2014) prepared PU-PMMA/titanium oxide blend, which showed excellent mechanical strength and good biocompatibility applied as dental implants. For drug delivery the blend of PMMA with polymers like gelatin has been tested for delivery of antibiotics in biological targets. Langer et al. (1997) in their study has prepared nanoparticles of MMA/sulfopropyl methacrylate and used them for topical ophthalmic application of muscarinic agonists arecaidine propargyl ester and (S)-(C)-aceclidine in rabbits. In another study Downes (1991) altered the polymer-to-monomer ratio, which markedly improves the drug release from PMMA−bone cement composite. It has been observed that higher ratio of polymer-to-monomer enhances the porosity in polymer structure and thus increase the drug release. Mechanical property of PMMA makes it suitable for repair of craniofacial deformities (Ali et al., 2015).

3.3.4 Poly hydroxyethyl methacrylate

Poly hydroxyethyl methacrylate (PHEMA)-based hydrogels are the first synthetic hydrogels for pharmaceutical applications. The hydrogels made of PHEMA are biocompatible in nature and possess tunable mechanical properties (Baker et al., 2009). Besides high load bearing ability, this polymer also exhibits the resistance to crack propagation 18. One of the disadvantages of this material is lower biodegradable property that limits its application in biological field 16. The degradation product of this polymer consists of high molecular weight long chain. By changing the pattern of cross-linking with short hydrostable dimethacryloyl hydroxylamine, the researchers have been able to improve the mechanical property and low molecular weight product after degradation (Moghadam and Pioletti, 2016). PHEMA in dry state is hard and brittle and after swelling it becomes flexible. This transparent hydrogel with porous structure permits the diffusion of liquids and oxygen. Such properties of this polymer make it suitable for fabrication of hydrophilic contact lens and artificial cornea (Kannan and Narayanan, 2015). PHEMA and chitosan blend is commonly applied in drug delivery. In this work, using UV free-radical polymerization technique, a pH-sensitive membrane with interpenetrating polymer network has been formed for delivery of targeted drug. Membrane fabricated by this method can modulate the controlled drug release at the target site (Bayramoğlu and Arıca, 2003).

There are various applications of PHEMA-based hydrogel in biomedical field. Besides controlled drug release and protein release, the hydrogel also has potential application in development of synthetic skin, breast augmentation, contact lens, etc. (Zahedi and Lee, 2007; Lu and Anseth, 1999). Photopolymerized hydrogels of this polymer are used in 3D scaffold formation for soft tissue engineering (Bártolo et al., 2009). Research group from Portugal has developed novel stereolithographic fabrication technique for the fabrication of multimaterial graded scaffolds using UV and thermal energy for cross-linking the polymer (Pj, 2008). The swelling property of PHEMA is moderate and can be modulated by changing the thickness of gel and by changing the degree of cross-linking (Mabilleau et al., 2006; Davis and Huglin, 1991). Thin hydrogel films are applied for sensing mechanism (Kikuchi and Okano, 2005; Blanco-López et al., 2004). Deposition of

polymer hydrogel on metallic implants improves the compatibility of implants and also develops controlled release device such as stent or microchips (De Giglio et al., 2011).

3.3.5 Polyurethanes

Polyurethane (PU) consists of a flexible long oligodiol part and a relatively rigid part containing diisocyanate. During synthesis introduction of ionic component transforms the PU into ionomers, which is easily dispersed in water (Eceiza et al., 2008). Synthesis of water-based PU is eco-friendly in comparison with conventional synthesis of PU and it has wide range of applications in development of wound dressing, surgical sutures, drug delivery vehicles, etc. (Cherng et al., 2013). Property of segmented PU depends on their molecular structure, which is controlled by the components use in polymerization process, and phase separation of these components takes place during formation of supramolecular structure (Kojio et al., 2007; Velankar and Cooper, 2000). PUs have array of commercial applications in different form, majorly classified in seven categories including flexible slab, foam, rigid foam, adhesive, sealant, carpet backing, etc. Modified PU by introducing different groups like sugar acts as insulator (heat or tremor), protective case, etc. (Kizuka and Inoue, 2016). The popularity of PU in biomedical application is due to its ability to act as thermoplastic elastomer. Although degradability of this polymer is a matter of concern, however, recently a wide range of modified PU with controlled biodegradable property has been reported in the application of regenerative medicine and drug delivery (Chen et al., 2013). The degradability pattern of this polymer is altered through introducing macrodiol of hydrolytically labile polymers such as poly(lactic acid) (PLA), PEG, PCT (polycyclohexylenedimethylene terephthalate), etc. (Fang et al., 2014; Guan et al., 2005).

PU is synthesized by the process known as polycondensation of diisocyanates with alcohols and amines 286. Rigid and flexible segments of this polymer allow it to bear the physical stress through microphase separation 287. It is widely used in development of prosthesis such as vascular shunt, tracheal tube, cardiac assist device, etc. (Uttayarat et al., 2010; Backman et al., 2009). NovoSorb (PolyNovoVR) is a commercial available polymer blends containing PU, which acts as bone cement and cured under physiological temperature. This product promotes cellular growth and proliferation (Bonzani et al., 2007). However, limited degradability limits its application in biological field. Recent studies reported that the degradation of PU can be modulated through blending with multidegradable polymer groups (Ulery et al., 2011). To speed up the degradation rate of PU, generally ester bond has been introduced in polymer backbone (Henry et al., 2007). Commercial products such as PolySorb and porous DegrapolVR are prepared by this method and applied for bone, cartilage, and tracheal regeneration, respectively (Yang et al., 2003; Brizzola et al., 2009).

3.3.6 Amino acid and polyamino acids

Polyamino acid (PAA) is eco-friendly polymer synthesized from bio-based polyesters such as poly(hydroxyalkanoate) and PLA (Numata et al., 2008). It has potential to

contribute to sustainable biomass-based society due to its biomass-based origin, physical properties, plasticity, and excellent workability (Chuah et al., 2013; Numata and Doi, 2012). PAA contains amino acid as monomer units. PAA endows remarkable biological activity such as target specificity and fast metabolism of the degraded products of such materials in biological system. Due to such advantages of the PAA, there are wide possibilities of application of this polymer as functional materials (Vandermeulen and Klok, 2004). However, the structure−function relationship is yet not fully explored, which limits its practical applications. PAAs contain different types of water molecules, which defines thermal stability and biological and mechanical integrity of the polymers (Numata et al., 2011). Based on the origin, the properties of PAAs may vary. As for example, the poly(hydroxyalkanoate) derived from various biomass such as lignin and carbon dioxide (Osanai et al., 2013; Tomizawa et al., 2014) possess excellent biodegradability and biocompatibility; however, due to lack of toughness their practical application is limited (Numata et al., 2009). Engineered biopolymer such as biopolyamide (nylon 4) has also explored as biomass-based engineering plastic (Tachibana et al., 2013). Major limitation of PAAs is limited synthesis methods reported till date. Till date the most common techniques are solid-phase peptide synthesis and recombinant DNA techniques, both these methods possess limited production capacity, difficulties in sequence regulation, and also financially not suitable (Shen et al., 2015).

Two most commonly used biopolymers are poly(c-glutamic acid) (cPGA) and poly(L-lysine). The compositions of biodegradabale polyamides, cPGA, are readily synthesized by the numbers of different bacterial populations (Yeh et al., 2010; Candela et al., 2009). To enhance its biological property, addition of different functional groups such as benzyl ester (Jeong et al., 2009), sulfide (Yoshida et al., 2009), sulfonate (Matsusaki and Akashi, 2005), and chemotherapeutic attachment (Van et al., 2010) to the reactive carboxylate group is present inside chain. cPGA has been used in delivery of vaccine, antibiotics, DNA, protein, etc. (Matsuo et al., 2010). Cross-linked hydrogel or composite of cPGA blended with PLA, PLGA, is commonly used in soft tissue engineering (Matsusaki et al., 2007; Deng et al., 2006). The poly(L-lysine) is produced by bacterial synthesis and popularly used as tissue engineering scaffold material or in drug delivery. It possesses intrinsic antibacterial, antiviral, and even antitumor activities (Matsusaki et al., 2007; Deng et al., 2006). However, high positive charge of this polymer sometime limits its application; and to overcome this, the blend of poly(L-lysine) with other degradable polymers such as PLA, PCA, cPGA, chitosan (Ulery et al., 2011; Itoh et al., 2008; Bertram et al., 2009) are frequently used for practical applications. To overcome the scarcity of natural PAA, synthetic PAA also studies to explore their possible applications in biomedical field (Ulery et al., 2011).

However, most of the synthetic PAA exhibits unfavorable mechanical property, high crystallinity, and immunogenicity (Bourke and Kohn, 2003). In this regard two PAA—poly(L-glutamic acid) (L-PGA) and poly(aspartic acid)—emerge as promising materials. In contrast to c-PGA, L-PGA is more flexible due to the presence of amide linkage as an alternative of c-carbon amine group. The biocompatibility of L-PGA is reported and they are susceptible to degradation by lysosomal enzymes

(Wadhwa and Mumper, 2010). Additionally, the negative charge of this polymer in physiological condition makes it a potential candidate for DNA delivery (Matsuo et al., 2010). Recently LBL films have been developed by blending with chitosan and poly(L-lysine) for drug delivery system (Jessel et al., 2006). It contains charged polymer layers that help in delivery of positively or negatively charged drugs to the target site. By modulating the side chain of this polymer, potent chemotherapy conjugates have been developed and marketed as OPAXIOVR (cell therapeutics) (Huang et al., 2010). Tian et al. (2011) in their study have developed L-PGA−gadolinium complex as the contrast agent in MRI.

3.3.7 Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is water-soluble synthetic polymer. It is inert, pH stable, temperature resistance, nonionic polymer. PVP exhibits excellent biocompatibility, reasonable solubility in most of the organic solvent, capability to interact with range of hydrophilic and hydrophobic materials, and low chemical toxicity makes this polymer a perfect choice for biomedical applications. It is commonly used in pharmaceutical, cosmetic, adhesive, electronic, and beverage industries. Its application in tissue engineering was first reported by Bognitzki et al. (Tian et al., 2011) and then fabricated electrospun scaffolds from PVP/chitosan blend. Xia et al. studied the fabrication of composite nanofibers containing PVP and TiO_2 (Li et al., 2003). Application of PVP scaffolds in regenerative medicine has been reported by several research groups. Scaffolds prepared with PVP and bioglass was applied as tissue constructs and their effect on mesenchymal stem cells has also been studied (Li et al., 2003). Such polymer composites are reported to enhance the mechanical property of scaffolds and also improve their degradation and biocompatibility (Ignatova et al., 2006). Chitosan−PVP hydrogel is also used in vascular application for activation and proliferation of endothelial cells (Subramanian et al., 2014).

3.4 Biomedical applications of synthetic gels

Application of synthetic gel in biomedical field can be categorized into three groups: (1) extracorporeal application that includes artificial skin, kidney, dialysis membrane, different types of dressing materials, etc., (2) permanent implant such as cardiovascular, dental, orthopedic devices, etc., and (3) temporary implants that include degradable implants, suture materials, tissue engineering scaffolds, temporary vascular graft, stent, etc. Biodegradable and bioabsorbable polymers are most interesting group of material for biomedical applications. There are certain synthetic polymers that possess good compatibility in physiological environment; however, their degradation profile is not appropriate for biological applications. To overcome such limitations, blending of synthetic polymers with biodegradable natural polymers of different compositions has been tested. Elastomer group of polymers is biodegradable polymer types, which are further categorized into two types based on their duration of interaction with physiological conditions (Du et al., 2016). Biodegradation of polymers depends on several factors such

as molecular weight of polymer, hydrophilic/hydrophobic properties, types of functional groups present in the structure, condition of surrounding environment (pH, temperature), presence of microorganisms and types, etc. (Spigelman et al., 1984). The ester linkage of biopolymers degrades faster under physiological conditions wherein degradation urethane, carbonate, and urea linkages are only enzymatically decomposed. One of the oldest commercially available synthetic polymer scaffolds is Ivalon, which is extensively used as embolic material (Tadavarthy et al., 1975). Regeneration of cartilage is bit difficult due to lack of vascularity and cellular components (Suciu et al., 2004). High water content of PVA hydrogel makes it suitable for cartilage regeneration. PVA-C is prepared from high molecular weight polymers (~30% PVA or more) for cartilage resurfacing. Wear resistance implants are required for orthopedic implants because such implant prone to undergo wearing and osteolysis. This not only loosens the implants but the wear particles often induce immunological reactions (Ingham and Fisher, 2000). Suciu et al. (2004) studied different compositions and thickness of PVA for knee joint reconstruction. They concluded that PVA with less water content causes smallest wear factor. A comparative study on the wear particles produces from PVA with that of ultrahigh molecular weight polyethylene showed that the PVA particles are less inflammatory in comparison with the other polymer (Oka et al., 2000). PVA hydrogel fabricated in saline (SalubriaVR, Salumedica, Atlanta, GA) has clinically evaluated for cartilage replacement in human (Oka et al., 2000; Falez and Sciarretta, 2015). In a clinical study with PVA implants showed that the patient with chondral defect was treated with PVA implants and MRI and X-ray results of postoperative periods showed that no loosening or dislocation of implant occurs even after 4 months after operation. In contrast to above observations few reports are available regarding the failure of PVA implants due to dislocation or loosening from the application site (Meyer et al., 2005). One of the reasons behind such failure was identified that possibly multiple devices have been implanted into a single site of defect. Generally it is believed that, in case of multiple implants if they are in contact with each other causing them free floating, it may cause expulsion with loading and time (Baker et al., 2012).

In pharmaceutical industry, the PMMA is one of the widely used polymers. Biocompatibility of such polymers makes it an important component for drug delivery system. It is also used as adjuvant for vaccine and suitable for delivery of drugs, antioxidants, etc., at the target site (Bettencourt and Almeida, 2012). PHEMA is a transparent polymer. Its application in ophthalmology especially in contact lens development has been studied in 1960 by the scientists Wichterle and Lim. Based on the elasticity, the contact lenses are categorized into hard and soft lens. Although hard lens are longer-lasting, they often suffer from poor adaptability. The hard lens are generally fabricated from PMMA and poly(hexafluoroisopropyl methacrylate), and for soft lens the hydrogels are commonly used (Wichterle and Lim, 1960).

The one that is most important in biomedical engineering is the development of appropriate wound dressings based on the types of wound beds. The basic requirements of a dressing material are optimum shelf life, conformability, cost-effectiveness, and intrinsic properties that are able to diminish the bacterial load and inflammation of the

wound bed (Halim et al., 2010). One of the oldest dressing materials is gauze. In modern days, gauze are modified by impregnation of active ingredients containing zinc, iodine, etc. (Vahidhabanu et al., 2017). However, such dressing suffers from unwanted adhesiveness with the underlying cellular structure. To overcome such problem low adhesive dressing material is employed as dressing materials. Maintaining the optimum moisture level at wound site is another prerequisite for successful healing process. Hydrogel-based dressings become popular due to their high water retention capacity (Lee and Mooney, 2012). Recent studies have revealed that in comparison to hydrogel, the hydrocolloid-based dressings are preferable as they are able to maintain optimum moisture level at the wound bed (Sood et al., 2014). Hydrofiber (ConvaTec) is one of such dressings that can retain the optimum moisture level at wound site (Barnea et al., 2010). In burnshield dressing (Levtrade International), burn wound is made of PU foam and in combination with other active ingredients, which provide cooling effect to the burn site due to their hydrating effect (Osti, 2006). The water-retaining capacity of wound dressing also accelerates the autolytic debridements, which in turn speed up the granulation tissue formation and rapid healing process. Cartmell and Sturtevant (1992) have developed transparent film of PEG, which was nonadhesive at center and transparent that allowed maintaining the gaseous permeability, and the healing progress can also be observed through this thin film. Holm et al. (2011) have developed a similar product containing hydrogel dressing pad with adhesive backing. Composite wound dressings comprising hydrogel with other bioactive materials were also developed to improve the healing potential. Thermoplastic polymers reinforced with fibrous structure were developed by Shah et al. (1996). The hydrogel part exhibited microphase separation and also facilitated the absorption of wound exudates and avoiding adhesion to wound surface.

Synthetic hydrogels attract noticeable interest in the field of drug delivery. Although many studies have performed on drug delivery potential of such materials, only few products are available commercially. The unique characteristics of hydrogels including high porosity, affinity of water, and easily adjustable degree of cross-linking are suitable parameters for drug delivery system (Hoare and Kohane, 2008). Moreover, the possibility of sustained release with hydrogel-based delivery vehicles increases the bioavailability of high concentration of active ingredients at the target site. The target-specific release of drugs can be achieved through various techniques controlled by diffusion, swelling, chemical process, and environmentally responsive method. The reservoir type delivery system is viable in the form of capsules, cylinder, or slab in which the drug is encapsulated in hydrogel and the concentration of drug is higher at center that allows constant release rate (Langer, 1994). Nho and Lee (2005) demonstrated the topical application of therapeutic hydrogel of PVA/PPP mixed with active ingredients from herbal products for the treatment of atopic dermatitis. Development of hydrogel-based smart delivery system is emerging, which can sense the surrounding microenvironmental factors and are able to adjust the drug release accordingly. Bae et al. (1991) reported application of pH or temperature-sensitive drug delivery device in which the hydrogel could be swelled responding to the altered pH, ionic strength, or glucose concentration. In the same direction, Park et al. (1997) proposed the smart gastric retentive device comprising superporous hydrogel of different cross-linked polymers such as (meth)acrylic

acid, carboxymethylcellulose, PVP, etc. Such superporous structure was made by gas blowing method consisting of simultaneous foaming and polymerization process. The study has confirmed the retention and drug release from such device in stomach up to 24 h.

Due to intrinsic biocompatibility, the synthetic hydrogels become indispensable candidate in the field of tissue engineering applications. Although several manufacturing methods are available for the fabrication of hydrogel-based tissue engineering scaffolds, however, an interesting fabrication process has been depicted in recent European patent (Wang et al., 2005b). In this process unsaturated self cross-linking polymer poly(propylene fumarate) was blended with biodegradable hydrogel microparticles composed of collagen or gelatin. The microparticles used in this process entrapped free radical initiators, which initiate the cross-linking process. The fabricated product described as "super-absorbent semisolid" microparticles showed remarkable water retention properties and were applied for correction of skeletal defects. Using self-assembling peptides, the nanofibrous and nanoporous hydrogel scaffolds were developed by Harris et al. (2004). Such scaffolds possess nonimmunogenic and biodegradable properties and are able to stimulate the tissue growth, drug diffusion, and vascularization. Self-assembling peptides contain alternating hydrophobic and hydrophilic amino acids and are commercially available as commercialized as "PURAMATRIX" (3D Matrix, Inc., Cambridge, Mass.). Besides tissue engineering scaffolds such products are also applicable for wound dressing purpose. Hydrogel scaffolds in the form of cell sheets were recently studied by Kumar et al. (Harris et al., 2004). In their study biodegradable PVA was mixed with phenylboronate-containing polymers. Cells of interest (keratinocyte and fibroblast) were cultured on the polymer scaffolds and cell layers were collected after 5−20 days of culture using saccharide solution, which induce the degradation of hydrogel underneath the cellular structure. Particularly the phenylboronate ligands were dissociated in the presence of saccharide solution. Blanchard et al. (Slaughter et al., 2015) used cross-linked keratin-based hydrogel for mimicking the endogenous cellular structure. Keratin is isolated from biological samples such as hair or nail of the patients and after chemical processing the scaffolds were prepared. Such material is not only biocompatible but also provides nutritional support to the attached cell layers. Radiation fusion technique was used by Song et al. (2010) for preparation of scaffolds from beta-glucan. Beta-glucan has excellent biocompatibility and also promotes cellular regeneration. In successful tissue engineering the most prevailing challenges are the formation of suitable vascular structure in the tissue-engineered constructs. Many researchers have explored the possibility to utilize hydrogel to effectively control the vascularization process (Ishihara et al., 2006). In such methods local delivery of proangiogenic factors using hydrogel as delivery vehicles has been investigated. To mimic the extracellular matrix (ECM) for increasing cell−material interactions different strategies have been followed by the research groups. To provide the cell binding ligands of short peptide sequences like RGD sequence are incorporated in the ECM structures. Various functional groups such as amine, carboxyl, thiol, azide, vinyl, etc., are also incorporated into the hydrogels, which functionalize the peptide sequence (Tallawi et al., 2015). Acrylation is one of such methods for modification of N-terminus of RGD-monoacrylate and RGD-PEG monoacrylate (Zhu and Marchant, 2011).

In tissue engineering bulk degradation of the polymer causes internal breakdown of scaffolds that reduce the molecular mass. For hard tissue engineering the stability of scaffolds is an important prerequisite. Due to good mechanical stability, synthetic polymers such as PGA and PLLA (poly-L-lactic acid) are commonly used for fixation of internal bone structure. PGA is also used in development of resorbable sutures due to its good fiber-forming properties. Nondegradable fibers of PLLA were used in replacement of ligament (Chen et al., 2003). Mechanical properties of synthetic polymers can be modulated by blending with more degradable polymers or inorganic bioactive agents for wide range of tissue engineering applications (Zhu and Marchant, 2011). Cellular attachment and proliferation also depends on surface topology, architecture, and macroscopic 3D structure (Viswanathan et al., 2015). Besides selection of materials, identification of suitable fabrication method is also an important criterion. For maintaining porosity in melt extrusion or inkjet molding, physical/chemical molding agents are employed. Researchers have proposed that using such agents the pore size and geometry can be controlled, which is suitable for growth of osteoblast cells and apparent bone formation (Siva and Ansari, 2015). Microstructure of scaffolds can also be controlled through employing techniques such as solvent phase separation, gas foaming, ice crystal formation, freeze drying, etc.; however, porous scaffolds made of PLA and PLGA are not suitable for delivery of cells due to hydrophobic structure. Inorganic components such as HA, TCP (1,2,3-Trichloropropane), and bioactive glasses are commonly used to overcome such limitations (Raucci et al., 2012); for development of vascular structure, PGA is commonly used polymer. High porosity of this material allows easy diffusion of nutrients that facilitates neovascularization process (Williams and Wick, 2005). Combination of other polymers along with the PGA is also used to improve the bioabsorbility.

3D printing method is an emerging technique in the field of tissue regeneration. In this method, the selected polymers (bioinks) are blended with the cell of interest to form the tissue construct in layered fashion. Development of suitable bioink is extremely challenging and it requires satisfying set of parameters of biomaterials because the material is in direct contact to the cells right from the beginning and should maintain optimum microenvironment for viability of the cell-laden constructs (Williams and Wick, 2005). Among synthetic polymers, PEG is used as bioink by modulating its mechanical properties through controlling the degree of cross-linking or exposure of UV radiation in photo-cross-linking process (Buwalda et al., 2017). Synthetic self-assembling molecules such as ultrashort peptides can assemble into hydrogel network, which contains considerable amount of water and their fibrous structure mimics the native ECM (Loo et al., 2015). Size of the peptides may vary from 2 to 20 amino acid length and by modulating their intrinsic properties (gelation time, amino acid sequence) and extrinsic factors such as pH, salt concentration, and temperature they can be used as perfect candidate for bioprinting process. Hauser et al. used ultrashort self-assembling hexapeptide hydrogels as bioinks for development of burn wound dressing (Hauser et al., 2011). These peptide hydrogels

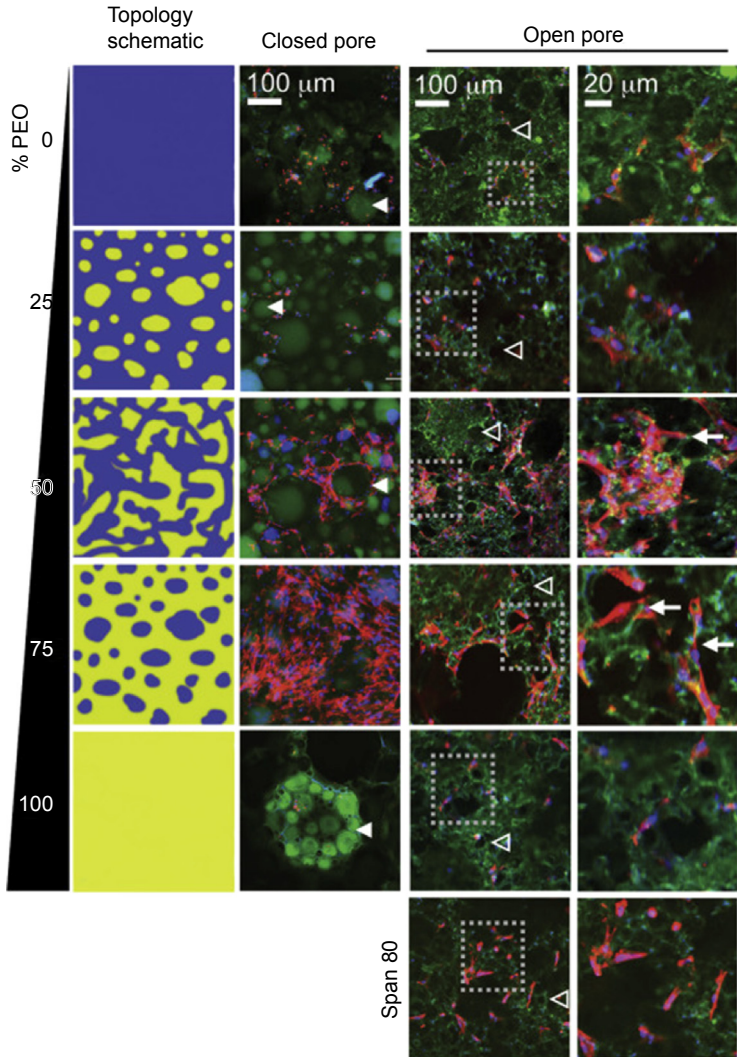

Figure 3.4 Depicts adhesion of human mesenchymal progenitor cells on poly(ethylene oxide) (PEO). The topologies of fabric at different concentration of PEO are represented. The alteration in cellular attachment is altered with topological modulations (Viswanathan et al., 2015).

combined with intestinal epithelial cells for development of organoid 3D cultures. It was also reported that embryonic stem cells are also able to maintain their pluripotent characteristics in this hydrogel bioink. Studies have reported that encapsulated mesenchymal stem cells in hydrogel are able to maintain their differentiation potential in response to the external stimuli (Loo et al., 2015). Attachment of human mesenchymal progenitor cells on polymerized high internal phase emulsion of poly(ethylene oxide) is represented in Fig. 3.4.

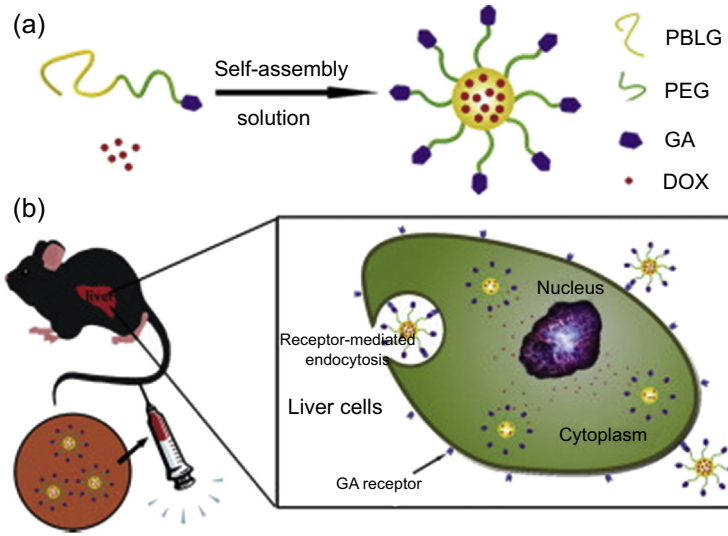

Figure 3.5 Shows (a) mechanism for self-assembly of glycyrrhetinic acid (GA)-modified poly(ethylene glycol)−b-poly(γ-benzyl L-glutamate) (GA−PEG−PBLG) block copolymers and (b) uptake of polymer composite by liver through endocytosis in mice model (Huang et al., 2010).

Gao et al. (2015) have prepared constructs using PEG-based hydrogels blended with acrylated RGD and matrix metalloproteinase and MSC (mesenchymal stem cells) through ink-jet printing. MSCs showed their differential potentials toward osteogenic or chondrogenic lineages under external stimuli (Gao et al., 2015). Abbadessa et al. (2016) used a number of copolymers, methacrylated PEG, poly[*N*-(2-hydroxypropyl) methacrylamide mono/dilactate] (polyHPMA-lac), and methacrylated hyaluronic acid (MeHA) and chondroitin sulfate, for constructing thermoresponsive hydrogels for cartilage regeneration. They have successfully maintained the viability of chondrocytes up to 95% even after 42 days of printing (Abbadessa et al., 2016). Another composite of PEG containing self-assembly of glycyrrhetinic acid (GA)-modified poly(ethylene glycol)−b-poly(γ-benzyl L-glutamate) (GA−PEG−PBLG) block copolymers and their application in delivery of drug in mice has been depicted in Fig. 3.5.

Due to excellent water retention property, hydrogels are also applied in agriculture and diaper industry as superabsorbent polymers (Abbadessa et al., 2016). The first synthetic hydrogel that was used in this purpose was cross-linked starch-g-polyacrylate (Masuda, 1994). Recently Osborn proposed two layered structure for sanitary napkins. The core of the product composed of monomers of acrylic acid, methacrylic acid, or 2-acrylamido-2-methyl propane sulfonic acid and two air-laid tissue sheets were used to enclose the hydrogel (Masuda, 1994). A more comprehensive enlisting of synthetic polymer gels and their biomedical applications are provided in Table 3.1.

Table 3.1 Synthetic polymers and their biomedical applications

Synthetic polymer	Biomedical application	Commercial product and company name	References
Poly (vinyl)alcohol (PVA)	Suitable for tissue mimicking, vascular cell culturing, and vascular implanting	Vascular Grafts (Axcelon Biopolymers Corporation)	Jiang et al. (2011)
Poly(ether urethanes)	Vascular grafts, heart valves, blood contacting devices, coatings	Implantable neurostimulator/medullary Precision Spectra (Boston Scientific)	Shastri (2003)
Polyphosphazenes	Implant devices, controlled drug delivery among tumor-bearing animals	Implantable cardioverter defibrillator ENERGEN (Boston Scientific)	Schacht et al. (1996)
Poly(ethylene glycol) (PEG)	Hydrophilic linear polymer used as antifouling coating on catheters, hydrogel, or as pore former in dialysis membranes	PEGDMA 1000 (Polysciences, Inc.)	Campoccia et al. (2013)
Poly(*N*-2-hydroxypropyl methacrylamide) (PHPMA)	PHPMA is a water-soluble, synthetic, vinyl-based polymer with singular nonimmunogenic and nontoxic characters. It has been broadly exploited for biomedical applications, to the extent that several PHPMA-based systems entered Phase I or II clinical trials for cancer chemotherapy applications Heterogeneous PHPMA hydrogels can be used for tissue repair and axonal regeneration in the injured spinal cord	Altrazeal (Sandor Medical Pvt. Limited)	Woerly et al. (1998)
Poly(lactic acid) (PLA)	Tissue engineering, wound management, drugs delivery, orthopedic devices	Stryker Craniomaxillofacial (Stryker) 4DMesh—semiresorbable implant for parietal treatment and reinforcement of inguinal and femoral hernia (Cousin Biotech)	Hamad et al. (2015)

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Table 3.1 Continued

Synthetic polymer	Biomedical application	Commercial product and company name	References
Polyvinylidene fluoride (PVDF)	Suture material or surgical mesh	Extracorporeal circulation filter/Blood/ Polyvinylidene fluoride (BRAILE BIOMEDICA) IPOM, CICAT, ENDOLAP, ENDOLAP 3D, IPST (Dynamesh)	Capperauld (1989) and Karakelle and Zdrahala (1989)
Polyethylene (PE)	Orthopedic sutures, artificial tendons, stable polyolefin, used as LDPE, HDPE, or UHMWPE	Force Fiber Suture (Teleflex Medical OEM) CLINIfibre (tendon and ligament repair) (Sutures India Private limited)	Maitz (2015)
Polypropylene (PP)	Heart valves	Biomesh P1, Premium (Cousin Biotech)	Kretschmer et al. (1992) and De Somer et al. (1994)
Polyurethane	Commonly used materials in the production of blood contacting devices such as heart valves or artificial veins and arteries	Bionate PCU (long-term use in the body and has been used in chronic implants) Biospan SPU CarboSil TSPCU (exceptional toughness and biocompatibility) Elasthane TPU (long-term implantation)	Burke and Hasirci (2004)
Polydimethylsiloxane (PDMS)	Silicones are highly inert elastomer, used for catheters, nucleus pulposus substitute, plastic surgery, intraocular lenses,glaucoma drainage devices, and dialysis membranes	Dow corning qp1-20 liquid silicone rubber kit (Dow corning)	Johnson et al. (2013) and McLaughlin et al. (1997)

Poly(lactide-co-beta-malic acid) (PLMA)	Cell and tissue carriers in tissue engineering techniques, degradable implants, fibers and threads, and drug carriers	Inion Spine system (Inion)	Bondì (2015)
Polylauryl methylacrylate (PLMA)	Cell and tissue carriers in tissue engineering techniques, drug carriers, degradable implants, fibers, and threads	μco Carrier (SNBL)	Maitz (2015)
Poly(N-isopropyl acrylamide) (PNIPAAm)	Thermosensitive injectable hydrogels	–	Maitz (2015)
Ethylene-co-vinylacetate (EVA)	Implantable drug delivery devices	Product name: Nuvaring Company name: Merck and Co.	Shastri (2003)
Poloxamer	Lung tissue engineering	Poloxamer 407 Synonym: Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)	
Polysulfone (PS)	Surgical and medical devices, clamps, artificial heart components, heart valves, component of hemodialysis membrane	CrocClamp (ITL Biomedical Healthcare)	Maitz (2015)
Polyvinylchloride (PVC)	Blood tubing, blood bags	NiproSet Blood tubing set (NIPRO)	Maitz (2015)
Polyetheretherketone (PEEK)	Dentistry products, rigid tubing, hard stable polymer for orthopedic applications or inner lining of catheters	Austin Moore Hip prosthesis, Broad stem, Regular Finish (Auxein)	Kurtz and Devine (2007) and Pruitt and Furmanski (2009)
Polytetrafluoroethylene (PTFE)	Tubing, endoscopes, cannullas, catheter linings, surgical sutures	TRINOX Trocar system (Xion medical)	Maitz (2015)

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Table 3.1 Continued

Synthetic polymer	Biomedical application	Commercial product and company name	References
Poly(dioxanone) (PDS)	Degradable polymer, frequently as copolymer with PLLA with comparable applications	–	Maitz (2015)
Polyethersulfone (PES)	Tubing, catheters, hemodialysis membrane	PUREMA, DIAPES, SYNPHAN (3M Science Applied to Life.)	Klinkmann and Vienken (1995)
Nylon	Nylon-6 nanofiber mat incorporated with 5, 5-dimethyl hydantoin (DMH) acts as an antimicrobial drug when electrospun from formic acid	Zytel (DuPont)	El-Newehy et al. (2011)
Poly(methyl methacrylate) (PMMA)	Bone cement used in joint replacements	Cemex Bone cement (Exactech)	
Poly(ethylene terephthalate)	Biostable polyester Dacron used for membranes, vascular grafts, surgical meshes, ligament, and tendon repair	EHIBOND EXCEL Polyester Suture (ETHICON)	Longo et al. (2010), Klinge et al. (2013) and Kannan et al. (2005)
Poly (hydroxyethyl methacrylate) (PHEMA)	Antifouling coating and hydrogel for intraocular lenses, frequently in copolymers with PMMA Antifouling coating and hydrogel for intraocular lenses, frequently in copolymers with PMMA	Contact lenses (Axcelon Biopolymers Corporation)	Venkatesan et al. (2010) and Bellucci (2013)
Butyryl-trihexyl-citrate (BTHC)	Alternative plasticizer of PVC in blood bags	BLOOD BAG 732-3XM-Series (HAEMONETICS) BLOOD BAG SUPPR (Limbs and Things)	Muylle et al. (1995)

High-density PE (HDPE)	Stiff polyolefin used for packaging, inner lining of catheters, or graft for craniofacial contour augmentation	Stryker MEDPOR Facial Contour Implants (Company name: Stryker)	Pruitt and Furmanski (2009) and Klinkmann and Vienken (1995)
Poly (amide) (PA)	Nylon, used as suture material, ligament and tendon repair, balloon of catheters, dialysis membranes	LINEX—MONOFILAMENT NYLON SUTURE (Dolphin Sutures) Ethilon Nylon Suture (Ethicon)	Pruitt and Furmanski (2009) and Klinkmann and Vienken (1995)
Poly (acrylonitrile) (PAN)	Dialysis membranes	Dialysis membrane tubing (San Jose Scientific)	Klinkmann and Vienken (1995)
Poly(carbonate) (PC)	Biostable polyester for dialysis membranes and containers	Makrolon (Covestro)	Klinkmann and Vienken (1995)
Poly (D-lactic acid) (PDLA)	Degradable polyester of D-lactic acid, similar spectrum as PLLA	BioDegmer (BMG)	Maitz (2015)
Poly(caprolactone diol) (PCL)	Diol for polyurethane formation	PURASORB PC 12 (**Corbion**)	Ferreira et al. (2008)
Poly(ethylene oxide) (PEO)	Antifouling coating of catheters	-	Campoccia et al. (2013)
Poly(lactic-co-glycolic acid (PLGA)	PLLA/PGA copolymer with similar application spectrum as PLLA	PURASORB PLG 8523(Corbion)	Gaikwad et al. (2008)
Poly (L-lactic acid)	Degradable polyester of L-lactic acid for orthopedic fixation tools, ligament and tendon repair, vascular stents	Carotid WALLSTENT Monorail Endoprosthesis (Boston Scientific)	Maitz (2015)
Poly (vinylpyrrolidone) (PVP)	Hydrophilic, soluble polymer as antifouling coating or in dialysis membranes	Serene Coatings (Surdomics)	Maitz (2015)

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Table 3.1 Continued

Synthetic polymer	Biomedical application	Commercial product and company name	References
Ultrahigh molecular weight PE (UHMWPE)	Stable and low friction polymer for joint prostheses	Accolade II (Stryker)	Maitz (2015)
Poly (glycolic acid) (PGA)	Degradable polyester with similar application spectrum as PLLA	Related products of PLLA	Kasser (2013)
Parylene	Implantable Pulse Generator for Deep Brain Stimulation • Neuroprosthetics • Cognitive prostheses • Catheters Nasal implants for nose reconstruction	Peri-Pyriform (Implantech)	Teo et al. (2016)
Polyhydroxyalkanoates	Artificial urinary sphincter implant	AMS 800TM Urinary Control System (Boston Scientific)	Shastri (2003)
SU-8	Implantable Pulse Generator for Deep Brain Stimulation Neuroprosthetics • Cognitive prostheses • Catheters		Teo et al. (2016)
Polyimides	Implantable Pulse Generator for Deep Brain Stimulation Neuroprosthetics • Cognitive prostheses • Catheters	Implantable Neurostimulator/Medullary (Boston Scientific)	Teo et al. (2016)

3.5 Conclusions

Like natural hydrogels, several synthetic gels are also biocompatible due to their optimum hydrophilicity and resemblance to native ECM microenvironment. Their main advantage lies in the ability to obtain gels with tailored degradation and mechanical and surface properties. Synthetic gels can also be produced at mass scale and have superior mechanical and chemical stabilities over natural hydrogels. Moreover, in comparison to natural gels, synthetic gels have higher reproducibility in terms of physicochemical properties and, hence, in biological properties. These have made them indispensable for wide range of biomedical applications, including emerging areas of bioprinting and microfluidics. Newer methods of synthesis to obtain more accurate network descriptions along with bioactive functionalization techniques are also emerging to expand their scope of applications. Application of click chemistry, supramolecular assemblies, and controlled radical polymerization is complimenting the methods of fabrication of scaffold with more tailored stiffness and network properties. Observation of smart and intelligent gels is another area which is poised to deliver solutions to several biomedical challenges. However, though commercial products in medical sector such as contact lens, wound dressings, and injectable carriers for stem cell therapy have already been introduced in clinical practice, future products in tissue engineering, drug delivery, and molecular biology are expected. Addressing regulatory concerns and high production costs will be key determinants for this translation (Caló and Khutoryanskiy, 2015). In tissue regeneration, controlling degradation rate of synthetic gels to match rate of growth of neotissues will be an important research direction.

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Semi-IPNs and IPN-based hydrogels

4

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4.1 Semi-IPNs and IPNs gels

Semiinterpenetrating polymer networks (semi-IPNs) and interpenetrating polymer networks (IPNs) have emerged as innovative materials for biomedical and pharmaceutical applications. The interest in these structures is due to the possibility of combining the favorable properties of each polymeric component of the IPNs or the semi-IPNs leading to a new system with properties that often differ from those of the two single components (Matricardi et al., 2013).

4.1.1 Definitions

The IPNs may be considered belonging to the class of polymer blends. Polymer blends are systems containing two or more polymer components, which may be classified into two categories: **mechanical blends** in which no chemical bonds are formed between the two polymers and **graft copolymers** containing primary bonds between the polymeric components. Graft copolymers are further divided into subclassed according to the presence or absence of cross-linking between the components. Particularly, when both polymers are cross-linked, the resulting materials are known as "interpenetrating polymer networks," IPNs (Banerjee et al., 2010b).

An IPN is defined by IUPAC as "A polymer comprising two or more networks which are at least partially interlaced on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken. A mixture of two or more pre-formed polymer networks is not an IPN" (Jenkins et al., 1996). Moreover, the IUPAC definition referred to semi-IPN as follows: "A polymer comprising one or more networks and one or more linear or branched polymer(s) characterized by the penetration on a molecular scale of at least one of the networks by at least some of the linear or branched macromolecules." Based on these definitions, semi-IPN differs from IPN because these linear or branched macromolecules are only dispersed into the polymer network(s), without forming a further interpenetrated network and could be separated from polymer network(s) without breaking chemical bonds (Jenkins et al., 1996). A schematic representation of mechanical blends, graft copolymers, block copolymers, semi-IPN, and full-IPN is reported in Fig. 4.1.

Polymeric Gels. https://doi.org/10.1016/B978-0-08-102179-8.00004-1

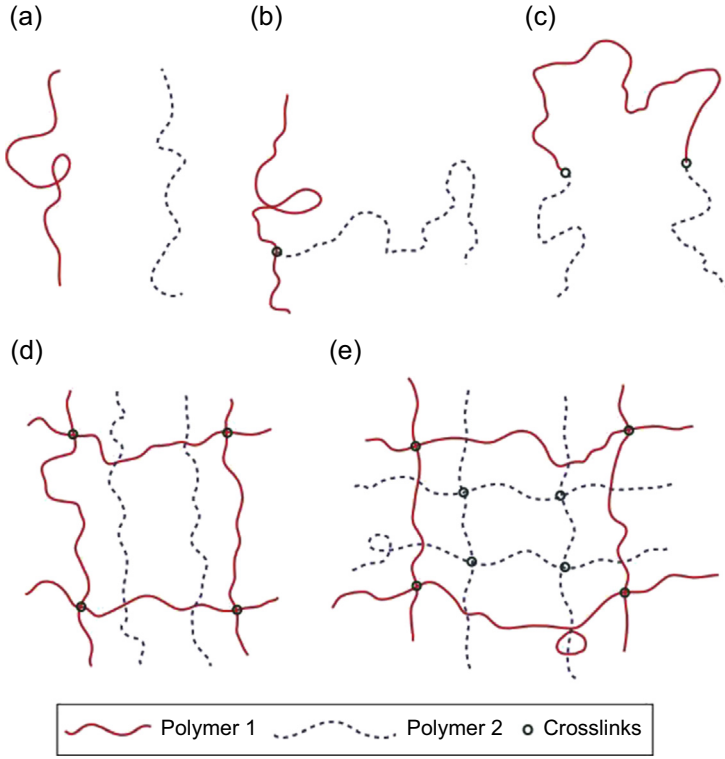

Figure 4.1 Schematic representation of mechanical blends (a); graft copolymers (b); block copolymers (c); semi-IPN (d); and full-IPN (e).
For details, see James, J., Thomas, G.V., Akhina, H., Thomas, S., 2016. Micro- and nano-structured interpenetrating polymer networks: state of the art, new challenges and opportunities, In: Thomas, S., Grande, D., Cvelbar, U., Raju, K.V.S.N., Narayan, R., Thomas, S.P. (Eds.), Micro- and Nano-structured Interpenetrating Polymer Networks: From Design to Applications. Wiley, 1–27. Figure reproduced with permission.

4.1.2 Historical overview

The development of the first synthetic IPN was generally attributed to Aylsworth, which in his patent described the preparation of an "improved rubber" material, composed of a mixture of natural rubber and phenol-formaldehyde resins cross-linked with sulfur (Aylsworth, 1914). However, the idea of IPN systems was rediscovered several times over the years (Sperling, 1977, 1994). In fact, some years later Staudinger and Hutchinson (1951) reported the preparation of thick sheets or blocks of a thermoplastic material suitable for optical use and composed of cross-linked polystyrene or polymethyl methacrylate that were swelled in a solution of the same monomer and then further polymerized (Staudinger and Hutchinson, 1946). Independently, in 1955, Solt polymerized one network containing anionic groups with a second network composed of cationic groups. Thus, obtaining a new ion exchange

resin. Only in 1960, the term "interpenetrating polymer networks" was coined by Millar, who prepared an IPN composed of two identical networks with the same chemical composition (Klempner, 1980). Interpenetrating polymer networks composed of two different polymers were developed later independently by the work of Frisch and Sperling. Thanks to the nature of the starting components, the network formed by Frisch resulting material was defined as interpenetrating elastomer network (Banerjee et al., 2010b; Frisch et al., 1969). Sperling and Friedman (1969) synthesized an IPN based on poly(ethyl acrylate) and polystyrene by UV polymerization.

4.1.3 Classification

There are mainly two different types of classification for IPNs systems. The first type of classification is based on the chemical bonds existing between the polymeric components of the resulting IPN network (James et al., 2016; Lohani et al., 2014). Therefore, based on the chemical bonding it is possible to distinguish:

- **Covalent semi-IPN**: a single polymeric network is formed by the two separate polymer systems that are cross-linked.
- **Noncovalent semi-IPN**: only one of the polymer systems is cross-linked.
- **Noncovalent full-IPN**: the two separate polymers are cross-linked independently.

A second classification is based on the synthetic procedure. In this context, it is possible to find

- **Sequential IPN**: In this type of networks, the term "sequential" suggests the time order of polymerization (Sperling, 1977). In sequential IPN, the polymer (I) is cross-linked and the resulting network is then swollen by the monomer of the polymer (II). Then, the polymer (II) is polymerized and/or cross-linked in situ in the presence of the cross-linker. These syntheses are simple: they only required that both the monomer (II) and coreactants swell properly in the polymer network I. Usually elastomers are used for network I because they swell easily (Lohani et al., 2014).
- **Simultaneous IPN**: A simultaneous IPN is formed in a one-step procedure by mixing and cross-linking together the monomers I and II with their respective activators and cross-linkers. Even this synthetic process is simple and requires only that the two polymerization routes do not interfering. Compared with sequential IPNs, simultaneous IPNs are better because the starting monomeric mixture is highly compatible and thus a higher degree of intermixing is obtained in the resulting network.
- **Latex IPN**: The first latex IPN was synthetize by Frisch et al. in 1969. These IPNs systems are also called interpenetrating elastomeric networks because both the latex components are normally elastomeric. In latex type IPN, both networks are included in a single latex particle, usually by polymerization of the second monomer together with the cross-linking agent and activator in the original seed latex of the first cross-linked monomer (Lohani et al., 2014). They often show a "core" and "shell" structure.
- **Thermoplastic IPN**: In the thermoplastic IPNs the cross-linking among the polymers is physical. There are three types of physical cross-link that can occur in a thermoplastic IPN. Particularly, in *ionomer formation*, cross-links are due to ionic groups present along the polymer chain, in *partially crystalline polymers* physical cross-links arise from crystalline regions and finally in *block copolymers* with ABA structure, the end blocks form a discrete phase and thus the cross-links are due to the glassy domains (Sperling, 1977).

Thanks to their nature, these materials flow at elevated temperatures but exhibit a typical IPN behavior at the use temperature because of the presence of cross-links (James et al., 2016).

- **Gradient IPN**: Gradient IPNs have compositions, which vary from location to location on the macroscopic level (Sperling and Mirshra, 1996). They can form by swelling the first polymer network in the network of the second monomer and before that the equilibrium is established, a rapid polymerization is carried out. In this way, the second monomer exhibits a gradient of concentration over the first polymer network in the resulting IPN.

Also a third classification of IPN is possible and it is based on the topological arrangements of the polymer chains (Sperling, 1977; Klempner et al., 1973).

4.1.4 Properties

IPN and semi-IPN networks are distinguishable from blends, block copolymers, and graft copolymers. The most important feature of IPN and semi-IPN is phase separation. Phase separation leads to the formation of a heterogeneous network. It is mainly due to the chemically different structure of the components forming the IPNs networks. However, the process of separation proceeds very slowly due to the high viscosity of the system and to entanglements between chains. Two are the mechanisms of phase separation that may occur during the IPN formation:

- *Nucleation and growth*: in this case, spheres of the second phase are formed within the matrix of the first phase. These spheres grow leading to an increase in their diameter.
- *Spinodal decomposition*: it is the most common mechanism of phase. Here, interconnected cylinders of the second phase are formed within the matrix of the first phase. These cylinders grow by increasing their wave amplitude. Later, coarsening and coalescence may cause important changes. However, these changes may be impeded by cross-links, which keep the domains small.

Phase separation affected the morphology of IPN networks. Particularly, when gelation occurs before phase separation, the resulting network exhibits smaller phase domain, which is the case of sequential IPN. While, if phase separation occurs before gelation, then the phase domain sizes will tend to be large. In addition, phase separation affected also the glass transition temperature of IPN (Lipatov and Alekseeva, 2007). In fact, IPN systems may exhibit two glass transition temperatures corresponding to the Tg of the single polymeric components, with or without inward shift. Alternatively, IPNs networks may present one broad or sharp Tg, intermediate to the glass temperatures of the components. In many cases, an inward shift or a merging of the Tg was observed and it is generally interpreted as an increase in the miscibility of the two polymers caused by the presence of cross-links, as Sperling and others have been reported. However, a single transition temperature cannot be considered, necessarily, as evidence of compatibility of two networks in IPNs. In fact, one glass transition point may be observed even in phase separated IPNs in the case of small domains of one of the phases (Lipatov and Alekseeva, 2007; Mathew, 2013). Semi-IPNs systems generally show a higher shift in transition temperature compared with the full IPNs because of a more complete phase separation in semi-IPNs as compared with full ones (Lipatov and Alekseeva, 2007).

Despite phase separation, IPN and semi-IPN exhibit several advantages. These include, improved mechanical properties compared with the mechanical properties of the single polymers forming the IPN or semi-IPN network. Therefore, a synergic effect of the all the components is observed in IPN or semi-IPN networks. On this base, by choosing appropriately the starting IPN or semi-IPN materials is possible to obtain networks with the desired mechanical properties (Matricardi et al., 2013; Banerjee et al., 2010b). Thermal stability and chemical resistance are other favorable properties presented by IPNs and semi-IPNs systems. In addition, compared with the other types of polymeric blends, IPNs swell without dissolving in solvents and can suppress creep and flow. Finally, the two polymeric networks forming an IPN scaffold cannot be separated unless chemical bonds are broken (Banerjee et al., 2010b; Mathew, 2013).

4.1.5 Characterization

IPNs are characterized mainly into physically (mechanical properties, morphological properties, and spectroscopic properties) and thermally.

4.1.5.1 Morphological characterization

The morphology of the IPNs systems is profoundly affected by the synthetic methodology, the compatibility of the single components forming the IPNs, the cross-link densities of the networks, the interfacial tension, and the rates of network formation. Some morphological aspects of IPNs can be observed directly by using transmission electron microscopy of stained and ultramicrotomed thin sections. A traditional staining method is based on the use of osmium tetroxide, which attacks reactive groups of polymers, such as diene type polymers containing double bonds in their networks. However, transmission electron microscopy is not useful with saturated or nonreactive polymers. With these techniques, it is possible to determine the distribution of phase domains, the shape, the structure in terms of micro- and nanoscale, and the degree of mixing. In this way, a complete and clear elucidation of the IPN architecture may be obtained. Also other techniques can be used to identify the morphology of IPNs. These include atomic force microscopy analysis, confocal laser scanning microscopy, X-ray scattering experiments, energy-dispersive X-ray analysis, and laser scanning confocal microscopy.

4.1.5.2 Thermal characterization

Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and differential thermal analysis are the most common techniques used for the thermal characterizations of IPNs and semi-IPNs (James et al., 2016). As above reported, DSC analysis performed on IPN or semi-IPN networks can show one or two glass transition temperature (Tg) values. In the case of two Tg, they could be shifted or not in comparison to the Tg of the single polymeric components. As Zhang reported, the Tg means the beginning of the polymer chains to exhibit mobility (Zhang et al., 2004). If the

interactions among polymer chains were strong, a higher temperature would be necessary to promote this chain mobility because of this polymer−polymer delay chain mobility. The presence of 2 Tg is due to the formation of two different phases and it is common in IPN and semi-IPN networks. Therefore, these thermal analyses allow to understand the interpenetration degree of the components network and to identify if any macro- or microphase separation occurs in the IPN or semi-IPN networks (James et al., 2016). Information about the thermal stability of IPN networks is obtained through TGA analysis. It is well known that TGA analyses reported the variation in the mass of a substance as a function of temperature or time as the sample is subjected to a controlled temperature program in a controlled atmosphere. In most reports, the thermal stability of the IPN has been found to be higher than their homopolymers.

4.1.5.3 Mechanical characterization

Mechanical properties commonly used to characterize IPN are tensile strength, elongation at break, Young's modulus, and hardness (James et al., 2016). Rheological, tensile, extensiometry, tearing, and compression tests are the most common mechanical tests used for IPN or semi-IPN characterization. Through rheological data, it is possible to investigate the structural characteristics of the networks, to determine the conditions required for their formation, to examine the effects caused by changes in chemical parameters, and to evaluate their potential applications (Lapasin, 2015). In addition, rheological measurements are useful for the elucidation of the kinetic properties of IPNs and semi-IPNs, through the determination of their gel point. Kinetic results are useful to optimize the polymerization conditions. Moreover, the determination of the gel points at different temperatures can be used to calculate the activation energy of cross-linking (James et al., 2016). Tensile tests allow to determine several mechanical properties, including Young's modulus, yield strength, and elongation at rupture. Another important property of IPNs and semi-IPNs systems is the fracture toughness that plays an important role for the possibility of using these materials as engineered tissue or drug delivery systems. This property can be determined by tearing tests (Lapasin, 2015). IPNs usually show mechanical properties intermediate of the single polymeric components, so the interaction of a less-swellable, stiffer hydrogel with a more-swellable, softer hydrogel can be used to tune the IPN mechanical properties and swellability. In many cases, IPNs show a higher toughness compared with the single constituents. Additionally, the most powerful method in the investigation of viscoelastic properties of polymeric systems is dynamic mechanical spectroscopy.

4.1.5.4 Spectroscopic characterization

The spectroscopic characterization is obtained through several analyses, including nuclear magnetic resonance (NMR), infrared, and electron spin resonance (ESR). NMR spectroscopy is used to estimate the interpenetration of polymers in IPN at the nanoscale level. In addition, this technique could provide information about morphology, miscibility, and microstructure of IPNs (James et al., 2016). IR and Fourier-transform infrared (FTIR) spectroscopic techniques are used when special

groups are presented in IPNs and they help to understand the completion of reaction during the IPN formation process. Finally, ESR analyses are useful for the study of the structure and the heterogeneity of IPN in a scale < of 5 nm.

4.2 Polysaccharide-based IPNs and semi-IPNs gels

4.2.1 Alginate

Alginate (Alg) is a linear polysaccharide derived from brown algae or bacteria and composed of 1 → 4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). Recent applications of alginate are linked to its gelling ability thanks to the interactions of G residues with divalent cations. These G/divalent cation interactions are responsible for the "egg-box" model formation. Alginate hydrogels are considered biocompatible materials with mucoadhesive properties. Due to these characteristics, Alg has been widely used in food industry and in biotechnological and biomedical fields.

4.2.1.1 Smart alginate IPNs and semi-IPNs

Stimuli-responsive hydrogels have attracted great interest recently due to their potential application in the field of drug delivery, tissue engineering, and biosensors. These polymers are able to be responsive to a number of stimuli, such as temperature, pH, and electrical or magnetic field. Among these stimuli, pH and temperature are the most extensively studied because they are the two important parameters for the human body functions. Several smart IPNs and semi-IPNs Alg-based systems are reported in literature in the form of hydrogels, beads, or microspheres. In many cases, these smart systems are responsive only to one stimulus. However, they can be also responsive to both temperature and pH and thus defined as dual-stimuli responsive gels. Pluronics are a class of polymers some of which exhibit a thermoreversible gelation below the body temperature and for this reason they are often applied in the biomedical and pharmaceutical fields. Examples of IPN and semi-IPN composed of Alg and pluronic are reported in literature (Lin et al., 2004). Also, natural polymers, such as gelatin, cellulose derivatives, and agarose, were widely used in combination with Alg to form thermosensitive IPN and semi-IPN (Liu et al., 2006; Nochos et al., 2008; Karewicz et al., 2010; Choudhary et al., 2011). Isiklan (2006) prepared thermoresponsive beads composed of sodium Alg blended with two other biodegradable natural polymers such as gelatin (Gel) and sodium carboxymethyl cellulose (NaCMC). Beads have been prepared by using glutaraldehyde (GA) as cross-linking agent. The prepared beads were characterized by FTIR spectroscopy and scanning electron microscopy (SEM). SEM confirmed the spherical nature and surface morphology of the particles. Bead characteristics, such as entrapment efficiency, particle size, equilibrium swelling degree, and release kinetics, were determined. It was observed that the carbaryl (an insecticide) release decreased with increase in cross-linking of network, whereas it increased with increase in Carb/NaAlg ratio and temperature. The release of carbaryl also increased with increasing in Gel or NaCMC content in the blend beads. From

these results, cross-linked alginate and blend beads appear to be very interesting as a system for agrochemical applications.

Poly(N-isopropylacrylamide), pNIPAAm, is one of the most widely studied temperature-sensitive polymers, exhibiting a temperature-dependent volume phase transition with the lower critical solution temperature (LCST) around 32°C (Shi et al., 2006). There are several examples of thermosensitive IPN and semi-IPN composed of alginate and pNIPAAm in literature. The use of this "smart" polymer in interpenetrating structures has been particularly investigated to improve the mechanical performances and the response to temperature to tailor the release of drugs. Table 4.1 summarizes some of these thermoresponsive networks.

In literature, there are also numerous examples of dual stimuli-responsive IPN and semi-IPN hydrogels composed by Alg and pNIPAAm. In this contest, Hernandéz developed a novel semi-interpenetrating polymer network of Alg and pNIPAAm responsive to both temperature and magnetic fields (Hernàndez and Mijangos, 2009). In their work, homogeneous porous hydrogels were obtained by copolymerizing *N*-isopropylacrylamide and bis-acrylamide in the presence of an aqueous alginate solution. Then Alg/pNIPAAm samples were immersed in aqueous solutions containing $FeCl_2$ and $FeCl_3$, and an in situ oxidation of the iron cations coordinated to the alginate network was induced. The advantage of synthetizing iron oxide nanoparticles in situ prevents their diffusion out of the Alg−pNIPAAm semi-IPNs. The porosity of the resulting samples was investigated and it was found that this porosity greatly increases with the introduction of iron oxide nanoparticles because of the partial hydrolysis of the alginate chains. Because of the alginate hydrolysis, the LCST of the semi-IPN shifts to lower temperatures with respect to pure pNIPAAm indicating that the hydrophobic interactions of the isopropyl groups are facilitated. Most studied

Table 4.1 Thermoresponsive interpenetrating polymer network (IPN) and semi-IPN Alg-based networks

Polymers	Type of network	Cross-linker	References
Alg + pNIPAAm	IPN hydrogel	*N*,*N*-methylenebisacrylamide (BIS)	Dumitriu et al. (2009)
Alg + pNIPAAm	IPN/semi-IPN membrane	*N*,*N*-methylenebisacrylamide (BIS)	Guilhermea et al. (2005)
Alg + NIPAAm	IPN/semi-IPN hydrogel	γ-ray irradiation	Lee et al. (2006)
Alg + pNIPAAm	Semi-IPN beads	−	Matricardi et al. (2008a)
Alg + pNIPAAm	Semi-IPN microspheres	Glutaraldehyde	Mallikarjuna Reddy et al. (2008)

are dual stimuli-responsive IPN and semi-IPN, which are responsive to temperature and pH because of the presence of carboxylic groups on Alg chains, which charge changes according to the pH of the medium (Ju et al., 2001; Shi et al., 2006). Shi et al. (2006) studied the effect of pH and temperature on the release of indomethacin, a nonsteroidal antiinflammatory drug, from semi-IPN beads based on CaAlg and pNIPAAm. They found that both the pH and the temperature affected the release behavior of the drug from the beads. Particularly, at 37°C the release was faster than at 25°C. At pH 7.4 and 37°C the drug was completely released within 3 h, whereas a very small amount of the drug was released from the beads at pH 2.1. The authors attributed this behavior to a leakage of the drug from the pNIPAAm hydrogel, due to the demixing of the semi-IPN when the temperature of the system was raised above the LCST: pNIPAAm chains were not cross-linked and consequently demixing was possible, depending on the chain length and the environmental conditions.

Also, IPN and semi-IPN networks composed of Alg in association with other polymers, containing carboxylic or sulfate groups that exhibit a sol−gel transition as a result of changing the external pH, were investigated for the development of pH-responsive systems. Ramesh Babu et al. (2006) prepared novel IPN microgels of sodium alginate and acrylic acid for the controlled release of ibuprofen. The IPN exhibited better encapsulation efficiencies than the plain sodium alginate microspheres. The release of ibuprofen from these IPN was investigated at pH 1.2 and 7.4 media. Experimentally, microgel network consisting of pure sodium Alg disintegrates in the intestinal fluid, whereas poly(acrylic acid) provides pH-sensitivity to the microgel network. The system developed in their study showed a pH sensitivity for the release of ibuprofen, which was attributed to the diffusion controlled release of the drug through the surfaces of IPN that undergo disintegration after swelling, depending on the chemical composition of microgels and pH of the medium.

Recently, Eswaramma et al. prepared dual responsive IPN microbeads composed of sodium Alg and modified guar gum (Eswaramma and Krishna Rao, 2017). First, guar gum was modified by graft copolymerization using N-vinylcaprolactam (GG-g-PNVCL) and then IPN hydrogel microbeads of sodium Alg and GG-g-PNVCL were prepared by water-in-oil emulsion cross-linking method using GA as cross-linker. Fig. 4.2 reported the schematic method formation of the microbeads. The resulting systems were characterized by FTIR spectroscopy, 1H NMR, SEM, DSC, and X-ray diffraction. The microbeads have a size of 100 ± 10 nm and present a semispherical nature having a rough surface. The surface also exhibits porous structure and few depressions, which might have been produced due to the contraction of the more hydrophilic part of the network during the particle hardening process.

An anti-HIV drug, Zidovudine, was encapsulated within the microbeads with an encapsulation efficiency of 68%. Swelling studies ascertained that microbeads were potentially sensitive to both pH and temperature. In vitro release studies were investigated in pH 1.2 and 7.4, the release time enhanced up to 34 h in pH 7.4 at 37°C.

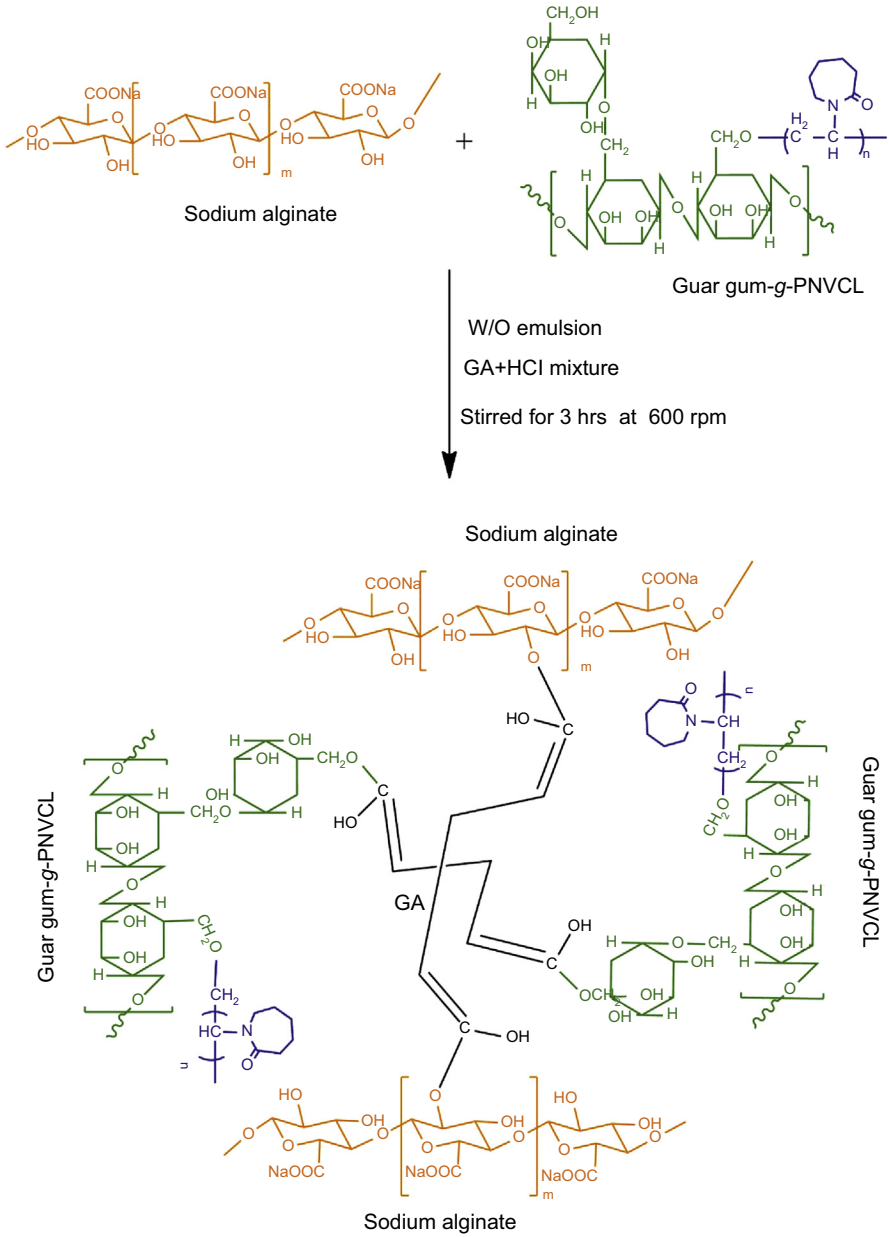

Figure 4.2 Schematic representation of sodium Alg and GG-g-PNVCL blend microbeads. *W/O emulsion*, water-in-oil emulsion.
For details, see Eswaramma, S., Krishna Rao, K.S.V., 2017. Synthesis of dual responsive carbohydrate polymer based IPN microbeads for controlled release of anti-HIV drug. Carbohydrate Polymers, 156, 125−134. Figure reproduced with permission.

4.2.1.2 Other alginate-based IPNs and semi-IPNs

This section deals with three different kinds of Alg-based IPN and semi-IPN networks. These include the following:

- *Physically cross-linked alginate IPNs and semi-IPNs:* physically Alg networks are often prepared by dispersion of the polymeric systems into a gelation medium in the presence of divalent cations such as Ca^{2+}. In fact, divalent cations can bind solely to guluronate blocks of the alginate chains. The guluronate blocks of one polymer then form junctions with the guluronate blocks of adjacent polymer chains in what is termed the "egg-box" model of cross-linking, resulting in a gel structure (Lee and Mooney, 2012). Several examples of physically cross-linked alginate IPNs and semi-IPNs were reported in literature (Lin et al., 2005; Jana et al., 2015). Lin et al. (2005) prepared IPN beads composed of a water-soluble derivative of chitosan (CH) (N,O-carboxymethyl CH, NOOC) and alginate. Experimentally, aqueous solutions of alginate−NOCC mixtures were dropped into a gently stirred calcium chloride solution thus immediately forming gel beads. These beads were evaluated as a pH-sensitive system for delivery of a model protein drug (bovine serum albumin). The swelling characteristics of these hydrogel beads were investigated showing that beads with alginate−NOCC weight ratio of 1:1 had better swelling properties. At pH 7.4, with increasing the total concentration of alginate−NOCC, the swelling ratios of test beads increased significantly due to a larger swelling force created by the electrostatic repulsion between the ionized acid groups ($-COO^-$). Therefore, these IPN carriers showed excellent pH sensitivity and could be a suitable polymeric carrier for site-specific bioactive protein drug delivery in the intestine.
- *Chemically cross-linked alginate IPNs:* Chemically cross-linked alginate IPNs show an increased resistance to failure and high mechanical strength compared with physically cross-linked alginate gels. However, a disadvantage of chemically cross-linked Alg network is that covalent cross-linking reagents may be toxic, and the unreacted chemicals may need to be removed thoroughly from gels. La Gatta et al. developed a semi-IPN network composed of Alg and a pHEMA through copolymerization of the cationic monomer 2-methacryloxy ethyltrimethyl ammonium chloride (La Gatta et al., 2009). Recently Hina et al. formulated a novel Na-alginate/PVA hydrogels employing 2-acylamido-2-methylpropane-sulfonic acid as monomer (Anwar et al., 2017). Zhang et al. (2015) prepared an IPN hydrogel composed of alginate and sericin by using calcium ion and GA as the cross-linking agents for alginate and sericin, respectively, as showed in Fig. 4.3. This hydrogel exhibits improved mechanical strength and has more stable degradation kinetics when compared with alginate hydrogels. The IPN hydrogel shows the excellent cell-adhesive property and effectively supports the proliferation and migration of mouse myoblasts.
- *Photopolymerized alginate IPNs:* Photopolymerization is an alternative approach for the formation of chemically cross-linked hydrogels and offers the possibility to obtain in situ formed systems by means of UV or visible light irradiation (Matricardi et al., 2013). In general, hydrophilic/water-soluble polymers with polymerizable groups, such as acrylate and methacrylate moieties, form a hydrogel when exposed to UV or visible light. Radicals that initiate the polymerization are generated when a photoinitiator undergoes homolytic bond cleavage on exposure to UV/visible light. At present, several photoinitiators are available that have a good cytocompatibility allowing their in vivo use. Wang et al. (2005) used photopolymerization to improve the stability of calcium−Alg microcapsules by introducing additional polymers to provide covalent linkages via photopolymerization. Vinyl monomers and a photoinitiator were allowed to diffuse into the initially formed calcium−alginate microcapsules, and an in situ photopolymerization in the presence of sodium acrylate and

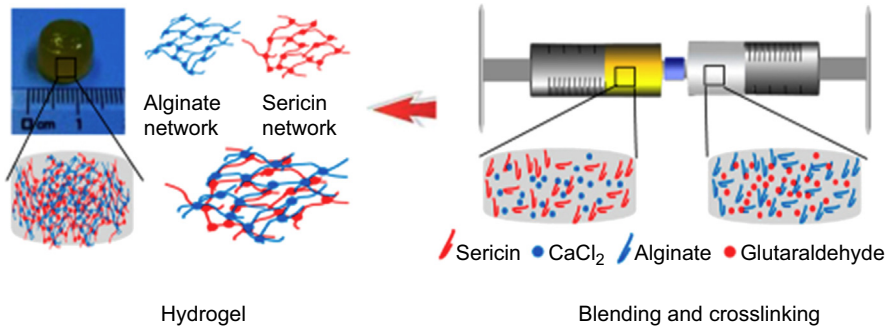

Figure 4.3 Scheme for the formation of sericin−alginate hydrogels.
For details, see Zhang, Y., Liu, J., Huang, Z., Wang L., 2015. Design and performance of a sericin-alginate interpenetrating network hydrogel for cell and drug delivery. Scientific Reports 5, 12374. Figure reproduced with permission.

N-vinylpyrrolidone was performed leading to an enhancement of the mechanical strength of the system. After 4 months of storage in saline, >70% of these capsules remained intact in the osmotic pressure test, whereas the unmodified alginate microcapsules totally disintegrated. Tests of their permeability to polyethylene glycol of different molecular weight and their ability to support cell survival showed that these properties remained unaffected by the photopolymerization. Hence, these microcapsules modified by adding a network of vinyl polymers are promising candidates to use for long-term delivery of recombinant gene products in this cell-based method of gene therapy. Photocrosslinkable IPN beads based on Alg and polyethylene glycol were reported for the encapsulation of Langerhans islets (Desai et al., 2000). Also in situ cross-linkable IPN hydrogel composed of CaAlg and dextran methacrylate was widely investigated (Pescosolido et al., 2010, 2011a; Matricardi et al., 2008b; D'Arrigo et al., 2012). Recently, Sun et al. (2012) prepared a series of hydrogels based on an IPN of methacrylate alginate and collagen to support preosteoblast spreading and proliferation as well as osteogenic differentiation.

4.2.2 Hyaluronic acid

Hyaluronic acid (or hyaluronan, HA) is a linear glycosaminoglycan composed of repeating disaccharide unit of D-glucuronic acid and *N*-acetyl-D-glucosamine, linked through alternating β-1 → 4 and β-1 → 3 glycosidic bonds. HA is one of the major components of the extracellular matrix and its biological functions have been widely investigated showing that HA is able to interact with specific cell receptors that can recognize and bind it selectively. Despite its advantages, there are some problems related to the use of this polysaccharide such as its degradation catalyzed by human hyaluronidases and its poor mechanical properties. To overcome these drawbacks and to tune the mechanical properties of HA, chemical derivatives of HA were obtained. Three are the functional groups of HA that usually are chemically modified, which are the glucuronic acid group, the primary and secondary hydroxyl groups, and the amine group (after deacetylation of *N*-acetyl group).

HA has been widely used in association with pH- and temperature-responsive polymers to synthetize stimuli-responsive IPN and semi-IPN networks. Some of these works focused on the use of HA with thermosensitive polymers such as pNIPAAm (Santos et al., 2010; Pasale et al., 2014), hyperbranched polyethylene glycol (PEG)−based copolymer with multiacrylate functionality (Dong et al., 2002), and pluronic F127 (Jung et al., 2017). HA and pluronic F127 semi-IPN were synthetized and investigated by Jung et al. (2017) as a new intraarticularly injectable hydrogel for the controlled release of piroxicam, a nonsteroidal antiinflammatory drug. The use of the thermoresponsive surfactant properties of pluronic F127 polymer allowed to disperse the drug within the hydrogel, but at the same time could erode the hydrogel in a very short time at physiological conditions. For this reason, HA was used and the authors found that HA reduced the amount of pluronic required for the gelation, enhanced the mechanical strength of the semi-IPNs, and reduced critical gelation temperature value of the hydrogel compared with pure pluronic F127 gels. The hydrogel exhibits both sustained drug release behavior and better bioavailability in physiological conditions, thus these IPN systems could be a promising hydrogel-based drug delivery platform for the treatment of arthritis.

HA has also been tested not only as material for drug delivery but also for tissue engineering applications. An example of this is the work of Tsaryk et al. (2015). They prepared a semi-IPN material composed of type I collagen and low-molecular weight HA, loaded with microspheres of gelatin. The material displayed a gellike behavior, it was easily injectable because of the presence of gelatin microspheres that in an appropriate amount effectively acted as a "reinforcement" thus improving the viscoelastic properties of the neat semi-IPN network. The composite hydrogel-based system supports the growth and chondrogenic differentiation of mesenchymal stem cells and nasal chondrocytes, whereas gelatin microspheres provide a suitable vehicle for growth factor delivery. Thanks to these properties, this semi-IPN was proposed as a candidate for nucleus pulposus regeneration. Also, Pescosolido et al. (2011b) developed a semi-IPN network as potential bioprintable material for tissue engineering applications. In their paper, the authors described the preparation of a chemically cross-linked semi-IPN based on high-molecular weight of HA and a dextran derivative, dex-HEMA (hydroxyethyl methacrylate−derivatized dextran), by UV-cross-linking of the methacrylate groups of Dex-HEMA in the presence of the photoinitiator Irgacure 2959 (Fig. 4.4). Kinetic studies of these semi-IPN hydrogels with different HA contents were performed evidencing that the cross-linking kinetics were almost instantaneous, as shown by the rapid increase of the storage modulus G' after 10 s of UV exposition. Also, the printability of these systems was studied confirming that the scaffold possessed high porosity. In addition, the architecture of the scaffold can be easily tuned by controlling the process parameters, such as fiber spacing and orientation.

Networks of fibrin and HA were synthetized as tissue engineering scaffold (Zhang et al., 2016; Lee and Kurisawa, 2013). Fibrin is a fibrous, nonglobular protein formed by the action of the protease thrombin on fibrinogen, which allowed it to polymerize during the clotting process. Fibrin gels are widely studied in biomedical applications because they support cell adhesion, proliferation, stem cell differentiation,

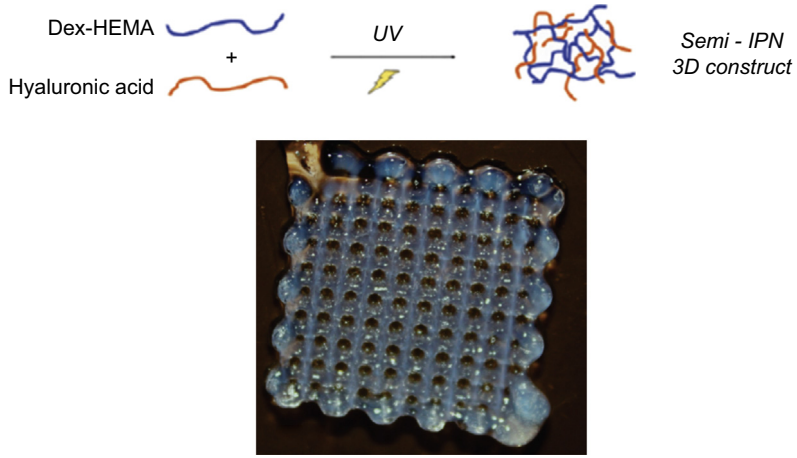

Figure 4.4 Schematic representation of the three-dimensional (3-D) hydrogel formation with the photograph of 3-D printed hydrogel.
For details, see Pescosolido, L., Schuurman, W., Malda, J., Matricardi, P., Alhaique, F., Coviello, T., van Weeren, P.R., Dhert, W.J.A., Hennink, W.E., Vermonden, T., 2011b. Hyaluronic acid and dextran-based semi-ipn hydrogels as biomaterials for bioprinting. Biomacromolecules 12, 1831−1838. Figure reproduced with permission.

and angiogenesis. However, this protein has poor mechanical properties, which often result in rapid contraction and degradation of the scaffold. To improve the mechanical properties of fibrin hydrogels, Lee et al. prepared an IPN hydrogel composed of fibrin and HA−tyramine (HA−Tyr) (Lee and Kurisawa, 2013). Experimentally, the derivative HA−Tyr was mixed with fibrinogen and then horseradish peroxidase (HRP), hydrogen peroxide, and thrombin were added. In the presence of HRP and H_2O_2, HA−Tyr conjugates form chemically cross-linked hydrogels via C−C or C−O bonds between the tyramine moieties. The formation of IPN hydrogels was characterized by rheological measurement and the results showed that varying the amount of H_2O_2 added, which controlled the cross-linking density of the HA−Tyr network, IPN hydrogels with different G' values were obtained. However, it was observed that an increase in the G' value of the IPN hydrogels prevented the cells from spreading. The decrease in cell proliferation with increasing G' was attributed to the increase in cross-linking density of the HA−Tyr network. Finally, these IPN hydrogels demonstrated improved structural stability in the presence of plasmin. Recently, another interesting IPN system based on fibrin and disulphide cross-linked HA was described by Zhang et al. (2016). In their study, HA was dually functionalized with hydrazide (HA-hy-SSPy) and thiol groups (HA-SH). The IPN system was then obtained through the mixing of mixtures *A* containing fibrinogen and HA-hy-SSPy and *B* composed of thrombin and HA-SH. In this way, the polymerization of fibrin was obtained thanks to the presence of thrombin, whereas the cross-link of HA was obtained via thiol−disulfide exchange reaction. The mechanical characterization of this novel IPN showed an increased stiffness in comparison to that of the pure fibrin gel and this is probably due to the entanglements between fibrin and HA networks.

As previously reported, HA can be easily modified to obtain HA derivatives with different and improved properties. Actually, several HA derivatives are employed for wound healing, drug delivery, and as scaffolds for tissue engineering. The most common chemical modification on HA polymers for the IPN formation is the introduction of acrylate moieties on HA chains, in the form of methacrylate, acrylate, or glycidyl methacrylate groups because of these derivatives can be prepared using very mild conditions (Park et al., 2003; Suri and Schmidt, 2009; Khoshakhlagh and Moore, 2015).

4.2.3 Chitosan

Chitosan is a linear cationic polysaccharide composed of β-(1 → 4)-2-amino-2-deoxy-D-glucopyranose and β-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose units, randomly distributed along the polymer chain. It is obtained by partial deacetylation of the insoluble naturally available chitin with an easy and inexpensive process. The deacetylation of chitin leads to the formation of a more water-soluble polysaccharide. Chitosan could be modified to change the mechanical properties of the polymer and to impart important biological functions such as solubility and bioadhesity (Ahmadi et al., 2015). The interest in CH for drug delivery and biomedical applications is mainly due to its muchoadhesive properties.

4.2.3.1 Chitosan-based IPNs semi-IPNs hydrogels

Many papers describe the development of IPN and semi-IPN based on unmodified CH combined with different polymers (Al-Mubaddel et al., 2016; Cui et al., 2015; Shanmugasundaram et al., 2001). In this context, Cui et al. fabricated innovative CH/gelatin IPN networks cross-linked in the presence of genipin. These porous materials were prepared by freeze-drying and investigated as adsorbents for removal of anionic dye from aqueous solutions (Cui et al., 2015). Specifically, in the field of biomedical applications, Shanmugasundaram developed a range of IPN networks by using different ratio of collagen and CH, respectively 3:7, 4:6, 5:5, 6:4, and 7:3 (Shanmugasundaram et al., 2001). In their work, GA was used as cross-linker. The scaffolds were characterized through FTIR, DSC, and TGA analyses and tested for their swelling behavior. In this way, a network with the best swelling properties was selected and tested for in vitro culture studies by using human epidermoid carcinoma cells. Pulat et al. prepared a semi-IPN material for the immobilization of lipase. Chitosan, acrylamide, and citraconic acid were used for the network preparation by using free radical polymerization (Pulat et al., 2014). Very recently, Sampath et al. (2017) used nanocellulose, CH, and GA for the formation of a semi-IPN network. First, cellulose nanocrystals (CNCs) were extracted from microcrystalline cellulose via sulfuric acid hydrolysis. Then these nanocrystals were homogenized through ultrasonication and CH was added and cross-linked with GA to form Schiff base linkages. The reaction scheme for the semi-IPN formation is showed in Fig. 4.5. SEM analyses revealed that CNCs were uniformly distributed within the CH matrix. The addition of CNCs (up to 2.5%) to CH hydrogels improved the maximum compression of the

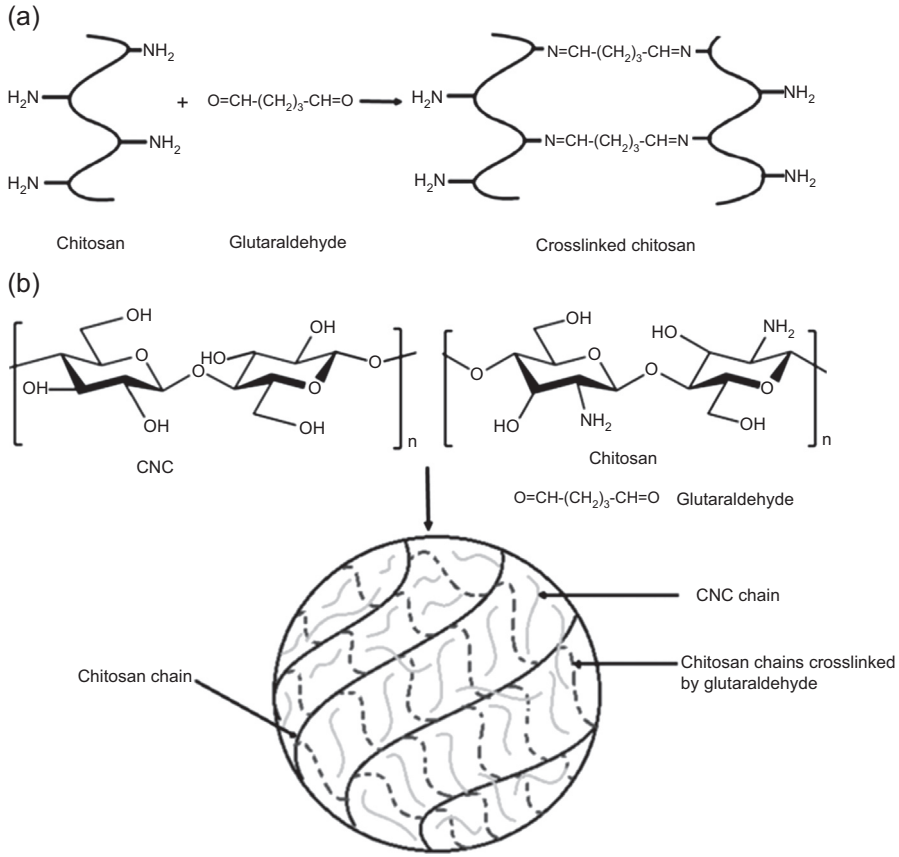

Figure 4.5 The schematic representation of the steps for the formation of CNC-chitosan hydrogel: crosslinking process of chitosan with glutaraldehyde **(a)**; proposed mechanism for the formation of semi-interpenetrating polymer network hydrogel **(b)**.
For details, see Sampath, U.G.T.M., Ching, Y.C., Chuah, C.H., Singh, R., Lin P.C., 2017. Preparation and characterization of nanocellulose reinforced semi-interpenetrating polymer network of chitosan hydrogel. Cellulose 24, 2215−2228. Figure reproduced with permission.

networks and led to the formation of a semi-IPN with several potential applications in the field of tissue engineering, pharmaceuticals, and drug delivery.

Chitosan can be also combined with specific responsive polymers with the aim to form systems responsive to temperature, pH, and enzymatic activity. In this contest, several studies focused on the formation of thermoresponsive IPNs based on CH and *N*-isopropylacrylamide or poloxamer (Fernández-Gutiérrez et al., 2016; Chung et al., 2009; Kim et al., 2000).

However, most of the work reported in literature reported the use of chemically modified CH for IPN and semi-IPN formation (Zhou et al., 2008; Yin et al., 2007; Chen et al., 2004; Guo et al., 2007). Chemically modification on CH has the advantage of production CH derivatives that do not require an acidic environment for their

solubilization, with benefits for their potential application in biomedical applications. Huang et al. (2016) prepared a novel semi-IPN hydrogel through the interpenetration of carboxymethyl CH with maleic acid and acrylamide by covalent bond cross-linking enhanced by ionic (carboxylic−Fe^{3+}) secondary cross-links. The second cross-linking had the advantage of enhancing the mechanical strength of the hydrogels. These hydrogels showed a good tensile stress of about 1.44 MPa and outstanding antibacterial activity because carboxymethyl CH had an inhibitory effect on *Staphylococcus aureus* and a general inhibitory effect against *Escherichia coli*.

4.2.3.2 Chitosan-based semi- and full-IPNs microspheres

Chitosan-based IPN systems have gained considerable attention as a vehicle for drug delivery systems. These drug delivery systems can be prepared in the form of beads, microspheres, discs, or tabs. Angadi et al. (2010) developed IPN microspheres of CH and hydroxyethyl cellulose using GA as cross-linker agent and loaded with isoniazid, an antitubercular drug. Morphological analyses performed on these systems confirmed the formation of microspheres, whereas TGA and DSC analyses confirmed the thermal stability of these carriers. Finally, release studies of isoniazid from the matrix were performed, showing a sustained release of the drug over 16 h depending on the extent of GA cross-linking and IPN blend composition. Recently, Ozerkan et al. (2013) reported the preparation of a set of CH/polyvinylpyrrolidone (PVP) semi-IPNs microspheres in different composition by water/oil emulsification method. In the formation of semi-IPN structures, CH was cross-linked with GA and PVP molecules were entrapped in the matrix thus forming a semi-IPN construct. As a model drug, 5-fluorouracil (5-FU) was loaded into the microspheres during the preparation process. Increasing the concentration of GA led to the formation of spherical and homogeneously sized microspheres, with diameter of 153 and 170 μm as the amount of PVP in the matrix raised. The release rate of 5-FU from microspheres increased as the amount of PVP increased in microspheres. In addition, mechanical test results showed that cross-linked CH−PVP semi-IPN films had enough strength to handle them. It was found that CH and CH−PVP semi-IPN microspheres are promising devices for the controlled release of 5-FU. By changing the ratio of CH/PVP, it becomes possible to adjust the release rates of drugs. A similar protocol was used also by Bulut for the preparation of semi-IPNs microspheres composed of CH and polyvinyl alcohol (Bulut, 2014). Even in this case, the microspheres were cross-linked with GA and ibuprofen was loaded as a drug within the matrix. Several other studies reported in literature were based on semi-IPNs microspheres of CH with other polymers such as polyethylene oxide-g-acrylamide (Agnihotri and Aminabhavi, 2006), acrylamide-g-dextran (Rokhade et al., 2007), and methylcellulose (Rokhade et al., 2009).

4.2.4 Dextran

Dextran is a bacterial hydrophilic polysaccharide consisting of consecutive α-1 → 6 linked D-glucopyranose units with a low percentage of α-1 → 2, α-1 → 3, and α-1 → 4 linked side chains. Dextran has been extensively explored in tissue

engineering and biomedical applications thanks to its biocompatibility. It is used in medicine as an antithrombotic agent to reduce blood viscosity and as a plasma expander. In addition, dextran possesses several pendant hydroxyl functional groups useful for chemical modification, to develop dextran derivatives with specific and desirable characteristics.

Dextran-based IPN and semi-IPN networks have been investigated for biomedical applications, especially in association with gelatin (Liu and Chan-Park, 2009; Li et al., 2015). Kosmala et al. (2000) developed IPN scaffolds composed of dextran and gelatin by using three different type of cross-linking, which are treatment with glyceraldehyde, thermal hardening and chelation of dextran by divalent metal cations. Gels prepared by using 2% glyceraldehyde were stable at 25°C and did not show phase separation; however, these gels dissolved completely at 37°C. When Ca^{++} was used as cross-linker, weaker gels were obtained, whereas thermal hardening did not successfully let preparing interpenetrating networks. Liu et al. synthetized a methacrylate- and aldehyde-bifunctionalized dextran (Dex−MA−AD) and prepared an interpenetrating network of Dex−MA−AD and gelatin (Liu and Chan-Park, 2009). The IPN was formed by UV cross-linking between the methacrylate pendant groups of Dex−MA−AD and Schiff base reaction between Dex−MA−AD and gelatin. The mechanical properties of this new hydrogel coupled with 2-D and three-dimensional (3-D) biocompatibility with vascular cells make this a promising material for 3-D scaffolds for vascular tissue engineering. More recently, a novel conductive IPN hydrogel composed of polyaniline-grafted gelatin, carboxymethyl CH, and oxidized dextran was prepared by Li et al. (2015). Conductive hydrogels have gained considerable attention in the field of tissue engineering combining conductivity properties with the possibility to form a 3-D network. These hydrogels can promote cell adhesion, proliferation, and differentiation. In that work, oxidized dextran was used as cross-linker, able to connect the amino groups of modified gelatin with carboxymethyl CH. The reaction was performed at 37°C, thus it is possible to obtain the formation of the network at the body temperature.

Other in situ IPN and semi-IPN dextran-based systems were also prepared by Matricardi et al. (2008b). In that work, the in situ network was formed by methacrylate dextran (dexMA) and Ca/Alginate (Alg/Ca). In more detail, the semi-IPN was composed of dexMA chains interpenetrated with the Alg/Ca network and it could behave like a weak and easily injectable gel. After the injection within the body, the authors proposed the transformation of a semi-IPN in an IPN network through UV polymerization of DexMA (Fig. 4.6). After UV curing, mechanical properties of the IPN were deeply different from those of the semi-IPN and the release behavior of model molecules loaded within the network. The same authors also described another in situ IPN for biomedical applications composed by hydroxyethyl methacrylate dextran (dex-HEMA) derivative and Ca/Alginate (Pescosolido et al., 2011c). Also, semi-IPNs networks based on dextran and HA were developed by Pescosolido et al. (2011b) as reported above.

IPN and semi-IPN microspheres and beads composed of dextran intended for drug delivery are widely reported in literature (D'Arrigo et al., 2012; Rokhade et al., 2007; Sullad et al., 2011). Al Kahtani et al. (Ahmed et al., 2009) developed semi-IPNs

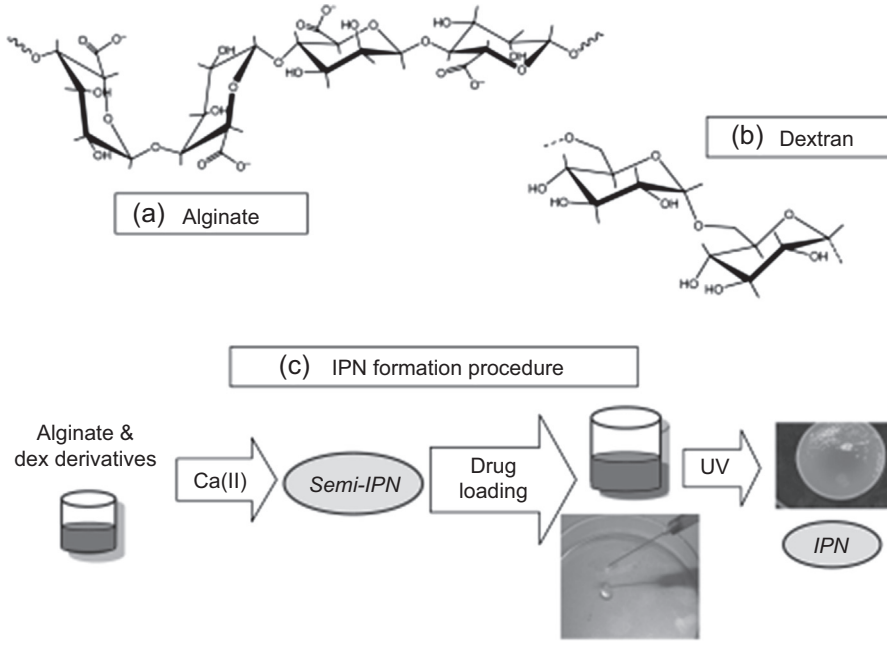

Figure 4.6 Repeating units of alginate (a) and dextron (b). Scheme of the procedure adopted for the semi-IPN and IPN formation (c).
For details, see Matricardi, P., Pontoriero, M., Coviello, T., Casadei, M.A., Alhaique F., 2008b. In situ cross-linkable novel alginate−dextran methacrylate IPN hydrogels for biomedical applications: mechanical characterization and drug delivery properties. Biomacromolecules 9, 2014−2020. Figure reproduced with permission.

microspheres of CH-(dextran-g-acrylamide). They prepared a dextran-grafted acrylamide (Dex-g-AAm) by aqueous free-radical polymerization using ceric ammonium nitrate as redox initiator. After the polymer derivatization, semi-IPN microspheres of CH and Dex-g-AAm were prepared by emulsion cross-linking method using GA as a cross-linking agent. SEM analyses showed that the microspheres are almost spherical in nature with smooth surfaces. The laser light scattering technique showed that the particles size was related to the % Dex-g-AAm content and the extent of GA used for cross-linking. In general, the size of particles ranged from 270 to 400 μm. The microspheres were then loaded with a water-soluble drug, theophylline, exhibiting an efficient encapsulation. The drug release from these carriers depends on the amount of GA and the % of Dex-g-AAm present into the microsphere and pH conditions.

4.2.5 Gellan gum

Gellan gum is a bacterial exopolysaccharide. Particularly, it has a tetrasaccharide repeating unit consisting of two molecules of D-glucose, one of L-rhamnose and one of D-glucuronic acid. Gellan is structurally a double helix, formed by two intertwined

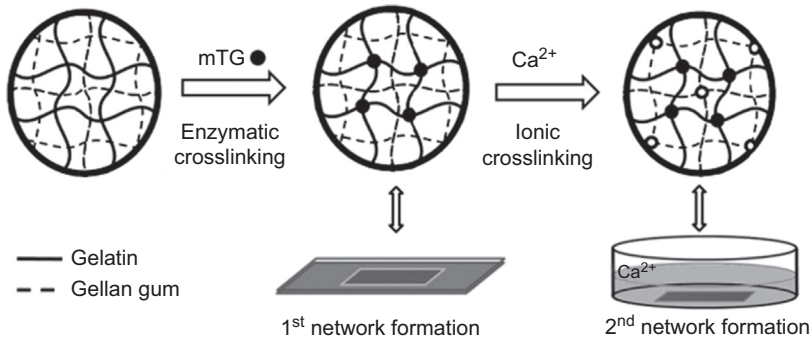

Figure 4.7 Fabrication scheme of IPN biohydrogels through a two-step process of enzymatic and ionic cross-linking approaches.
For details, see Wen, C., Lu, L., Li X., 2014a. An interpenetrating network biohydrogel of gelatin and gellun gum by using a combination of enzymatic and ionic crosslinking approaches. Polymer International 63, 1643−1649. Figure reproduced with permission.

left-handed, threefold helical chains. This helical geometry is promoted by the (1 → 3) linkage in the gellan repeating unit. It is well known that gellan gum can form gels in the presence of cations because of their electrostatic interactions with the carboxylate groups of the polymer chains. The gelation of gellan gum is harder with divalent cations compared with monovalent cations because they promote the aggregation by site binding between pairs of carboxylate groups on neighboring helixes. Thanks to its characteristics, gellan gum is used for a great variety of applications in the field of food, pharmaceutics, and biomedicine. Wen et al. (2014a) prepared an IPN scaffold based on gellan gum and gelatin, using a combination of enzymatic and ionic cross-linking approaches. In fact, gelatin was enzymatically cross-linked in the presence of microbial transglutaminase and ionically cross-linked gellan gum with Ca^{2+} ions (Fig. 4.7). The improved mechanical properties of the final network, the reduction in the degradation rates and the decrease in the swelling ratios are important advantages exhibited by the resulting IPN network. Another interesting feature of this IPN is its cytocompatibility suggesting a potential application as tissue engineering scaffold.

Also, the work of Bellini et al. (2015) falls within the field of tissue engineering. In this work, the authors investigated the disturbing effect of HA on the gellan gum-Ca (GG-Ca) hydrogels. The semi-IPN HA-Ca-GG hydrogel was characterized through rheological, dynamomechanical, and swelling-degradation analyses. High HA concentrations made the hydrogels very weak with a more rapid degradation, whereas high amount of calcium led the hydrogels stronger and more stable hydrogels. In vitro adhesion tests also revealed that this semi-IPN promoted cell survival and osteoblastic progression. All the analyses conducted on this network confirmed its potential use as scaffold for bone regeneration of osteochondral defects. Amici et al. (2000) developed a new type of IPN based on gellan gum and agarose by simple mixing. The two components appear to form their own individual ordered conformations at the appropriate temperatures; however, microscopy and turbidity data confirmed that the two networks interpenetrate on a molecular length scale.

Recently, a large number of IPN microspheres composed of gellan gum in the presence of other polymers for drug delivery purpose have been developed, as previously reported (Mundargi et al., 2010; Agnihotri et al., 2005; Kulkarni et al., 2011a). Agnohotri et al. (2005) developed gellan gum−polyvinyl alcohol (GG-PVA) interpenetrating microspheres through an emulsion cross-linking process. Particularly, they prepared a series of microspheres by changing the ratio of GG:PVA and the extent of the cross-linking and loaded carvedilol, an antihypertensive drug, within these microspheres. The formation of INPs microspheres was confirmed by FTIR spectroscopy, whereas SEM analysis confirmed their spherical nature with smooth surfaces. Thanks to the formation of a huge number of connections among the polymer chains, the IPNs microspheres exhibited a tensile strength fivefold or sixfold higher compared with microspheres of pure GG. Also release studies were performed on these drug delivery systems, showing a release rate depending on the extent of cross-linking of the matrix and the amount of gellan gum present in the matrix. A higher release was observed for microspheres with lower cross-link density and lower amounts of gellan gum in the matrix.

4.2.6 Carrageenan

Carrageenan is a family of sulfated polysaccharides extracted from edible red seaweeds. All carrageenans are composed of repeating galactose units and 3,6 anhydrogalactose, both sulfated and nonsulfated. The units are joined by alternating α-1 → 3 and β-1 → 4 glycosidic linkages. There are three basic types of carrageenan: kappa (κ), iota (ι), and lambda (λ). K-carrageenan forms strong and brittle gels in the presence of potassium ions; ι-carrageenan forms soft and elastic gels in the presence of calcium ions; λ-carrageenan is not able to form gels. Lohani et al. (2016) prepared pH-sensitive IPN beads of κ-carrageenan and sodium carboxymethyl cellulose using $AlCl_3$ as a cross-linking agent by water-in-water emulsion gelation process and loaded with ibuprofen. The reaction scheme for the synthesis of IPN beads is showed in Fig. 4.8. SEM analyses revealed a spherical morphology of the beads, whereas DSC

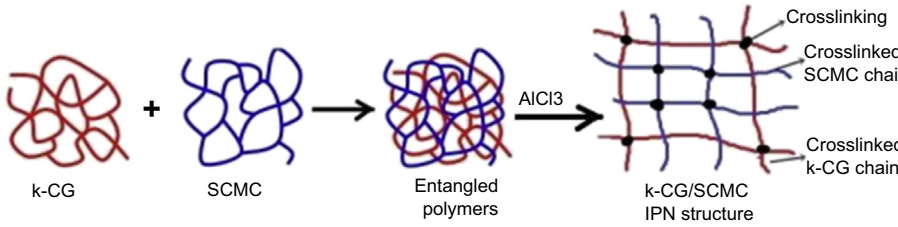

Figure 4.8 Reaction scheme for the synthesis of IPN composed of k-carrageenan (k-CG) and sodium carboxymethyl cellulose (SCMC).
For details, see Lohani, A., Singh, G., Bhattacharya, S., Hegde, R.R., Verma, A., 2016. Tailored-interpenetrating polymer network beads of κ-carrageenan and sodium carboxymethyl cellulose for controlled drug delivery. Journal of Drug Delivery Science and Technology 31, 53−64.
Figure reproduced with permission.

analyses confirmed the presence of molecular dispersion of the drug within the polymer matrix. Swelling and drug release studies revealed that ability of these IPN beads both to reduce the drug release in acidic medium and control the release in alkaline medium.

IPN beads of κ-carrageenan were also prepared by Mohamadnia et al. (2007), using sodium alginate as second polymer network. The formation of IPN beads was due to the presence of Ca^{2+} and K^{+} ions as cross-linkers. In their study, betamethasone acetate was used as model drug. Kulkarni et al. (2011b) prepared an ion exchange resin based on compressed IPN matrix tablets of SA and CG for controlled release propranolol hydrochloride. Several IPN and semi-IPN hydrogels reported in literature are composed of carrageenan (Gizem Makas et al., 2010; Chen et al., 2008; Demirel et al., 2006). Wen et al. (2014b) fabricated semi-IPN and IPN networks as potential scaffold for tissue engineering. The IPN bioscaffold was prepared by enzymatically cross-linking gelatin with microbial transglutaminase followed by physical cross-linked κ-carrageenan in the presence of potassium ions. Indeed, for the preparation of the semi-IPN, gelatin was crosslinked with microbial transglutaminase, avoiding the physical crosslinking of the second polymer network with potassium ions. The combination of physical and chemical cross-linking for these biomaterials led to the formation of thermostable hydrogels, whose mechanical and swelling properties could be easily modified by varying the amount of carrageenan in the network. Moreover, these biohydrogels demonstrated a biodegradable nature and excellent cytocompatibility.

4.2.7 Scleroglucan

Scleroglucan (Sclg) is a natural nonionic polysaccharide produced by microorganisms. It consists of a main chain of (1 → 3)-linked b-D-glucopyranosyl units where every third unit bears a (1 → 6)-linked b-D-glucopyranosyl monomer. In an aqueous solution, this polysaccharide adopts a stable triple-stranded helical conformation held together by hydrogen bonds. Thanks to its resistance to hydrolysis and its rheological behavior, scleroglucan is widely investigated in agriculture, in cosmetics, in the field of food additives, and in pharmaceutics. Matricardi et al. (2006) prepared semi-IPN tablets composed of Sclg/borax and Alg as a carrier able to deliver myoglobin, a protein, in the intestine and to protect the same from the degradation in the gastric environment. Experimentally, a solution of Alg and Sclg with myoglobin was prepared and borax was used as the cross-linker. The resulting hydrogel was then freeze-dried and used for the preparation of tablets. Alg modified the physicochemical properties of the Sclg/borax hydrogel and was able to prevent the release of a protein in simulated gastric conditions, preserving, at the same time, its activity. However, at the same time, a faster release of the protein at neutral pH was obtained by increasing the amount of Alg in the semi-IPN network. Aalaje et al. developed two semi-IPN hydrogels based on Sclg as entangled polymer (Aalaiea et al., 2009; Alvand et al., 2018). In both studies, chromium triacetate was used as cross-linker, while the cross-linking polymer was, respectively, partially hydrolyzed polyacrylamide and sulfonated

polyacrylamide. In both studies, rheological and swelling properties were investigated. Finally, Corrente et al. (2013) synthetized an injectable and in situ cross-linkable hydrogels composed of methacrylate dextran (DEX-MA) and scleroglucan (Scl), both in its native form or carboxymethylated. Both unmodified and modified Scl tuned the mechanical properties of DEX-MA hydrogels that became harder and more elastic. All the hydrogels investigated were able to swell in contact with biological fluids showing a consistence similar to natural tissues. The systems released very fast small molecules but were able to modulate the release of $VitB_{12}$ and to behave as depot delivery systems.

4.3 Applications of IPNs and semi-IPNs gels

The patent literature reveals that there are many products based on IPNs and semi-IPNs systems, including adhesive, optically smooth surfaces, damping materials, and ion exchange resins (Mathew, 2013). However, drug delivery and tissue engineering are two of the growing and interesting fields in which IPNs and semi-IPNs are widely used. The increasing interest in these materials is mainly due to the possibility of improving the mechanical properties of the single components by combining different kinds of natural and synthetic polymers, sterilization of the resulting networks because of the thermal stability of these materials compared with other types of hydrogels and a switch from laboratory to large-scale production (Lohani et al., 2014).

Drug delivery: IPN and semi-IPNs systems are mainly used for the controlled release of drugs (Raveendran and Sharma, 2016). The design of a controlled release system requires controlling the site where the drug has to be released and the kinetics of the release. In this scenario, IPNs and semi-IPNs exhibit the advantage of delivery drugs at predetermined rate for a specific time period and also show a low toxicity, thus they are good candidates as drug delivery systems. Several drug delivery systems were developed by using IPNs and semi-IPNs systems, these include

- *Microspheres*: these carriers were studied for the delivery of several drugs, such as antiinflammatory, antibiotics, hypoglycemic, antineoplastic, and vasodilator drugs (Quadri et al., 2015). Table 4.2 summarizes IPN microspheres composed of only natural polymers or a mixture of natural/synthetic polymers.
- *Membranes or patches*: Kulkarni et al. (2010) prepared an IPN membrane composed of sodium alginate and polyvinyl alcohol for the systemic delivery of prazosin hydrochloride through skin. More recently, Mallikarjuna et al. developed an IPN membrane composed of CH and gelatin by using GA as cross-linker. Thanks to its nature of bifunctional cross-linker, glutaraldehyde is able to bond the amine group of CH with the amine groups of gelatin. The membrane was tested for the controlled release of 5-FU (Mallikarjuna et al., 2015).
- *Films:* Polymeric films are also used as drug carriers especially when the mechanical properties of the carriers become important, such as the mechanical strength. Gunbas et al. (2012) prepared a semi-IPN film composed of CH and PEG by using GA as cross-linker. Mayet et al.

Table 4.2 List of the drugs delivered through IPN-based microspheres

Microspheres composition	Type of network	Cross-linker	Drug loaded	References
Sodium alginate + PVA	IPN	Glutaraldehyde	Diclofenac sodium Naproxen	Banerjee et al. (2010a) and Solak (2011)
Sodium alginate + *N*-isopropylacrylamide	Semi-IPN	Glutaraldehyde	5-fluorouracil	Mallikarjuna Reddy et al. (2008)
Chitosan + Hydroxy propyl cellulose	IPN	Glutaraldehyde	Chlorothiazide	Rokhade et al. (2009)
Chitosan + Gelatin	IPN	Glutaraldehyde	Isoniazide	Angadi et al. (2011)
Chitosan + Hydroxy ethyl cellulose	IPN	Glutaraldehyde	Isoniazide	Angadi et al. (2010)
Chitosan + Polyvinyl pyrrolidone	IPN	Glutaraldehyde	Amoxicillin	Risbud et al. (2000)
Chitosan + Acrylamide grafted dextran	Semi-IPN		Acyclovir	Rokhade et al. (2007)
Gellan gum + poly (N-isopropylacrylamide)	Semi-IPN		Atenolol	Mundargi et al. (2010)
Gellan gum + PVA	Semi-IPN	Glutaraldehyde	Carvedilol	Agnihotri et al. (2005)
Pullulan + PVA	IPN	Glutaraldehyde	Pirfenidone	Soni and Ghosh (2018)

developed a semi-IPN film based on CH, citric acid, hypromellose-hydroxymethylcellulose for the delivery of curcumin. Experimentally, CH was dissolved in a solution of citric acid and cross-linked with genipin, whereas hypromellose-hydroxymethylcellulose solution containing curcumin was added to the blend previously formed (Mayet et al., 2014). Several other IPN and semi-IPN films are reported in literature (Mohanan and Vishalakhi, 2009; Karaaslan et al., 2012; Kim et al., 2005).

- *Hydrogels*: Hydrogels are 3-D, hydrophilic, polymeric networks capable to retain large amounts of water or biological fluids, with properties and consistency very similar with living tissue (Dragan, 2014). The advantage of IPN or semi-IPN hydrogel compared with another hydrogel is the enhancement of the mechanical properties of the single polymers. Among IPN and semi-IPN hydrogels, especially those that are responsive to

environmentally stimuli, such as temperature or pH, have gained considerable attention. The following section shows several examples of IPN and semi-IPN hydrogels useful for biomedical applications.

- *Nanoparticles*: nanoparticles raise a great interest among scientists because of the wide range of benefits they offer over conventional dosage forms (Somya et al., 2014). Xia et al. designed IPN nanoparticles composed of polyacrylic acid and poly(N-isopropylacrylamide) that form a gel at the body temperature. In this way, drugs could be mixed with the IPN nanoparticles and remained entrapped within the jellifying network of nanoparticles in the body (Xia et al., 2005).

Tissue Engineering: The term tissue engineering describes a "set of tools at the interface of the biomedical and engineering science that use living cells or attract endogenous cells to aid tissue formation or regeneration, and thereby produce therapeutic or diagnostic benefits" (Schoen, 2004). Hydrogels have been widely investigated as scaffolds for tissue engineering because of their biocompatibility and their composition similar to native tissue. Porosity, pore size, and pore structure are important factors that should be considered during the development of a tissue-engineered scaffold. Also, the mechanical properties of the scaffold should be similar to the tissue or organ intended for regeneration. In this context, synthetic polymers are preferred for their strength and elasticity. However, a crucial issue of synthetic scaffolds is their lack of biocompatibility. Recently, in the field of tissue engineering, great interest was obtained by IPN and semi-IPN networks. In fact, the possibility to prepare a network composed of one synthetic polymer and one natural polymer or composed only of natural polymers leads to the advantage of tune the mechanical properties of the single components, making the scaffold very similar to the native tissue. In addition, the presence of natural biomaterials can provide an environment that supports tissue growth. Table 4.3 shows several scaffolds developed for tissue engineering applications based on IPN or semi-IPN networks. Most of them are described in the previous sections.

4.4 Conclusions

Interpenetrating polymer networks (IPNs) have been studied extensively since their advent. The development of IPN systems by using a combination of polymers with different properties offers the opportunity to obtain materials with a range of improved properties and that will overcome the disadvantages of the single individual polymer network. IPNs have several applications and their success is mainly due to their cross-linked structure that provides better thermal stability, mechanical properties, and chemical resistance. In fact, IPNs are traditionally used as damping materials, impact resistant materials, and adhesives. However, more recently they are used alone or in association with nanoparticles to produce systems useful for biomedical applications, such as responsive hydrogels, medical implants, porous scaffolds, and micro- or nanocarriers for the delivery of drugs.

Table 4.3 List of IPN and semi-IPN scaffolds developed for tissue engineering applications

Hydrogel composition	Type of network	Application	References
Alginate + Pluronic F127	IPN	Antiadhesive agent after surgery	Choi et al. (2015)
Alginate + Fibrin	IPN	In vitro growth of ovarian follicles	Shikanov et al. (2009)
Alginate + pHEMA	Semi-IPN	Biomedical applications	La Gatta et al. (2009)
Alginate + Chitosan	Semi-IPN	Cartilage scaffold	Tigli and Gumusderelioglu (2009)
Methacrylated alginate + Collagen	IPN	3D-preosteoblast spreading and osteogenic differentiation	Sun et al. (2012)
Hyaluronic acid + DexHEMA	Semi-IPN	Potential bioprintable scaffold	Pescosolido et al. (2011b)
Hyaluronic acid + Fibrin	IPN	Potential scaffold	Zhang et al. (2016)
Methacrylated hyaluronic acid + Diacrylated polyethylene glycol	IPN	Cartilage repair	Park et al. (2003)
Hyaluronic acid glycidyl methacrylate + PuraMatrix	IPN	Neurite growth and extension	Khoshakhlagh and Moore (2015)
Chitosan + Collagen	IPN	3-D scaffold for carcinoma cells	Shanmugasundaram et al. (2001)
N-carboxyethyl chitosan + 2-pHEMA	Semi-IPN	Wound dressing	Zhou et al. (2008)
Dextran methacrylate and aldehydebifunctionalized dextran + Gelatin	IPN	Potential vascular scaffold	Liu and Chan-Park (2009)
Oxidized dextran + Gelatin graft polyaniline	IPN	Potential scaffold	Li et al. (2015)
Gellan gum methacrylate + Gelatin polyacrylamide	IPN	Regeneration of load bearing tissue	Shin et al. (2012)
Gellan gum + Hyaluronic acid	Semi-IPN	Bone regeneration of osteochondral defects	Bellini et al. (2015)
	IPN sponge	Skin wound regeneration	Cerqueira et al. (2014)

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Entrapment of essential oils in hydrogels for biomedical applications

5

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5.1 Introduction

In this modern era, the pace of technological progress and tremendous innovations in the field of biomedical research has revolutionized clinical practice to obtain sustainable health-care solutions for the developing world (Ullah et al., 2016; Vedadghavami et al., 2017). However, in this dynamic field, selection of biomaterials that interface with biological systems for implantation and delivery of drugs to treat human diseases remain as key challenges. Accordingly, the design and development of novel biomaterials for wound care have garnered much attention for acceleration of wound healing processes and maintenance of public health (Anitha et al., 2014).

One among the appropriate biomaterial used for this purpose in the human body is a hydrogel (Kopeček, 2007). These are a class of biomaterials with three-dimensional networks capable of displaying significant physicochemical changes with minor alternation in the surrounding medium. These variations in the biomaterial are reversible while the networks are held together by molecular interactions and secondary forces and would return to initial state after the removal of the trigger placed. Also, covalently cross-linked networks would result in the formation of permanent hydrogels prepared through cross-linking of water-soluble polymers and through conversion of hydrophobic polymers to hydrophilic polymers. Thus, with a wide range of promising applications in the pharmaceutical, biotechnology, biosensor, agriculture, and cosmetics sectors apart from the biomedical field, these are currently subject to considerable scientific research (Farhat et al., 2017; Gyles et al., 2017; Ullah et al., 2015).

A thorough literature survey finds that there has been a substantial increase in the application of hydrogels over the last two decades as a suitable carrier agent for the drug release, apart from its profound usage in tissue engineering and wound dressing matrices. Moreover, its characteristic properties make it potential agent to mimic the living tissue (Croisier and Jérôme, 2013; Wathoni et al., 2016). On the other hand, antimicrobial compounds such as essential oils have been reviewed to possess wound healing applications.

This chapter aims to critically review the current research, offering insights into the method of entrapment of essential oils in hydrogels. Also, this article would especially highlight the aspect of the inhibitory effect of antimicrobial hydrogels on pathogenic organisms and along with their diverse applications in the biomedical field.

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5.2 Mechanism of entrapment

Hydrogels posses a highly porous structure, which can easily be controlled by alteration of the density of cross-links present in the gel network. This high porosity also allows entrapment of oil-based drugs into the hydrogel matrix (due to hydrophobic interactions) and also allows the subsequent release of these drugs at a controlled rate. Hydrogels can be designed in various ways so that they can release drugs via various stimuli: enzymatic, hydrolytic, or environmental (e.g., pH, temperature, etc.). However, complete degradation of hydrogel structure depends on the timescale and location of the drug delivery device and is not always desirable.

Thermoresponsive hydrogels are the class of hydrogels in which gelation occurs on application of a particular temperature. Even a small disturbance in the thermal equilibrium of the structure can result in contraction or expansion of the network structure. Fig. 5.1 illustrates the mechanism of delivery of an oil-based drug in the thermoresponsive hydrogel. The thermal impact can either be internal or external or both and can also be due to some compromised metabolism, such as during infection. Despite natural polymers, some synthetic polymers such as cellulose and acrylamide (along with their derivatives) are also commonly utilized for the synthesis of temperature-sensitive polymers.

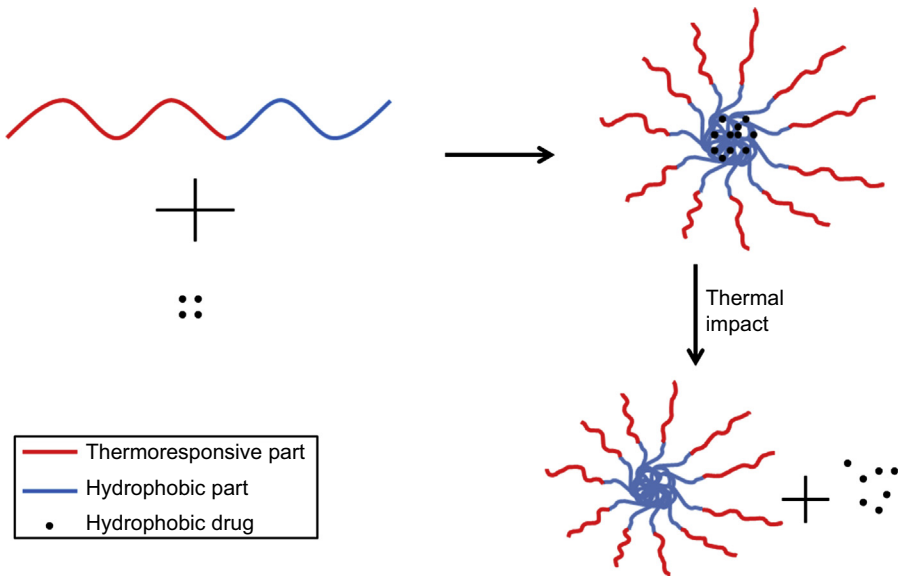

Figure 5.1 Mechanism of delivery of an oil-based drug in thermoresponsive hydrogel.

5.3 Production of hydrogels

The methods of production of such materials have also modified in recent years with special focus on physical and chemical cross-linking processes to obtain hydrogels with outstanding characteristics. However, physical hydrogels are found be more biodegradable and less toxic than chemical hydrogels (Shinde et al., 2013).

Various methods of preparation of cross-linked hydrogels are discussed in Fig. 5.2. Due to the presence of cross-linking in the structure, hydrogels do not disintegrate on swelling. Cross-linking in hydrogels can occur either during their preparation or after their application at a desired location in the human body. Chemical cross-linking occurs by reaction of a low-molecular weight cross-linking agent with the hydrogel-forming polymer. In the absence of cross-linking in the hydrogel, the hydrophilic linear polymer chains dissolve in water on achievement of thermodynamic compatibility of the elements. However, in the presence of cross-linking points, the solubility

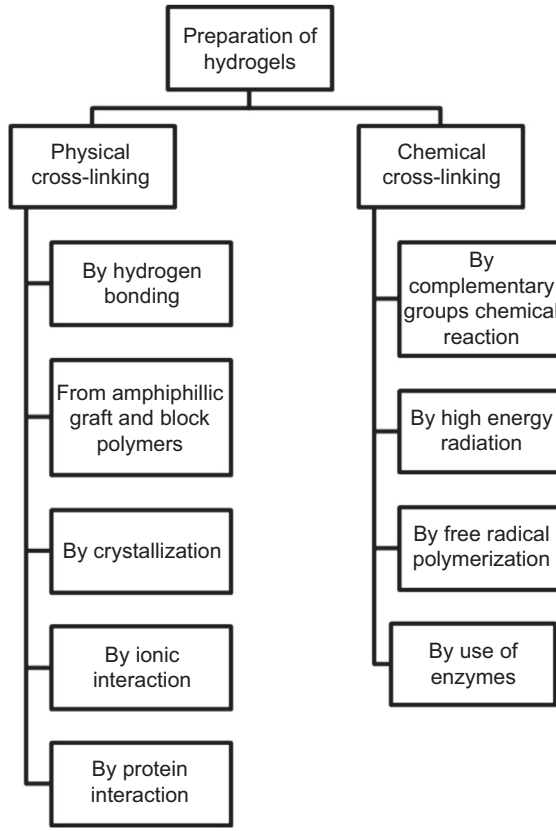

Figure 5.2 Various methods of preparation of cross-linked hydrogels.

of the hydrophilic chain in water is counterbalanced by the retroactive force at the cross-linking points in the network. Hydrophilicity of hydrogels is generally due to the presence of various hydrophilic groups such as amine, carboxyl, hydroxyl, amide, and sulfoxyl groups in the polymeric structure. In swollen state, the three-dimensional structure is maintained in equilibrium state by the presence of both physical and chemical cross-linking points (Ullah et al., 2015).

The chemical process of hydrogel synthesis is accomplished using a variety of methods, some of which involve simple, single-step processes, including the polymerization and simultaneous cross-linking of multifunctional monomers. On the other hand, other methods may involve several phases, such as the production of polymer molecules with reactive compounds and subsequent reticulation using specific cross-linking agents.

Hydrogels are classified based on various parameters such as source of polymer, physical state of hydrogels, configuration, type of cross-linking, degradability, ionic charge, and various responses as depicted in Fig. 5.3. Depending on the type of polymers used in the preparation of hydrogels, the hydrogels are categorized as homopolymeric, copolymeric, and multipolymeric. Homopolymeric hydrogels are the ones with only one class of polymer, whereas copolymeric hydrogels are composed of two different classes of monomers (generally with varying hydrophilic and hydrophobic properties) (Jain et al., 2008; Ullah et al., 2015). Multipolymeric hydrogels, also known as hybrid networks, involve two or more polymers and include both interpenetrating and semiinterpenetrating networks. These are usually prepared by addition of the prepolymerized hydrogel into polymeric network solution of monomers along with the addition of a polymerization initiator. Interpenetrating networks are produced in the presence of various cross-linking agents and in their absence semiinterpenetrating networks are formed.

5.4 Types of hydrogels and their biomedical applications

Based on the source of the biopolymer, the hydrogels are found to have various potential applications in biomedical field as illustrated briefly in Table 5.1.

5.4.1 Natural hydrogels

Hydrogels that are synthesized using naturally obtained polymers are called natural hydrogels. The type of polymers used in the preparation of hydrogel depends on the final use of prepared hydrogel. For instance, biocompatible, biodegradable, and nontoxic hydrogels are used for controlled release of drugs, so the polymers used for the preparation of such hydrogel must possess these characteristics (Ikada and Tsuji, 2000; Zu et al., 2012). Some of the natural polymers used for hydrogel synthesis and their biomedical applications are as follows:

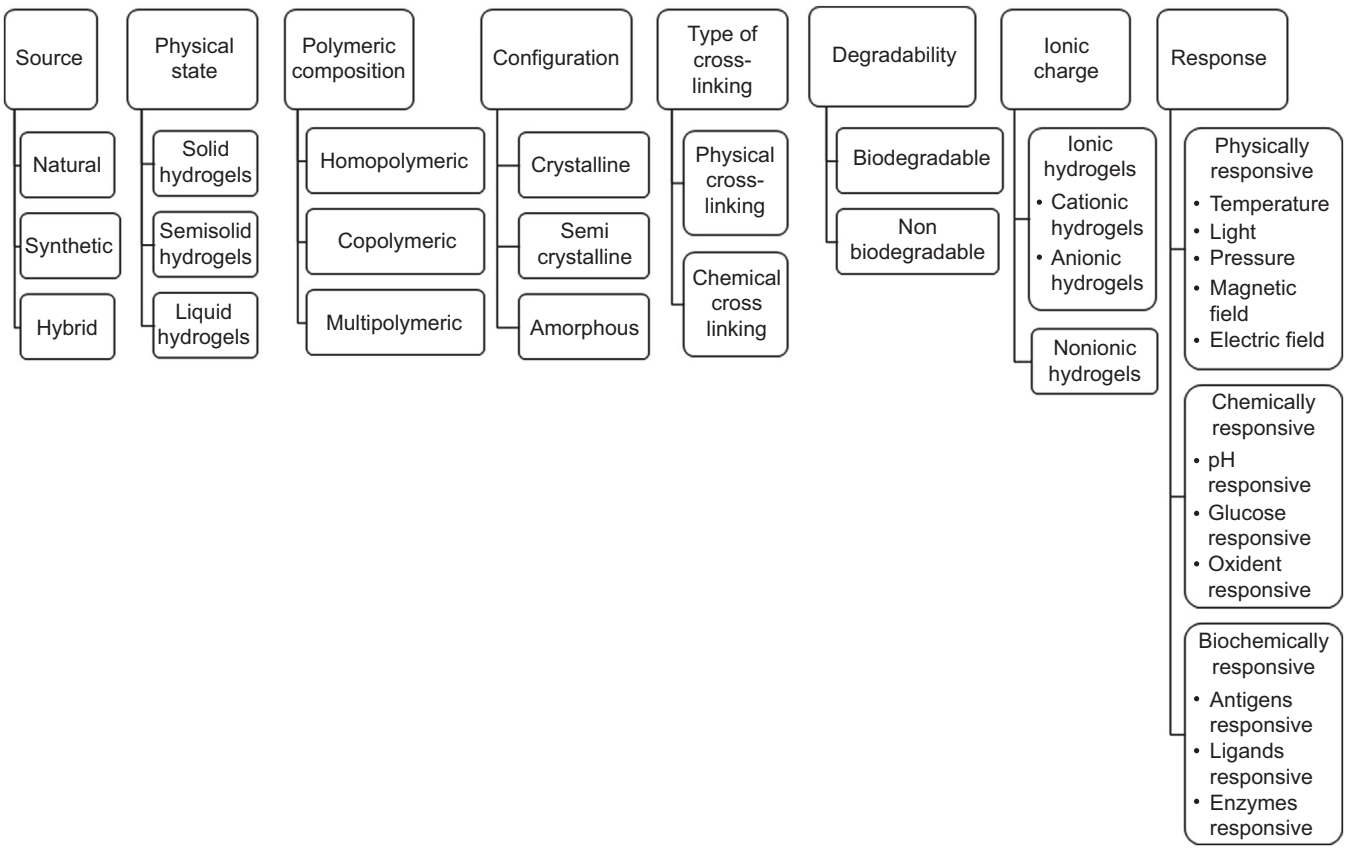

Figure 5.3 Classification of hydrogels based on different properties.

Table 5.1 Potential biomedical applications of some common hydrogels

Name of polymer	Type of hydrogel	Potential applications in biomedical field
Hyaluronic acid	Natural	Tissue healing, treatment of osteochondral dysfunction, injectable hydrogel, thermosensitive gel, tissue regeneration
Cellulose	Natural	Thermosensitive gels for drug delivery, tissue scaffolds, regenerative medicines, promotion of angiogenesis, burn wound regeneration, and cellular encapsulation
Dextran	Natural	Antithrombolytic agent, site-specific delivery vehicle for drugs and proteins, tissue engineering, drug encapsulation
Chitosan	Natural	Drug delivery vehicle, injectable hydrogels, bone tissue engineering, self-healing adhesives, ophthalmology
Polyethylene glycol	Synthetic	Tissue engineering, bone prostheses, improved wound healing, scaffolds for cartilage regeneration
Poloxamer	Synthetic	Delivery vehicle for oil-based drugs, site-specific drug accumulation, spinal cord regeneration, buccal drug delivery
Polyvinyl alcohol	Synthetic	Artificial grafts, wound dressings, artificial implant for meniscal tissue

5.4.1.1 Hyaluronic acid–based hydrogels

Hyaluronic acid (HA) is an anionic, linear copolymer of D-glucuronic acid, and 2-acetamido-2-deoxy-D-glucose. The anionic characteristics allow it to form strong electrostatic interactions with other components present in the hydrogel system. Various chemical alterations increase flexibility in its physical structure and allow it to exist in several forms, including firm hydrogels also. HA hydrogels increase the cell propagation and migration, development of new blood vessels, and control of inflammatory response when used in tissue healing (Burdick and Prestwich, 2011; Dahiya and Kamal, 2013; Nolan et al., 2006). They are also applied in the regeneration of cartilages and treatment of osteochondral dysfunction. HA is also applied to drug release mechanisms and has been investigated for diverse methods of drug administration through the ophthalmic, nasal, pulmonary, parenteral, and topical routes (Nusgens, 2010).

Jung et al. (2017) developed an injectable thermosensitive hydrogel synthesized using HA and pluronic F127 for the long-term sustained release of piroxicam. The formulation was found to have improved mechanical properties, sustained release of piroxicam, and also high bioavailability in physiological environments. The HA hydrogel is applied to various areas ranging from drug delivery to tissue regeneration; however, these applications require its efficient cross-linking for production of hydrogels that can retain their structural integrity.

5.4.1.2 Cellulose-based hydrogels

Cellulose is a hydrophobic, biodegradable polymer, which is found in plants, natural fibers, and is also biologically synthesized by some bacterial species (e.g., *Acetobacter xylinum*) (Sannino et al., 2009). Cellulose obtained from *Acetobacter* species possesses typical fibrous nanostructure, which accounts for its ideal mechanical and physical characteristics. One of the most distinct characteristics of cellulose is its excellent biocompatibility, which is one of the many reasons that cellulose is such an attractive component in biomedical devices (Czaja et al., 2007).

Generally, cellulose is chemically modified (esterification or etherification of some hydroxyl groups) to form cellulosic compounds such as methylcellulose, which has better hydrophilicity. Cross-linking of ethers within cellulose molecules results in the formation of more stable hydrogels, which can be reversible or irreversible. Some of the commonly used cellulosic polymers used for hydrogel synthesis are methylcellulose, ethylcellulose, hydroxyethylcellulose, methylhydroxypropylcellulose, carboxymethylcellulose, and sodium carboxymethylcellulose (Czaja et al., 2007).

Methylcellulose hydrogels have limited applications due to low mechanical strength; however, its combination with polyacrylamide results in the formation of a denser matrix. These hydrogels are also used as thermosensitive hydrogels and are widely used as vehicles for drug delivery (Aouada et al., 2010; Nasatto et al., 2015). Due to the high resemblance of hydrogels with biological tissues, they are widely applied in the fields of tissue engineering and regenerative medicines. Cellulose-based hydrogels are also used as fillers or scaffolds for tissue regeneration due to their suitable mechanical properties, biocompatibility, and biodegradability. They are used in cell transplantation, promotion of angiogenesis, burn wound regeneration, and cellular encapsulation (Caló and Khutoryanskiy, 2015).

5.4.1.3 Dextran-based hydrogels

Dextran has glucose molecules as its monomeric units, which are linked with a linear 1,6-glycosidic bond with branching at 1,3-linkage. They are widely used as an antithrombolytic agent and as a vehicle for drugs, proteins, and other bioactive agents. Li et al. used dextran hydrogels for preparation of a photocrosslinked tissue adhesive gel, which can be used in the rapid healing of surgical wounds. Dextran hydrogels are also found useful in the site-specific controlled delivery of various insoluble drugs such as ibuprofen, paclitaxel, and dexamethasone. However, their application as protein delivery systems is limited, and due to their high permeability, they cause an early release of the entrapped drugs (Artzi et al., 2009). Bae et al. (2015) reported that a sustained and gradual release of PEGylated protein could be achieved (along with preservation of curative properties and increased half-life) using dextran hydrogel microstructures. Jin et al. (2007) reported dextran−tyramine hydrogels cross-linked with enzymes possessed high potential for their applications as protein transporters and in tissue engineering with more effective gelation process. Sericin/dextran injectable hydrogel is synthesized using sericin and hydrazone (as a cross-linking agent), which enables its monitoring in real-time in vivo along with the elimination of even traces of

photoluminescent materials and final disintegration of the hydrogel. The hydrogel could successfully entrap the drug doxorubicin and release it in controlled form with active suppression of tumor growth (Liu et al., 2016).

5.4.1.4 Chitosan-based hydrogels

Chitosan-based hydrogels have a wide range of applications in biomedical areas where they can be used for the preparation of wound dressings, scaffolds in tissue engineering, as a vehicle for delivering various drugs, and in preparation of contact lenses.

5.4.1.4.1 Drug delivery vehicle

The main benefit of using chitosan-based hydrogels as a vehicle for drug delivery is that they offer time-controlled and site-specific delivery of drugs with a wide range of molecular weight along with enhanced biosafety and efficacy of these drugs. They also improve the stability of therapeutic compounds against degradation by enzymes and chemicals (Chandy and Sharma, 1990).

Wei et al. (2010) reported that chitosan nanogels grafted with salicylic acid could deliver paclitaxel more effectively. Chitosan-based nanogels loaded with insulin were found to have enhanced nasal absorption along with a reduction in pain. These gels were also found to have successful parenteral and mucosal delivery of antigen vaccines and enhanced immune responsive characteristics of cytokines (Van Der Lubben et al., 2001).

5.4.1.4.2 Injectable hydrogels

Administration of various drugs, genetic materials, and live cells can also be achieved by use of injectable hydrogels. Injectable hydrogels formed by the combination of chitosan and HA with cross-linkage with β-glycerophosphate and genipin have the potential to entrap live cells for cartilage regeneration (Sung et al., 2001). Cross-linkage of chitosan-based injectable hydrogels with genipin results in a reduction of toxicity of the hydrogel and can deliver lipophilic molecules such as curcumin along with a significant increase in its bioavailability (Songkroh et al., 2015).

In the treatment of arthritis, chondrocytes growth can be maintained by use of injectable photopolymerized chitosan hydrogels (Kim et al., 2015). Modification of chitosan-based hydrogels and their augmentation with bioactive glass nanoparticles results in the formation of thermosensitive hydrogels with a gelation temperature of 37°C and a high potential for their use in bone tissue bioapplications.

5.4.1.4.3 Bone tissue engineering

In bone tissue engineering, chitosan-based hydrogels have been used widely to promote the growth of cell (Seol et al., 2004). They can be used as an additive to enhance injectability and efficacy in bone cement for clinical application (Leroux et al., 1999). Hydrogel prepared from chitosan, alginate, and hyaluronan has latent in cartilage regeneration (Hsu et al., 2004). In a sheep model, articular cartilage defects can be repaired by the combination of chitosan and hydroxyethylcellulose hydrogel. Regenerative responses were improved in the facial nerve of rabbits by the delivery of progesterone in chitosan

hydrogel (Chávez-Delgado et al., 2003). Thermoresponsive hydrogel scaffolds prepared from a combination of chitosan and hydroxyethylcellulose were applied to repair articular cartilage defects in a sheep model (Hao et al., 2010).

5.4.1.4.4 Self-healing adhesives

Chitosan-based could be used for site-specific delivery of curative drugs into the integumental or skin wounds. When incorporated with fibroblast growth factor, these hydrogels could be used to prepare scaffolds, which can accelerate wound healing (as compared to gauze control) in the treatment of chronic ulcers and gelatin microparticles containing these hydrogels were clinically tested in aged mouse model (Park et al., 2009).

5.4.1.4.5 Ophthalmology

Chitosan hydrogels can replace synthetic polymers for the synthesis of contact lens due to their better optical clarity, mechanical strength, wettability, and immunological compatibility.

They also provide desirable properties (antimicrobial and wound healing properties) for their use in preparation of ocular bandage lenses.

The nanocomposites formed by dispersion of chitosan−glutathione and preactivated chitosan−glutathione could be used for in vivo studies of precorneal retention (Qin et al., 2015). Thermoresponsive hydrogels prepared from a combination of chitosan and gelatin possessed the potential to deliver latanoprost in a continuous manner control for controlling ocular hypertension (Cheng et al., 2014).

5.4.2 Synthetic hydrogels

Hydrogels that are prepared from synthetic polymers are known as synthetic hydrogels. They have a wide range of applications in biomedical field as they are generally hydrophobic, have better chemical stability and mechanical strength, and have reduced degradability in comparison with natural hydrogels (Garnica-Palafox and Sanchez-Arevalo, 2016). Some of the most commonly used synthetic hydrogels having the potential to be used in biomedical areas are polyethylene glycol, polyacrylamide and its derivatives, and polyvinyl alcohol hydrogels (Gulrez et al., 2011).

5.4.2.1 Polyethylene glycol hydrogels

Polyethylene glycol is an oligomer of ethylene oxide and is chemically similar to polyoxyethylene, polyethylene oxide but is having less molecular weight (<10,000 units) as compared with the latter compounds. They are generally amphiphilic and have good solubility in both aqueous and nonpolar environments (Akhtar et al., 2016). Low-molecular weight polyethylene glycols (<1000 units) are generally more viscous and colorless.

Food and Drug Administration has approved usage of polyethylene glycols in biomedical applications due to their good biocompatibility, nonimmunogenicity, and resistance to get adsorbed by protein molecules. They have been applied to a

wide range of areas such as tissue engineering, bone prostheses, wound healing, and much more. They have the potential to form insoluble networks alone, nonetheless, however, the addition of functional groups such as amine, carboxyl, acrylate, and thiol can enhance the functionality of the cross-linking networks and can help in the formation of a biomaterial with improved mechanical strength and reduced biodegradability (Yu et al., 2014).

Polyethylene glycol is widely used in sol−gel chemistry and for drug delivery due to its ability to release the drug at a specific site in a controlled manner with preservation of its bioactivity. The advantage with the usage of these hydrogels is that they can easily swell up after getting injected inside the body on getting the required triggering (Smeds and Grinstaff, 2001). The hydrogels, which can be swelled up in situ, are classified into two main groups:

1. Light sensitive hydrogel systems that are created on exposure to ultraviolet or visible light irradiation but not able to assemble of their own.
2. Hydrogel systems that can self-assemble on exposure to suitable physiological, environmental conditions. These are generally synthesized voluntarily on physical or chemical triggering such as pH or temperature change (Hoffman et al., 2000; Kumar et al., 2007).

Lin and Anseth (2009) reported that hydrogels prepared by a combination of polyethylene glycol and polyvinyl alcohol could be used in cartilage tissue engineering, which can be self-developed after photopolymerization and could temporarily replace the damaged cartilage with the developed one.

Zhang et al. (2016) created a macroporous IPN hydrogel from a hybrid of PEG and gelatin polymers for application as cartilage regeneration implants in tissue engineering. The IPN formulation improved the mechanical and structural properties of the hydrogel, necessary for application in tissue engineering, whereas the macroporous structure enhanced cell−cell interaction and tissue formation. The results showed that this formulation has great potential for applications as scaffolds for cartilage regeneration.

These hydrogels can also be efficiently applied as wound dressings by manipulating the molecular weight of polymers by irradiating it with various electronic beams. This was found to enhance swelling property, mechanical properties, and water vapor transmission rate of the resulting hydrogels. Their efficacy as a wound dressing was determined by experimenting them on mice wounds, and the results indicated that these hydrogels have a better healing potential than gauze or commercial treatments (Kim et al., 2014).

5.4.2.2 Poloxamer hydrogels

Poloxamers are the group of polymers having a hydrophobic block surrounded by two hydrophilic blocks. For instance, poloxamer 188 is a copolymer comprising two blocks of polyethylene glycol surrounding a block of polypropylene glycol. During gelation, the hydrophobic parts are paired with hydrophilic segments either by use of an amphiphilic polymer or by copolymerization technique. The mechanism of gelation of hydrophobic blocks is shown in Fig. 5.4. The amphiphilic polymers are water

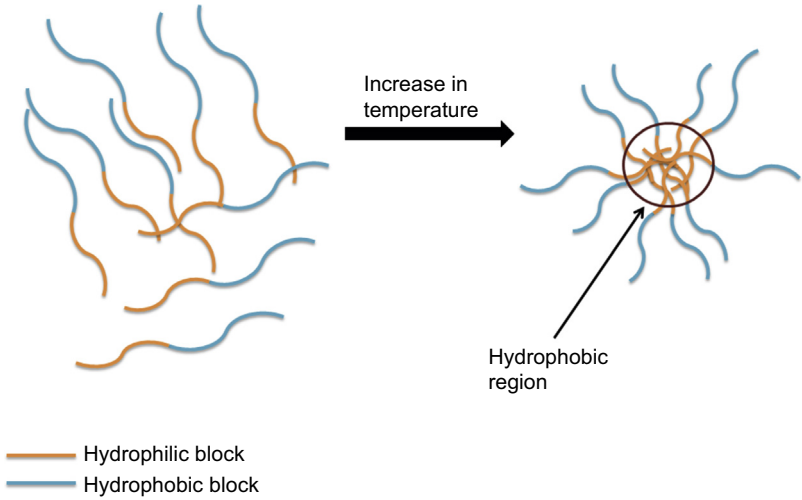

Figure 5.4 Mechanism of physical gelation of hydrophobic blocks in poloxamer hydrogels.

soluble at ambient conditions, but on exposure to high temperature, the availability of structured water is reduced, and entropy of solvent is increased. The temperature at which gelation occurs depends on the chemical structure and chain length of the hydrophobic polymer (Taheri et al., 2011).

Currently, optimization of the hydrogel−drug interaction and their release kinetics is a major area of study. Nanogels are widely accepted as a delivery vehicle for oil-based drugs due to increased solubility, decreased cytotoxicity, site-specific drug accumulation, and improved stability of bioactive agents.

Zhao et al. designed heparin−poloxamer, which is a thermoresponsive hydrogel and can boost spinal cord regeneration. Poloxamer−HA thermoresponsive injectable hydrogels could be used for bone regeneration by stimulation of extracellular matrix of the bone. The release of drug from these hydrogels depends on the drug loading content, water content, and ionic properties of the drug (Zhao et al., 2016). The hydrogels synthesized by a combination of salbutamol sulfate and poloxamer also possess the potential for buccal drug delivery (Zeng et al., 2015).

5.4.2.3 Polyvinyl alcohol hydrogels

Polyvinyl alcohol is a synthetic polymer having good biocompatibility, biodegradability, and hydrophilicity and finds applications in numerous biomedical fields. Polyvinyl alcohol hydrogels possess great potential for their use in tissue engineering and can also act as a feasible alternative of current artificial grafts. Their high mechanical strength is imparted by the presence of cross-linking hydrogen bonds. Efficient wound dressings can be prepared by gamma irradiation of chitosan−gelatin−polyvinyl alcohol hydrogels. These hydrogels were found to have enhanced pH sensitivity, swelling ability, and water vapor transmission, thus, confirming their application as wound dressing (Fan et al., 2016).

Hayes et al. reported the potential application of salt-modified polyvinyl alcohol hydrogels as an artificial implant for a meniscal tissue. An aqueous solution of polyvinyl alcohol was dehydrated with Na_2SO_4 solution form malleable hydrogel, which was then physically cross-linked to form the hydrogel. A meniscal-shaped mold was then created from the gel and was then directly compared with the human meniscal tissue. This hydrogel was found suitable for a replacement for critically damaged meniscal tissue (Hayes et al., 2016).

5.5 General biomedical applications of hydrogels

Hydrogels possessing entrapped essential oils have been various applications in the field of biomedical that include oral hygiene management, drug delivery, wound dressing, and tissue engineering.

5.5.1 Oral health

Lack of oral hygiene has been observed as a predominant factor leading to tooth loss (Gyles et al., 2017). In recent times, cure of periodontal disease includes the application of commercial products such as mouthwashes that are applied on a regular basis, systemic antibiotics, and various carrier agents targeted for local delivery of bioactive.

Treatment of oral diseases, including periodontal disease, usually becomes complex owing to high moisture content and the natural secretions of oral cavity such as saliva that dilute the applied drugs leading to mitigate the inhibitory effect. Thus, to achieve suitable treatment, application of drugs is a prerequisite.

Usage of biodegradable scaffolds as delivery vehicles was explored to possess multiple oral health applications especially cure of dental cavities, treatment of periodontitis, and maintenance of oral hygiene owing to better patient compliance and acceptance (Gyles et al., 2017). In recent research findings, chitosan hydrogels were evaluated as a biodegradable scaffold and were employed as a possible carrier vehicle for bioactive compounds such as thymol. The aforementioned compounds were found to possess promising characteristics for oral therapy. These hydrophobic compounds are sparsely soluble in water and need a medium such as a hydrogel as an oral delivery device for their successful application.

In a research as an alternative treatment method for periodontal disease, chitosan-based hydrogels were developed by spraying method to produce suitable matrices possessing requisite physical properties such as porous matrix, rheological properties, and swelling behaviour feasible for delivery of hydrophobic compounds such as thymol. An alkali solution spray was used incorporated with thymol promoting a sol−gel transition resulting in the production of hydrogels. To assess the biocompatibility of the delivery units, cytocompatibility assay was performed with [3T3] mouse fibroblasts. Furthermore, the release kinetics of thymol entrapped in the polymeric matrix of the chitosan hydrogel along with the antioxidant and antimicrobial properties were evaluated and were observed to fulfill the requirement of dual therapy and multiple actions as a treatment for periodontal disease.

5.5.2 Wound management

It has been examined that the antimicrobial agents with a single site of action could not overcome the resistant microorganisms. Different strategies have been developed to limit the development of resistance against the antimicrobial compounds (Low et al., 2016). Antibiotic resistance is one among the major factors that hinder the treatment of chronic wound infections. In a recent research aimed at effective wound management, chitosan-based hydrogels were ionically cross-linked with individual and combination of antimicrobial compounds for the effective management of wound-infecting pathogens. To achieve higher antimicrobial activity and reduce the occurrence of antibiotic resistance, chitosan-based hydrogels were loaded with a combination of antimicrobial agents (tea tree oil) and metal ions (Ag^{+}) that would possess different intra- and extracellular target sites. It was investigated that the usage of hydrogels as controlled release system for delivery of combined antimicrobials has inhibited the growth of highly antibiotic-resistant microorganisms including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans.* However, it was observed that the antimicrobial activity was dependent on the viscosity of the cross-linked chitosan and the concentration of the loaded antimicrobial agents (Low et al., 2016).

Many researchers have focussed to develop wound dressing films consisting of thymol. In one such research finding, composite wound dressing films were produced from a blend of chitosan (80%w/w) and PEGF (20%w/w) with varying concentration of thymol such as 0%(v/v), 0.6%(v/v), 1.2%(v/v), and 1.8%(v/v) as an additive to the blend solution. The wound dressing films produced from the formulations were evaluated for various tests such as Fourier-transform infrared, water vapor transmission rate, scanning electron microscope, and antibacterial tests. It was observed that the blend film possessing 1.8% thymol showcased optimum properties having greater mechanical properties, superior swelling level, and suitable water solubility. In addition, the films demonstrated excellent antibacterial properties against both gram-positive and gram-negative bacteria. The antibacterial test was conducted by colony forming assay against *S. aureus* and *Escherichia coli*. It was observed that the blend films with 1.8% thymol had the highest antibacterial activity against *S. aureus* and *E. coli*, 98.4% and 99.9%, respectively (Koosehgol et al., 2017).

5.5.3 Skin burn treatment

It has been reviewed that excessive exposure of skin to UV radiation can lead to various skin-associated damages and structural changes as well, including cell injury and premature skin aging. On exposure to ultraviolet B (UVB) (280−320 nm) radiation leads to skin burn and cytotoxic effects (Matsumura and Ananthaswamy, 2004). One among the alternative approaches applied for treatment of cutaneous UVB radiation−induced damages is the application of topical formulations of hydrogel possessing silibinin-loaded pomegranate oil-based nanocapsules. To carry out this study, an animal model such as mice induced with skin injury by UVB radiation was selected. Also, gellan gum was applied over the nanocapsules suspension as a gel-forming agent. It was observed that the application of hydrogel topical formulation was

successful in reducing the mice ear edema and leukocyte infiltration. Overall, the results of the application of these hydrogels demonstrated prolonged antiedematogenic effects comparable performance to that of traditional therapeutic remedies such as silver sulfadiazine (Marchiori et al., 2017).

5.6 Conclusion

Hydrogels have great potential to be employed for biomedical applications for delivery of bioactive compounds. Thus entrapment of hydrophobic compounds such as oils in hydrogels was demonstrated to possess good mechanical properties, degradability, improved retention and release of bioactive compounds, porous structure, antioxidant, and antimicrobial properties. Therefore the hydrogels play a vital role in the biomedical field and provide a supportive role to act as delivery vehicles for treatment of various human diseases.

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Particle-loaded gels

6

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6.1 Introduction to disperse systems and colloids classification

Since the last decade, considerable interest has been noticed in exploring the drug delivery mechanism through the disperse systems due to their potential to transport drug at the target site and negligible side effect with minimum alterations in drug pharmacokinetics. The common disperse system in this regard includes liposomes, nano- or microparticles, emulsion, etc., the smaller particle size of these carriers (in order of micron size or less) facilitates in drug transport through the tiny vessels of human body (Washington, 1990). In colloidal dispersion, colloidal particles are dispersed in continuous phase. The dispersed system contains internal and continuous phases based on particle size of dispersed phases and is categorized as molecular dispersion, colloidal dispersion, and coarse dispersions (Manoharan et al., 2010). In the year of 1980, the sol−gel method has become an attractive method for formation of hydrogel, aerogel, or monolith (Brinker and Scherer, 2013). The silicon-based systems are one of the oldest products based on the sol−gel chemistry (Bartczak et al., 1999). Ebelmen has reported the effect of reported the slow hydrolysis of an ester form of silicic acid for formation of transparent material. The sol−gel process helps to control the mechanism and the kinetics of reaction steps that leads to formation of hierarchical materials (Aegerter and Prassas, 2011). Colloidal dispersion is available in different forms such as—foam, liquid aerosol, emulsion, gel, aerosol, colloidal suspension, etc. In macromolecular colloids, certain molecules with larger dimensions and higher molecular masses are dispersed in suitable dispersion medium. Lyophilic solution is type of macromolecular colloids. Naturally occurring macromolecules such as gelatin, protein, starch, etc., are macromolecular colloids, whereas synthetic polymers, including polyethylene, propylene, rubber, etc., also form macromolecular colloids when dispersed in appropriate media. Besides these, another type of colloid called associated colloids or micelles exhibits colloidal property at high concentration, however, at low concentration they act as strong electrolytes. Aggregate particles of this type form micelles at higher concentration and thus show the colloidal behavior. For micelles formation, certain minimum concentration of particles is required and such concentration is termed as micellization concentration (CMC). Value of CMC varies depending on the dispersed phase (Mandavi et al., 2008).

Polymeric Gels. https://doi.org/10.1016/B978-0-08-102179-8.00006-5

6.2 Control of physical properties

6.2.1 Mechanical property enhancement

To improve the mechanical stability of polymers and to widen their application fields, different inorganic fillers, including SiO_2, glass, $CaCO_3$ particles, carbon nanotubes, etc., are added to the polymer composite. Studies have revealed that such fillers can dramatically improve the physical and mechanical properties of composites (Ray and Okamoto, 2003; Wang et al., 1996). Incorporation of layered silicate improves the mechanical stability of particle−polymer nanocomposite. The stiffness of micro- or nanoparticles is higher in comparison to polymer compositions and thus incorporation of such particles readily improves the Young's modulus of polymeric samples (Zhu et al., 1999; Amdouni et al., 1992). In composite system, the nanoparticles (NPs) provide superior yield strength and higher rigidity. Fu and Wang (1993) and Bartczak et al. (1999) showed the enhancement of toughness in polyethylene after addition of calcium carbonate particles. In pseudo-ductile polymers, enhancement of impact properties has been achieved by addition of inorganic particles (Sumita et al., 1983). Particle size of filler materials has obvious impact on mechanical properties of polymers. The smaller particle size of calcium carbonate is reported to provide higher mechanical strength of polypropylene composite (Lau et al., 2006). Sumita et al. (1983) reported that the replacement of microscale silica by nanoscale particles further improves the mechanical properties of polymer composite. The effect of smaller particle size of calcium carbonate can modulate the fracture toughness of high-density polyethylene (Bartczak et al., 1999). Besides particle size, particle−matrix interface adhesions and particle loading are important factors that for controlling the mechanical property of polymers. With increase in particle−matrix interface adhesion, the tensile strength of polystyrene composite containing the glass beads is reported to improve (Dekkers and Heikens, 1983). In another study with 10 wt% increase in particle loading, the strength of polyimide/silica composites has remarkably increased; however, beyond this percentage further addition of silica particles reduces the mechanical strength of polymer composite (Zhu et al., 1999). Such observation has also observed with addition of glass beads in epoxy composite in which after certain limit further addition of glass beads shows no effect on mechanical properties of polymer (Kinloch et al., 1985; Fu et al., 2008). So, it has now been established that addition of nanoscale filler particles even in small scale is able to provide large impact in mechanical properties of polymers (Tjong, 2006; Münstedt and Triebel, 2011). In this direction, Tjong (2006) reported that addition of different nanofillers, including carbon nanotube or other NPs, remarkably influences the mechanical properties of amorphous and semicrystalline polymers. The interaction between the nanotube and polymer matrix is showed in Fig. 6.1.

Based on the several experimental results, it has been opined that the enhancement of polymer properties depends on surface chemistry of polymers and nanofillers and dispersion quality of filler materials (Ju et al., 2014; Goren et al., 2010). Recent research trend is focused on structure−property relationships of polymer composites

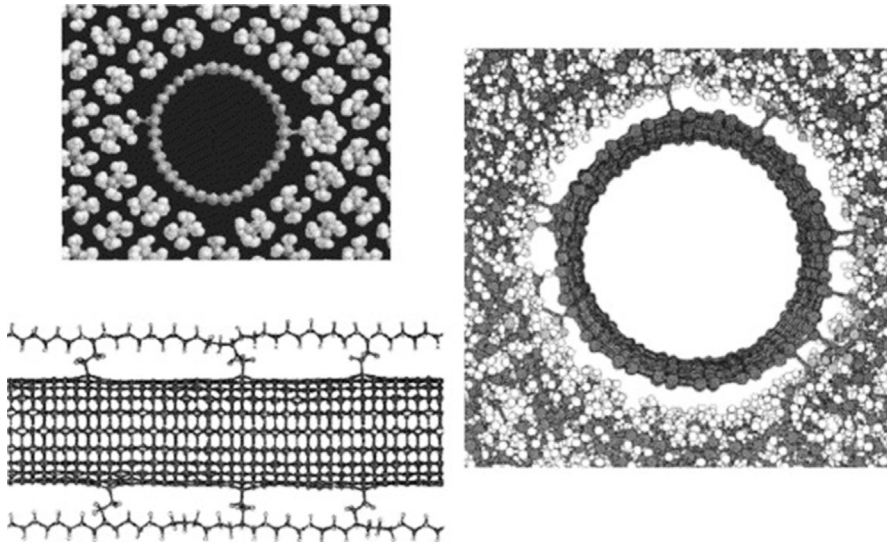

Figure 6.1 Represents the cross-linked structure of polymer matrix with impregnated nanotubes (Lau et al., 2006).

and their interfacial interaction for understanding the mechanism of reinforcement (Jancar et al., 2010; Senses and Akcora, 2013). Recent studies indicate the enhancement of polymer properties through polymer−filler interaction is due to formation of noncovalent interaction between them (Wu et al., 2013; Rose et al., 2014; Xia et al., 2013). The filler materials also improve thermal and electrical properties of polymer composites. Thermal propagation through any solid depends on the elastic modulus of the material and by changing the elastic moduli through addition of nanofillers, thermal properties can also be modulated (Zaragoza et al., 2015).

Wichterle and Lim (1960) for the first time have demonstrated the synthetic hydrogels with controlled properties. The cross-linked polymers were sensitive to surrounding environment in terms of solvent composition, pH, temperature, light, etc. (Loh et al., 2013; Appel et al., 2012). The schematic representation of stimuli-sensitive polymer is represented in Fig. 6.2. The NP-loaded hydrogels gradually find applicability in different fields of biomedical applications. Incorporation of NPs in hydrogels not only generates structural diversity but also improves the intrinsic properties of polymers and their responsiveness to external cues. After incorporation of silica NPs the cellular adhesion, biocompatibility, and mechanical stability of polyethylene glycol has reported to improve in comparison to the polymer without NP fillers (Liu et al., 2014). Poly *N*-isopropyl amide hydrogels show improvement in mechanical and thermal properties after incorporation of gold NPs (Liu et al., 2014). Hence, the composite materials comprising the NPs and hydrogels lead to formation of advanced materials with potential for application in numerous fields (Liu et al., 2014).

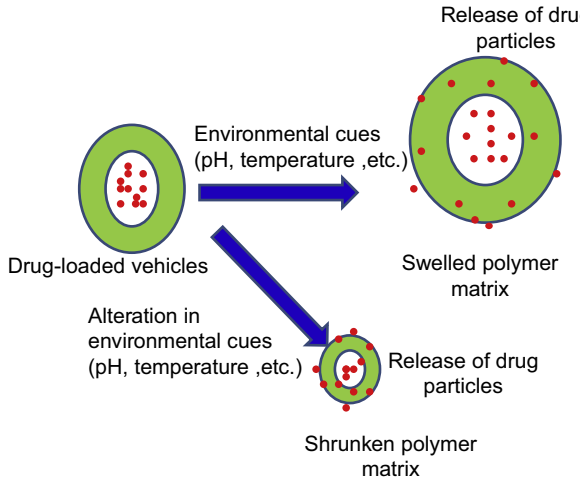

Figure 6.2 Shows the schematic representation of drug-encapsulated polymer matrix and release of drug in response to different environmental stimuli.

6.2.2 Control of optical properties

Optical property of nanoparticle-embedded polymers is an important parameter for its biomedical application (Böhmer et al., 2001). Nanocomposite structures containing optically active materials dispersed in polymer matrix exhibit interesting optical. Previous studies have reported that optically functional materials can be created by incorporating nanocomposite structure. For homogenous distribution of NP, different in situ synthesis processes are reported; however, one of the most common limitations in these methods is difficulty in maintaining homodisparity especially in the composite containing high concentration NP content (Kozuka and Sakka, 1993; Kobayashi et al., 2001). For optical application, quantum confined semiconductor, small molecules, polymers, etc., are dispersed in matrix materials (polymers, copolymers, etc.). Such nanocomposites show nonlinear optical properties and laser amplification properties. In addition to this, other optical properties such as absorption, luminescence, fluorescence, etc., have also been studied with such compositions. The size of NPs and refractive index (RI) mismatched between matrix and particles are two important factors, which instruct the undesirable optical scattering of these composite polymers. The RI mismatch does not possess much impact for very small particles with particle size <25 nm, however, for larger particles, the RI mismatches have potential role in scattering (Beecroft and Ober, 1997).

Incorporation of enzymes or other protein molecules in sol−gel matrix and their potential optical applications has been studied by several research groups (Ellerby et al., 1992; Avnir et al., 1994). Bacteriorhodopsin is one of such light-transducing protein used as a component of optically coupled devices, optical-based ion sensors, and real-time holographic medium (Weetall et al., 1993). Phycoerythrin is another light-transducing protein, and after incorporation in silica gel, the absorption and fluorescence properties have confirmed that the optical properties of protein have

been retained and also show better stability toward photodegradations (Beecroft and Ober, 1997). Application of these NPs has advantage in medical imaging because their cytotoxic effect is comparatively less in comparison with other molecular probes. The NPs do not suffer from nonspecific binding with cellular biomacromolecules, and handling of these particles is comparatively simply than the fluorescence probes. Silica NPs (SiNPs) are the first materials used for high-throughput bioimaging (Wang et al., 2013a). According to the extensive review by Wiesner regarding application of SiNPs in biosensing, composition of different NP with different polymers through various synthesis process has been studied (Ma et al., 2012). The advantage of SiNPs is that it can be easily doped with different range of organic, metallic fluorophores, and typical range of emission in between 300 and 1000 nm (Mader et al., 2008). However, emission range greater than 600 nm often interferes with the emission range of cellular autofluorescence. Fluorescence coating of SiNPs is commonly used although it may suffer from aggregation of NPs accompanied by the self-quenching. However, the emission wavelength of such NPs can be modulated through incorporation of dopants. The decay and size of such dopant are widely tunable. PAA hydrogels containing NPs of free amino groups were prepared through copolymerization of acrylamide, bisacrylamide with 3-aminopropyl−acrylamide, and the terminal amino group was labeled with pH-sensitive fluoroprobes such as Alexa 633, fluorescein, etc. The resulting sensor can cover a wide pH range, which is required for certain cellular imaging (Sun et al., 2011). Two-step fabrication process through free radical precipitation polymerization of polyacrylamides (PAAs) was studied in which the core−shell contained the indicator (Zhou et al., 2014). Application of Pluronic hydrogel has also been reported for fabrication of fluorescence-based imaging tools (Wang et al., 2013b). The hydrophobic polystyrene NPs doped with apolar fluorophores exhibited the emission spectra beyond 1000 nm. Doping of lipophilic groups is preferable because ionic probes are not readily soluble in hydrophobic NPs and have tendency to leach out. Polystyrene NPs are less toxic to cells, they have better cellular permeability, and slow excretion rate from biological system. Additionally in biological system, they are slowly coated with intracellular proteins and not attacked by body's immune system. The swelling and aggregation behavior of such NPs depends on the zeta potential values. Polystyrene nanoparticles with approximately 85 nm size can be used to image the cellular oxygen when loaded with oxygen-quenchable luminescent ruthenium complex (Ramos et al., 2014). Fluorophore-doped polyacrylonitrile (PAN) NPs exhibit suitable emission range, fairly biocompatible, and do not provoke any immune reaction. These doped PAN−NPs are widely used in temperature or pH measurements of cells with submicron level spatial resolution (Ramos et al., 2014; Wolfbeis, 2015).

Willner et al. (Pardo-Yissar et al., 2001) have reported the simplest method for forming an NP−hydrogel composite containing gold NPs (Au NPs) immobilized in polyacrylamide (PAAm). These nanocomposites are reported to use in development of optically responsive optomechanical nanocomposites. Other research groups such as S.R. Sershen et al. (2002) prepared Au NPs *N*-isopropylacrylamide/acrylamide (NIPAAm/AAm) composites, whereas using the same fabrication technique, Ravi et al. (2005) incorporated three different types of NPs in polymer matrix for application

in intraocular lens formation. However, the major drawback of this fabrication process is probability of leaching the NPs from the hydrogel matrix in case of low cross-linking density (Holtz and Asher, 1997). Liu et al. have reported the advanced application of titania nanosheet composites fabricated through similar manner (Holtz and Asher, 1997; Liu et al., 2013).

6.2.3 Antimicrobial properties

In particle-loaded gels, most common approach to obtain the antibacterial efficacy is doping with silver nanoparticles, which has proven bactericidal effect. Silver nanoparticles in different configurations, such as nanorods, nanotubes, etc., posses high surface to volume ratio and enhanced antibacterial efficiency (Sharma et al., 2009). Silver particles incorporated in inorganic matrix have been studied by different research groups (Panáček et al., 2009). Antibacterial property of these composites depends on the ability of trapped silver to release ions (Rivero et al., 2011). Initially silver NPs of ~5 nm size were embedded into dextran to enhance the radiotherapy efficacy for killing the tumor cells (Coll Ferrer et al., 2013; Lu et al., 2012). However, the cytotoxic effect of silver NPs limits its bactericidal activity and to reduce the exposure, the concept of hybrid nanogels (NGs) has been emerged. A recent study has reported the development of comparatively inert but biocompatible NGs containing lysozyme core and dextral cells along with silver NPs (Ferrer et al., 2013). The aim of such product is to encapsulate the NPs with NG to prevent there uptake by somatic cells. The degradable and biocompatible nature of natural polymers such as dextran and lysozyme make such NGs more acceptable to cells. Moreover, another advantage of such polymers is that they can be easily processed in aqueous media without addition of any harmful chemicals (Ferrer et al., 2014).

To reduce the bacterial infections from commonly used medical devices, the coating of device surface with Ag-NPs or development of certain devices with the polymers impregnated with NPs is commonly employed (Abou El-Nour et al., 2010). These NPs kill the bacterial cells through two ways (1) they can penetrate the bacterial cells (Morones et al., 2005) or (2) deposited on cell wall thereby modulate the membrane permeability of bacterial cells and alter the membrane transport mechanisms (Abou El-Nour et al., 2010). The Ag-NPs interact with the membrane proteins of bacterial cells and simultaneously activated the signaling cascades and ultimately stalled the cellular proliferation (McShan et al., 2014; Brzóska et al., 2015). In the year of 1998, for the first-time silver impregnated hydrogel coating was used for venous catheter (Gatter et al., 1998) to prevent the onset of catheter-related infections (McShan et al., 2014). However, such system failed to provide the long-lasting protection. Generally, the planktonic bacteria initiate the biofilm formation on the surface of device. Such bacterial contamination often evokes further complications such as inflammation, oral infections, implant loosening, or even detachment (Maitz et al., 2013). Recently, the Ag-NPs impregnated within swollen hydrogel networks have been used for catheter coating (Thomas et al., 2007; Stevens et al., 2011). The antibacterial functionalization of biomaterials often compromises the hemocompatibility parameter because silver ions show undesirable adverse effect on

mammalian cells (Thomas et al., 2007; Stevens et al., 2011). To overcome such limitation, in a recent study the star-shaped poly(ethylene glycol) (PEG)−heparin has been coated on thermoplastic polyurethane and incubated with $AgNO_3$ solution (Maitz et al., 2013). PEG−heparin coating is widely used for modification of biomaterial surface due to their hemocompatibility and anticoagulation activity and such coating is covalently linked with the biomaterial core. Fig. 6.3 shows the hemocompatibility of heparin-loaded hydrogel. Further modulation has been employed dual coating on polyurethane surface in which the internal coating contained the silver-loaded hydrogels, whereas the outer coating contained silver free hydrogels, which provide diffusion barrier from excessive release of silver ions and prevent direct interaction with coating-blood interface (Maitz et al., 2013).

Due to lack of antibacterial activity, it limits the application of otherwise suitable biomaterials with preferable properties for tissue engineering applications. For example, polyetheretherketone (PEEK) is widely used as biomaterial for fabrication of dental implants, however, lack of bactericidal effect limits its applications. It has been observed that lack of antibacterial properties is an important reason behind the failure of implants (Mouhyi et al., 2012). The PEEK composite containing nanofluorohydroxyapatite facilitates the antibacterial activity of polymers along with the osseointegration property of original materials (Kurtz and Devine, 2007; Rivard et al., 2002). Further to enhance the bioactivity, hybrid materials have been developed by incorporating the hydroxyapatite in PEEK (Sun et al., 2013). To improve the antibacterial property, some research groups have employed Ag-doping or Ag-contained HA coating on the surface of PEEK implant (Wang et al., 2014a; Kakinuma et al., 2015).

6.2.4 Stimuli-responsive properties (pH, temperature, and electroactive)

Surface reconstruction of polymer is essential to improve its biological responsiveness. However, reconstruction process needs long duration, which may cause the migration of polymers constituents to surface from bulk (Koberstein, 2004). Rapid response can be achieved through stimuli-responsive thin polymer coating. Such coating method involves grafting of macromolecules on surface of bulk polymers (Luzinov et al., 2008). For development of adaptive and responsive interface, the “grafting to” (Luzinov et al., 2008) and “grafting from” (Santer et al., 2006; Abu-Lail et al., 2006) approaches have been employed for reversible switching by external stimuli. The nanostructured thin network films bring the opportunity to exploit the stimuli-responsive properties for tissue engineering applications. In comparison to the bulk gel, such thin gel network posses faster swelling and shrinking kinetics in response to the external environment (Tanaka and Fillmore, 1979). These polymer gels exhibit anisotropic swelling behavior and volumetric expansion occurs in the perpendicular direction of the substrate plane (Toomey et al., 2004). Crowe and Genzer (Crowe and Genzer, 2005) have demonstrated that chemical grafting on poly(vinylmethylsiloxane) network with alkanethiols can modulate the response time of polymer network. Due to their ability to undergo dynamic changes in response

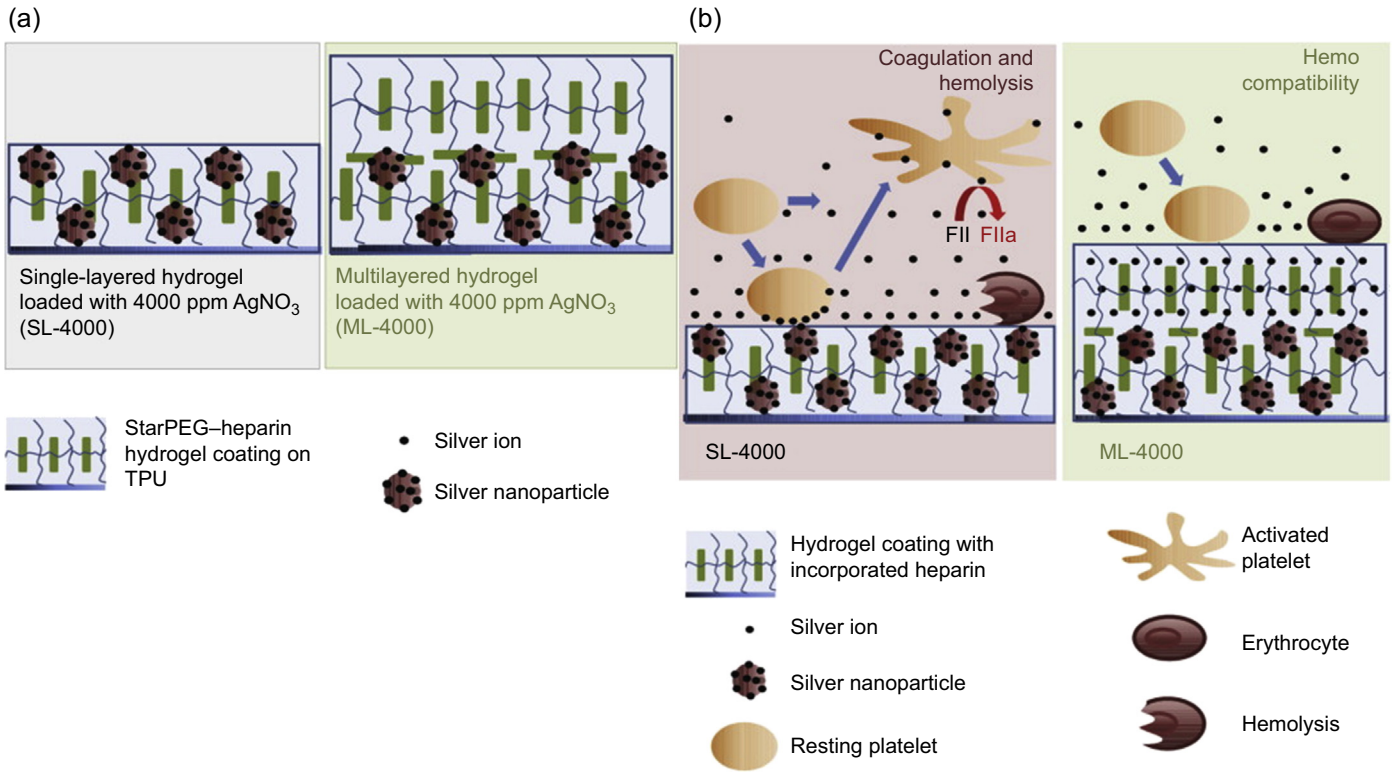

Figure 6.3 Shows (a) multifunctional coating with hydrogel (b) hemocompatibility of hydrogel-containing heparin (Fischer et al., 2015).

to the changing environment of biological system, these gels are applicable to numbers of biotechnological fields (Bajpai et al., 2008). There are several advantages regarding the stimuli-responsive polymers including (1) it helps in switching the adhesion between the polymers, proteins, and cells, which helps in bioseparation and tissue engineering (Lutolf et al., 2003); (2) possibility of certain functional moieties being exposed or masked at the biointerface, which may facilitate to modulate the regulatory signaling cascades for controlling cellular biomolecular activity (Hayashi et al., 2007); (3) facilitates the dynamic transport of chemicals through nanoporous membrane (Tokarev et al., 2009; Lue et al., 2008). Ebara et al. (2004) have studied the stimulus-responsive poly(*N*-isopropylacrylamide) (pNIPAAM) for controlling the RGD sequence for controlling cellular binding. Stimuli-responsive polymers also used in drug delivery through macro-/nanomembrane based on the membranous pore size, molecular weight of polymer, grafting density, etc. (Lue et al., 2008; Wong et al., 2009). The stimuli-responsive polymers (light, pH, and temperature responsive) are also reported to apply in macro- and nanoactuation (Edmondson et al., 2006). There is broad scope of application of stimuli-responsive polymers for in vivo drug delivery system. For example, the pH-responsive polymers can be used in vivo because the different location of human body exhibits substantial change in pH during normal physiological condition and pathological manifestations. Nanocapsule-based delivery systems can be used for storing and sustained drug release at target site. In this direction for enhancing the efficacy of chemotherapy, the stimuli-responsive polymer-based carrier system has been used, which can sense the alteration in pH, glutathione concentration, or presence of specific enzyme at the cellular vicinity and therefore release the drug molecules accordingly. Micelle such as NPs composed of polymers and proteolytic enzymes can reach up to submicron level into the living system. In similar study, linear dendritic copolymers such as PEO and enzyme such as polylysine or polyester dendron has been applied for pH-sensitive NP micelles in drug delivery in vivo (Gillies et al., 2004). It has been reported that in micelles containing the proteolytic enzyme, electrostatic force holds the nanoparticle−enzyme assembly together (Lee et al., 2008). Bae et al. (Lee et al., 2008) have developed virus-resembling NGs of drug-in-polymer core encapsulated by folate-conjugated PEO−albumin double shells and such structures have reported to bind the cellular receptor complex efficiently (Stuart et al., 2010). The stimuli-responsive polymers and their application are presented in Fig. 6.3. The pH-responsive polymer-based drug delivery systems are commonly used for different physiological locations that trigger the pH response, including the gastrointestinal tract (Fallingborg, 1999), blood vessels, intracellular vesicles (Steichen et al., 2013), vagina (Lang, 1955), or at tumor microenvironment and inflammatory site and wound bed (Dissemond et al., 2003). The presence of ionizable pendant groups at polymer backbone modulates the pH-responsive behavior of polymers (Gupta et al., 2002). In presence of aqueous media with appropriate pH, the pendant groups are ionized and formed a fixed charge along the polymers. Such electrostatic repulsive charge decides the swelling or deswelling process of the polymer network (Gupta et al., 2002; Sharpe et al., 2014). In anionic pH-responsive hydrogels, the pendant group ionized at pH higher in comparison to acidic dissociation constant (pKa) wherein in

cationic type, the pendant group is ionized at pH lesser than the pKa value. Several synthetic polymers such as methacrylic acid (MAA), dimethylaminoethyl methacrylate, AAm (Lowman and Peppas, 1999), etc., and natural polymers such as albumin, alginate, chitosan, gelatin (Kim et al., 2000) can exhibit the pH-responsive behavior. The isoelectric point (pI) defines the swelling behavior of pH-responsive polymers. Generally, if the pH value of the solution differs from pI, the protein will acquire the electrostatic repulsion that results swelling of the network (Gupta et al., 2002). The natural pH-responsive polymers are advantageous in comparison to the synthetic polymers because the natural polymers are biodegradable in nature, which make them ideal for circulating drug delivery carrier or implant materials (Schmaljohann, 2006). Other than drugs, the pH-responsive materials have also been used for delivery of proteins, small molecules, genetic materials, chemotherapeutics (Schoener et al., 2013; Lowman et al., 1999; Peppas et al., 2004), etc. Peppas et al. have developed anionic hydrogel consisting MAA backbone along with grafted PEG for oral delivery in small intestine. The pendant group of MAA undergoes protonation and deprotonation based on the pH value and control the swelling behavior, whereas the hydrophilic PEG increases the rate of water imbibitions (Bell and Peppas, 1996). John Klier et al. (1990) have shown oral delivery of protein through P(MAA-g-EG) hydrogel network. Some mucoadhesive molecules such as wheat germ agglutinin, etc., have been incorporated into the hydrogel network of P(MAA-g-EG) (Wood et al., 2008) and used for insulin delivery (López and Peppas, 2004), therapeutic molecules such as interferon-β (Kamei et al., 2009) and calcitonin (Torres-Lugo and Peppas, 2000), etc., composite of P(MAA-g-EG) and hydrophilic copolymer *N*-vinyl pyrrolidone (NVP) has been developed by Carr et al. (Carr and Peppas, 2009) in which NVP exhibits mucoadhesion property and minimal toxicity.

In addition to synthetic polymers, natural polymer such as alginate shows the pH-dependent swelling/deswelling behavior (Chen et al., 2004). Calcium cross-linked alginate beads are used for oral delivery of vaccine or small molecules through the oral route and alginate encapsulation is able to withstand in the GI tract low pH environment and deliver the molecules at the target site (Romalde et al., 2004). Recently, chitosan derivate of hydrogel network, NOCC, has been developed for oral drug delivery. In a composite of NOCC and alginate, both the materials exhibit favorable charge interactions and can be cross-linked through natural cross-linker genipin (Chen et al., 2004). The model experiment for delivery of bovine serum albumin encapsulated through such composite has been successful implemented, which depicted that the only pH change can trigger the release of cargo through hydrogel network (Koetting et al., 2015).

The thermoresponsive polymer (Schmaljohann, 2006), pNIPAAm, has received and been investigated due to its lower critical solution temperature (LCST) at 32°C close to physiological temperature (Schild and Tirrel, 1990; Tacheuchi et al., 1993). The LCST can be modified by incorporating hydrophilic/hydrophobic moieties (Wata et al., 1991). Besides other properties, the porosity of hydrogel network is an important phenomenon, which can be controlled through changing cross-linking density and their affinity toward aqueous media. Thus based on this property, the release of drug from polymer network can be controlled (Hoare and Kohane, 2008). Smart

hydrogel-containing NPs exhibit stimuli-responsive behavior (Chen et al., 2004; Koetting et al., 2015) and in addition of pH, temperature, or other chemical cues, magnetic field, light, etc., can modulate the responsiveness of polymer network and facilitate in remotely controlled drug delivery (Zhao et al., 2011). NPs developed from melanin showed UV-induced photothermal heating (Ninh et al., 2014). Composite containing such NPs dispersed within heat degradable hydrogels can easily be disintegrated under the UV radiation. In terms of in vivo application, the near-infrared (NIR) light is comparatively safer in comparison with UV light and NIR can penetrate deeper inside soft tissue without any adverse effect (Satarkar and Hilt, 2008). Keeping this fact in mind, gold nanorod−doped thermoresponsive polymer network (viz. glycidylmethacrylated chitooligosaccharide, methoxylpoly(ethylene glycol)-poly(ε-caprolactone)-acryloyl chloride, *N*-isopropylacrylamide, etc.) has been developed, which can release the drug under NIR irradiation (Qu et al., 2015). Likewise, the heat producing ability of superparamagnetic particles has been explored for "on demand" drug release in which the particles dissipated local heat under the application of alternating magnetic field (Liu et al., 2008). In one of such study iron oxide (Fe_3O_4), NPs were dispersed into NIPAAm-based matrix and under continuous AFM the drug release was found to be increased, whereas under pulse AFM the drug release profile showed pulsatile behavior (Qu et al., 2015).

6.3 Particle−hydrogel interactions

From the above discussion, it is now clear that the particle-loaded gels will have a wide range of applications in the field of biotechnology novel diagnostics, implantable devices, and consumer products. The entrapped particles inside polymer network may differ in their size from micro- to nanodepending on the specific application types. In this regard, understanding the interaction of particles with polymer network is important for exploring the novel application area. It has been observed that the nanoparticles posses synergistic and hybrid properties, which make them a suitable choice for drug delivery system. Moreover, the loading requirement in NPs is comparatively low than the traditional additives. The entrapped particles show light scattering property, which reduce the light transmittance and thus the optical clarity. Efficient dispersion of particles requires optimum interfacial interaction with polymer network. Recent trend has focused on development of carbon nanotubes due to their inherent conductivity even at very low loading dose and also able to improve the matrix electrical or mechanical and properties (Bernholc et al., 2002). By tailoring the surface properties of nanotubes, their interaction with polymer matrix can be modulated (Sinnott, 2002).

Effective dispersion of particles in polymer matrices is an important parameter. It has been observed that polymeric film containing oriented carbon nanotubes employed shear orientation at nanoscale level (Poulin et al., 2002). Preferential orientation is obtained by flow-induced alignment of nanotubes in network (Vigolo et al., 2000). The degree of shear orientation can be determined through Raman spectroscopic method (Frogley et al., 2002). Morphological features of hydrogel depend on the composite particles and it may differ with hard inorganic particles to soft

polymeric particles. The NPs are reported to modulate the glass transition of polymers (Kobayashi et al., 2002) along with the alteration in phase behavior of composite blend (Karim et al., 2002). The colloidal and rheological properties of impregnated particles have received considerable attention (Abou El-Nour et al., 2010). The transition of polymer from liquid hexagonal to lamellar phase has been studied on Pluronic block copolymer and clay (Castelletto et al., 2003). Lal and Auvray (2001) have studied the adsorption of PEO on Laponite clay at low concentration through small-angle neutron scattering method. In two separate studies, Smalley et al. (2001) and Swenson et al. (2001) employed the neutron diffraction method for understanding the interlayer and ordered structure of polymers around each clay platelet.

Particle−hydrogel interactions depend on several factors. The interfacial bonding between them depends on morphology, particle types, polymer types, surfactant, and processing methods (VanderHart et al., 2001; Tanaka and Goettler, 2002). The particle−hydrogel adhesive force is measured through atomic force microscopy (AFM). In an experimental study, the mica particles were coated with three different polymer types and three nanoplatelets on colloidal probes. Force at the polymer−particle interface was measured by colloidal−probe technique, which showed that two configuration probes coated with nanoplatelets and substrate coated with polymer produced less scatter in comparison to the opposite configuration. The imperfection in solid surface was identified as the reason behind the scatter in measured force adhesion value (Chung and Amo, 2011). To enhance the compatibility and affinity to polymer matrices, the surface of metal NPs is modified by passivation in which appropriate organic groups are attached on polymer surface, which alter the affinity of NPs toward either polar or nonpolar solvent (Rozenberg and Tenne, 2008). This aspect is particularly important for composite preparation and homogenous dispersion of NPs on polymer matrices. Homogeneous distribution enhances the mechanical and optical properties of composite (Nicolais and Carotenuto, 2005). Nonhomogenous distribution of NPs in aggregated form is exhibited repulsive electrostatic interaction with polymer (Rozenberg and Tenne, 2008). The homogeneous and nonhomogeneous distribution of NPs shows different optical properties. In homogenously dispersed NPs in dielectric polymer matrices, the conducting electrons collectively oscillate along the transversal direction of electromagnetic field, which can be characterized by single surface plasmon absorption in UV−Vis region (Bohren and Huffman, 2007). On the other hand, in aggregated system, electron oscillation takes place in both longitudinal and transverse directions of electromagnetic field. The dipole−dipole interactions between such NPs result in broadening of the extinction spectrum. The broadening of the peck depends on the size and shape of aggregates (Longo et al., 2011).

The major limiting factors in drug delivery system are poor bioavailability, limited effectiveness, lack of selectivity, and side effects. The aim of sustained delivery is to prevent rapid degradation and thus it may enhance the concentration at the target site. Such system is especially applicable where discrepancy between drug concentration and therapeutic or toxic effect exist. Size reduction of formulation proves effective in drug delivery system, and different types of nanostructures have been developed by size reduction method, which poses unique physicochemical and biological

characteristics. Several advanced microscopic systems, including scanning electron microscopy (SEM), AFM, and transmission electron microscopy (TEM), are useful for characterization of size distribution, morphology, and surface properties of NPs (Bhatia, 2016). In particle size characterization, the size, morphology, and distribution of particles are evaluated through electron microscopy. The size of NPs has profound effect on drug release profile. Generally, the smaller particle size exhibits larger surface area and faster drug release. In smaller particle, however, the aggregation may occur during storage and transportation (Redhead et al., 2001). Moreover, the particle size also affects the degradation rate of polymer. It has been observed that larger particle size causes faster degradation of polymer matrix in physiological system (Betancor and Luckarift, 2008). Other than electron microscopy, other methods for particle size determination are photon correlation spectroscopy and dynamic light scattering are used to determine the size of particles in nano- and submicron ranges. Brownian motion of particles causes Doppler shift when exposed under monochromatic light and based on this concept the size distribution of colloidal particles can be determined (de Assis et al., 2008). SEM helps in the direct visualization of particles and also provides morphological information. However, this method is not efficient enough to provide the size distribution and population average. For SEM analysis, suitable sample preparation is required and samples are maintained under vacuum during measurement. Fine beam of electron focused on samples and the secondary electron emitted from samples provides the surface characteristics of particles (Jores et al., 2004).

TEM is another technique required for analysis of structures. TEM provides the information regarding size, diffraction, and spectroscopic information with sufficient spatial resolution. TEM in combination with other advanced techniques, such as nanodiffraction, nanometer resolution X-ray energy-dispersive spectroscopy, etc., provides fundamental understanding regarding the nanoscience. AFM provides information about the surface properties of study samples (zur Mühlen et al., 1996). In AFM, the samples are scanned either in contact or noncontact mode. In contact mode the topographical map of sample is generated by tapping the surface. AFM is also able to acquire image even for the nonconducting samples even without any specific treatment (Shi et al., 2003). Zeta potential is an important feature for stability of colloidal particles. Surface charge of particles defines the interaction between polymer and dispersed particles. It has been observed that the high zeta potential prevents the particle aggregation and enhanced their stability. It is also helpful in evaluating surface property such as hydrophobicity and nature of materials used for coating or encapsulation (Otsuka et al., 2003). Sophisticated methods such as X-ray photon correlation spectroscopy facilitate in determining the surface hydrophobicity and identification of chemical groups on the surface of NPs (Scholes et al., 1999). After delivery of drug at the target site, the supersmall NPs are excreted through renal system through endocytosis. To evaluate whether the excretion process interacts with the renal system or not, fluorescent tagged dextran-based NPs were injected in mice model. The result demonstrated both filtered and internalization by renal cells in dose and time-dependent manner. The effect was analyzed through immunohistological methods for observing specific biomarkers (Nair et al., 2015).

6.4 Biomedical applications of microparticle-loaded gels

Microparticle-loaded hydrogels are commonly used in diagnostics, tissue engineering, and drug delivery system (Eun et al., 2014; Eng et al., 2013; Pregibon et al., 2007). Multifunctional microparticles are usually introduced in living cells, which helps in large-scale culture of anchorage-dependent cells, production of antibodies, stem cell−related components, etc. (Wang et al., 2014b). Microparticles incorporated living cells are also used in development of self-assembled tissues (Du et al., 2010). For clonogenic screening, cell sorting, and understanding the cell microenvironment interaction, the cell-adhesive microparticles have been tested (Shah et al., 2014). Emerging technology such as stop flow lithography has been employed for production of hydrogel microparticles with complex pattern (Bong et al., 2009, 2012; Dendukuri et al., 2006). However, the compatibility of microparticles with in vitro cells is yet not established. Few recent studies have indicated that the PEG particles prepared by this method are repellent for cellular adhesion (Bong et al., 2015).

On the other hand, microparticles (MPs)−loaded gels (microgel) exhibit excellent biocompatibility due to combination of hydrophilic network and structural features of colloidal particles (Das et al., 2006; Gaulding et al., 2013; Abdilla et al., 2016). Application and compatibility of microgels depends on several factor, including size of particles, their distribution, composition and functionality. Droplet-based microfluidics is an emerging technique for development of microgels (Kumacheva and Garstecki, 2011) and the droplet generation can be easily controlled by different cross-linking mechanisms (Tumarkin and Kumacheva, 2009). Traditional cross-linking mechanisms with radical polymerization or glutaraldehyde treatment (Tumarkin and Kumacheva, 2009; Utech et al., 2015) often show detrimental effect on biological environment. To overcome this issue, presently novel mild and efficient cross-linking methods have been adopted. In contrast to commonly employed covalent cross-linking, the physical gelation provides more dynamic network formation. Physical cross-linking of natural polymers such as alginate, chitosan, hyaluronic acid with charged homopolymers or multivalent ions (Zhou et al., 2007) provides a mild way to encapsulate biological cargo (Kim et al., 2014). Due to their compositional variation and inhomogeneous distribution pattern of ionic cross-linker, the hydrogel network can selectively tune the inherent characteristics and thus release of cargo (Sikorski et al., 2007). Recently, such cross-linking concept has also been applied for synthetic polymers. The synthetic network is advantageous than that of natural polymers in terms of tunable internal structure (Wang et al., 2016a). Alginate-loaded dendritic cell vaccination has been depicted in Fig. 6.4.

Alginic acid and its derivatives have widely studied due to their excellent cytocompatibility, biodegradability, chemical versatility, and sol−gel transition property (Lee and Mooney, 2012). Application of alginate for development of micro- and nanodrug delivery system has been extensively evaluated (Swain et al., 2012). Incorporation of alginate microparticles in polymer network can be performed through number of methods such as emulsification, atomization or complete formation with counterion polymers (Builders et al., 2008; Saravanan and Rao, 2010; Huang

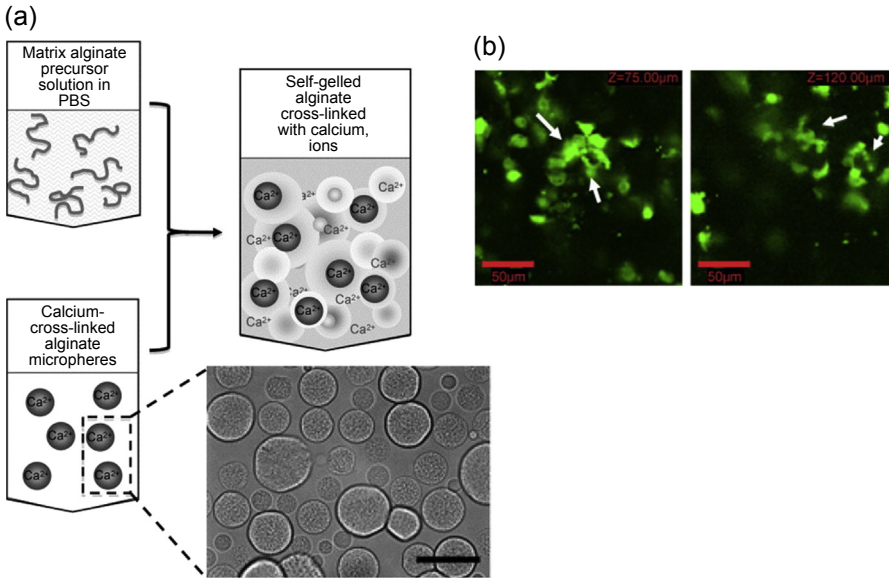

Figure 6.4 Represents (a) calcium-loaded alginate microspheres (b) activated dendritic cells encapsulated in alginate spheres (Hori et al., 2008).

et al., 2011). For fabrication of MPs−hydrogel network, commonly employed methods are spray-drying, inkjet/drying process, impinging aerosols (Suksamran et al., 2009; Iwanaga et al., 2013), etc., to enhance the mechanical stability and control the swelling behavior the alginate microparticles are coated with chitosan or poly-L-lysine (Matricardi et al., 2008; Urbanska et al., 2012). Coppi et al. (2004) developed ALG microparticles (<3 μm) by spray-drying method, and their uptake by M cells of the Peyer's patches has been investigated. Recently, Urbanska et al. (2012) encapsulated antitumoral drug within chitosan-coated alginate microparticles for treatment of colorectal cancer and their result exhibited such particles significantly prolonged the survival of mice when tested on in vivo model. Mladenovska et al. (2007) have developed 5-aminosalicylic acid−loaded alginate MPs and coated with chitosan for treatment of inflammatory bowel disease. Tetanus toxoid−encapsulated MPs are reported to develop through emulsification technique used for mucosal immunization (Tafaghodi et al., 2006). This fabrication method produces the MPs of ~1.34 μm diameter and low surface porosity. To improve the mechanical stability, alginate is blended with polycationic polymers such as chitosan, gelatin, pectin, etc., using the same strategy; Jaya et al. (2009) have used pectin−alginate microspheres for delivery of aspirin. The release profile was controlled over a wide pH range between 1.2 and 8.2, which covers the conditions of gastrointestinal tract. Moreover, the release rate of drugs from this polymer composite can be tuned by altering the ratio of pectin. Alginate−polysaccharide composites have been explored by Ramesh Babu et al. (2007) in which ALG/methylcellulose microparticles have been developed by water-in-oil emulsion method for delivery of nifedipine. Blended microbeads of

alginate and sodium carboxymethyl cellulose with chitosan coating were developed Angadi et al. for controlled release of amoxicillin in the stomach (Ghosh et al., 2012). Chitosan coating on this polymer composite enhanced the encapsulation efficiency, reduced the burst effect, and improved the sustained release for more than 8 h under the range of acidic pH environment. Mennini et al. (2012) have developed remedies for arthritis by incorporating celecoxib−cyclodextrin−polyvinylpyrrolidone into alginate/chitosan microspheres. Valdecoxib as cyclooxygenase-2 enzyme inhibitor was also impregnate into alginate and Eudragit S100 for colonic release (Thakral et al., 2011). Recently, pH and thermoresponsive MPs of alginate and poly(*N*-isopropylacrylamide)—guar gum have been reported to develop through emulsion and chemical cross-linking method for encapsulation of antituberculosis drug isoniazid (Kajjari et al., 2012). Experiment showed sustained release profile for 12 h and with environmental pH strongly modulates the nature of release. To reduce the burst effect, alginate MPs are embedded into hydrogel structure. In this direction, Zhu et al. (2011) used alginate spheres for encapsulation of natural drug berberine hydrochloride using emulsification/gelation method and finally entrapped within carboxymethyl chitosan hydrogels. Incorporated MPs have increased the compression strength of hydrogel. In 1980, Lim and Sun first demonstrated alginate microcapsules for cellular encapsulation. Afterward, Yannas et al. (1989) developed burn dressing incorporating collagen and cartilage isolated from shark in the polymer network. Gelatin MPs are studied for delivery of large bioactive molecules and amplification of cells. However, the NPs of gelatin are commonly used for drug delivery in different target region, including the brain. The large surface area of MPs is advantageous for sufficient exchange of nutrients and metabolic wastes (Tan et al., 2009). The mechanical properties of such MPs although poor in aqueous media, which accelerate the release of drugs, however, to overcome such limitation, different cross-linkers such as formaldehyde, genipin, gluteraldehyde (Dinarvand et al., 2010) are commonly used. Solorio et al. used gelatin microspheres for development of self-assemble system for delivery of TGF-b1 in mesenchymal stem cells (hMSCs) culture. Other biomarkers, such as VEGF, BMP-2, are also delivered through gelatin MPs and their release kinetics can be controlled through modulating the cross-linking density (Patel et al., 2008). Gelatin MPs are also used in delivery of drug in pulmonary system and Rifampicin and isoniazid are encapsulated in gelatin spheres for treatment of tuberculosis (Manca et al., 2013). The common limitation of anticancer drugs is poor bioavailability and high effective dose (ED50) index. Researchers are relentlessly searching novel strategy for sufficient drug uptake by tumor cells without affecting the surrounding healthy cells. One of such strategy includes sterically stabilized gelatin microassemblies containing anticancer drug noscapine and in vitro release of this drug showed natal burst release followed by sustained delivery. The result demonstrated the gelatin self-assembly was remarkably extended the drug release along with threefold lower IC50 in comparison to free noscapine (Madan et al., 2013). Propolis is commonly used in development of wound dressing, tissue engineering, rheumatism and sprains, periodontal diseases, influenza, and cold (Lue et al., 2008) etc. Gelatin microspheres loaded with propolis prepared by spray-drying method have been studied for drug delivery system (Bruschi et al., 2003).

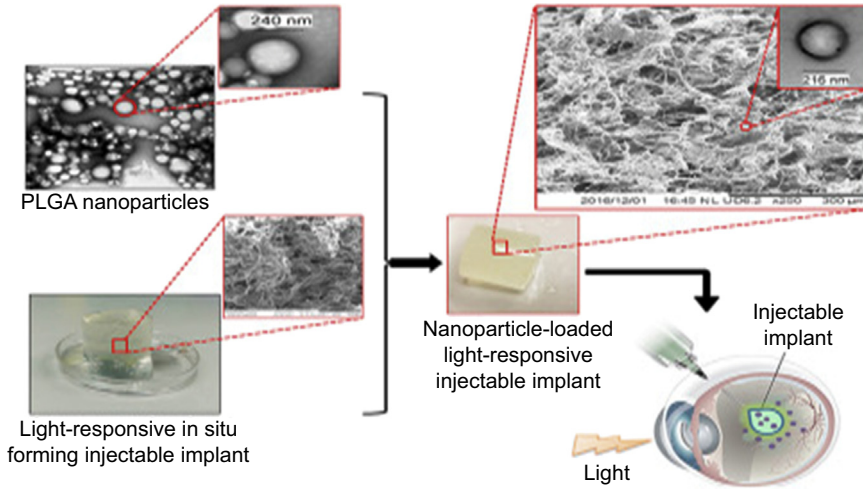

Figure 6.5 Shows the in situ application of poly(lactic-co-glycolic acid) (PLGA) nanoparticle−loaded stimuli-responsive polymer (Bisht et al., 2017).

Chitosan core−coated microspheres have been developed by Lorenzo-Lamosa et al. for colonic drug delivery system. Such microspheres were suitable for sustained drug release over a period of time at 7.4 pH (Chen et al., 2004). The solubility of chitosan can be improved by the process of phosphorylation. The application of phosphorylated chitosan for development of pH-sensitive gel has been explored by Win et al. (2005) for release of drug in gastrointestinal fluid.

Simvastatin−chitosan MPs into polyvinyl alcohol (PVA) have been developed for encapsulation of drug for prolonged release in physiological condition. In this process, the ratio of chitosan and surfactant was varied for optimizing the encapsulation efficiency and drug−polymer interaction (Wang et al., 2016b). Besides polymer, the MPs of several anticancer drugs such as doxorubicin and 5-fluoro uracil were reported for deliver in affected organs (Kumar et al., 2011). In addition to drug delivery, MPs are also commercially available for diagnosis, analysis, gene delivery, adjuvant therapy (Park and Yeo, 2006). To improve the process of neovascularization, the intravitreous injection poly(lactic-co-glycolic) acid (PLGA) MPs encapsulated with ranibizumab have been developed for sustained drug release. The coaxial electrospray method was used for fabrication of MPs and such method has been found advantageous due to high encapsulation rate of drug molecules and lower bioactivity (Zhang et al., 2015). Fig. 6.5 shows the in situ applications of stimuli-responsive PLGA NPs.

6.5 Biomedical applications of nanoparticle-loaded gels

The nanoparticle-loaded gels have a wide range of applications in biomedical engineering (Aryal et al., 2011). For drug delivery, NPs are advantageous in terms of high drug loading capacity, controlled, and sustained release of drug molecules,

prolonged stability, etc. For controlled drug delivery and to enhance the therapeutic index, the NP−hydrogel composites have been developed (Davis et al., 2008; Blanco et al., 2015). The NP−hydrogel composites have a wide range of application in the field of biomedical and biotechnology. For drug delivery NP composite gels can be used for (1) passively controlled drug delivery, (2) stimuli-responsive drug delivery, (3) site-specific delivery, and (4) detoxification (Baumann et al., 2010). In medical imaging, nanoparticles have been incorporated into polymer network like cellulose. Range of nanoparticle-loaded polymers such as polyurethanes, pHEMA, commercial poly(ethylene glycol)-co-poly(ethylene oxide) (Pluronic), etc., has been widely used in drug delivery (Ramos et al., 2014; Wolfbeis, 2015). The main problem in drug delivery is poor solubility and limited bioavailability of most of the drug molecules. To overcome these limitations, different drug carrier systems have been developed (Hommoss, 2008) and in this direction various nanodispersed systems such as nanoemulsion, liposomes, lipid NP, etc., are gaining importance (Jain, 2001). The nanoparticle vehicles have initiated in the year of 1990 when solid lipid nanoparticles (SLNs) were used as an alternate of traditional carrier such as emulsion, liposome, etc. (Hirlekar et al., 2011). In comparison to all these carriers, the SLNs have enhanced bioavailability, target-specific delivery system, and ease to production in large scale. Uner et al. (Üner and Yener, 2007) reported that the SLN-based system for topical administration of drugs. However, the SLN suffers from limitations such as limited drug loading, drug leakage, and gelation during storage (Üner and Yener, 2007). To overcome such problems, the new generation lipid system such as nanostructured lipid carriers (NLCs) has been developed, which consist mixture of different lipid molecules such as solid lipids blended in oil. The unique feature of such mixture is that the melting point of lipid particles is less in comparison to original solid lipids; however, the matrix can maintain the solid state at normal physiological temperature Müller et al. (1997). Both SLN and NLC are composed of biodegradable lipid carriers and used for controlled delivery through oral route. For topic application, such systems provide additional advantage by creating film on the skin surface, which facilitate the transepidermal loss of water and enhance the penetration of drugs through stratum corneum (Saupe et al., 2005). Müller et al. have shown that occlusion of these carriers depends on the particle size of lipid molecules. It has been reported that, smaller lipid molecules remain in closer contact with stratum corneum and enhance the drug penetration through mucosa or skin. In NLC formulation, besides the solid lipid components by alternating the liquid lipid amount, the drug release profile can be modulated. Hence by changing the composition, it is possible to control the drug release for prolonged period of time and the concentration of delivered drugs can also be controlled especially if for those drugs that produce allergic reactions at high concentration (Uprit et al., 2013).

The P(MAA-co-NVP) is an important pH-responsive polymer composite that exhibits anionic swelling behavior necessary for oral delivery of drugs. At lower pH, such composites are more tightly collapsed. For insulin delivery, this composite system exhibits no delivery of insulin at low pH, whereas it starts releasing insulin at neutral pH. Moreover, such composite nanoparticles do not evoke any cytotoxicity when studied on in vitro model (Koetting et al., 2015). By exploiting the pH

responsiveness, the nanoparticle-based sensor has reported to use in continuous monitoring of pH level in bacterial culture. In agar film, such nanosensor is incorporated into agar films and by monitoring the change in pH value the growth and proliferation of bacterial cells can also be monitored. Recently, dually responding nanoparticles capable of measuring the oxygen and pH of cytosol have been reported by the study group. Such sensor is developed from organic−inorganic composites (Pluronic reinforced with silica) (Wolfbeis, 2015). Combinations of hydrogels with NPs (metal, nonmetal, polymeric moiety, etc.) provide improved functionality and can be improved in biosensing, nanomedicines, catalyst, etc. Hydrogel−NP composites show plurality in property enhancement of each component like improved mechanical strength of polymers and decreased NPs aggregation. Wichterle and Lim (1960) for the first time reported the swelling and deswelling of hydrogels in response to the alteration of microenvironment. In silica NP−hydrogel silica, NPs are loaded with polyethylene glycol have demonstrated excellent cellular adhesion and biocompatibility in comparison to the hydrogel without NP impregnation (Liu et al., 2014). Silver (Ag) NPs have antibacterial property and widely used in dental filling, wound dressing, etc. Ag-NP−hydrogel composites facilitate in sustained bactericidal activities. Due to this property, such composites are commonly employed for providing functional coating for various applications (Nair and Laurencin, 2007). Various research groups have explored the preparation of Ag-NPs impregnated hydrogels with a range of synthetic polymers, including polyacrylic acid (PAA), PAAm, methyl methacrylate, PVA, etc. (Bardajee et al., 2012; Juby et al., 2012). Recently, the trend has been shifted toward utilizing natural polymers such as chitosan, gum acacia, gelatin to develop biocompatible composite for development of implantable dressings (Bardajee et al., 2012; Juby et al., 2012). Sustained release of NPs reduces the need of frequent removal of dressing. In an experimental study, Tokarev et al. (2010) reported that Ag-NPs able to enhance the activity of surface plasmon resonance−based sensors. Ag-NPs and pH-responsive hydrogels composite along with the enzyme glucose oxidase can monitor glucose concentration. Incorporation of Ag-NPs is able to enhance the electrical conductivity of the hydrogel network. The concentration of Ag^+ ions precursor and swelling−deswelling ratio of hydrogel can modulate the conductivity parameter. For example, high Ag^+ ions concentration facilitates in better conductivity but reduced the swelling ratios (Tokarev et al., 2010). However, recent study has exhibited that incorporation of Ag nanowires rather than Ag-NPs may modulate the hydrogel conductivity without altering the swelling ratio of hydrogel (Abdel-Halim and Al-Deyab, 2014). The external stimuli such as temperature or pH can alter the interparticle distance of such composites and thus affect the conductivity of hydrogel.

In the similar way, several researches have been conducted regarding the efficacy of gold NPs (Au NPs) in biomedical applications (Wang et al., 2011). Although the Au NPs are bit costlier in comparison with Ag-NPs, but Au NPs incorporated temperature-sensitive hydrogels have a wide range of applications. Irradiation of light at the plasmonic peak of Au produces localized enhancement of temperature, and these phenomena are applicable in remote-controlled drug delivery. By reaching the LCST of hydrogel, the network structure is started collapsing with burst release of

impregnated drugs within it. Shiotani et al. (Wang et al., 2011) have explored this concept and demonstrated that fast and reversible shrinking and reswelling of Au nanorod−NIPAAm composite with heat generation within polymer matrix. Based on the thermal response of Au NPs, the NP−hydrogel thermoresponsive composites have also applied in fabrication of remote-controlled microfluidics valves. The working of these valves depends on the excitation wavelength of irradiated light and plasmonic peak of Au NPs. For example, irradiation with 532 nm wavelength matches the plasmonic peaks of Au NPs with diameter 3−10 nm and the hydrogel network collapse at this wavelength with opening of the valve. However, if the hydrogel contains NPs with different plasmonic peaks, the valve remains unaffected at this excitation wavelength (Sershen et al., 2005). Besides Ag or Au NPs, other metallic or magnetic NP−hydrogel composites have also been reported. Platinum NPs in an amphiphile hydrogels were found to act as catalyst for p-nitroaniline hydrogenation. Copper (Cu) NPs can be used as alternate for Au or Ag-NPs and in a study by Cometa and colleague have shown the antibacterial efficiency of Cu-NP-poly(ethylene glycol diacrylate) hydrogel. Magnetic NPs made from cobalt (Co) or nickel (Ni) have incorporated in hydrogels and they function to generate magnetic field driven actuator, which applicable to biomimetic muscle (Stoppato et al., 2013; Maity et al., 2013). The bimetallic NPs provide nanocomposite hybrid materials. Like bimetallic Fe−Co, NPs were reported to form composite with PAAm hydrogel that can be applied for waste removal. Polymer NPs in forms of micelles, core−shell particles, hyperbranched polymers, nanogels, etc. (Li et al., 2013; Moughton et al., 2012) have exhibited multifunctionality with wide range of applications, including drug delivery and biosensing. Zhong et al. (Abdel-Halim and Al-Deyab, 2014) have improved the stability of collagen in biological environment by incorporation of polyamidoamine dendritic NPs and the modified collagen with enhanced mechanical property was able to support the proliferation of conjunctival fibroblast cells. To enhance the drug loading and sustain deliver, Zhang et al. (2013) have developed hyperbranched polyamine ester NP−hydrogel network, which allowed the controlled drug release over more than a week. The polymeric NPs reinforced hydrogels have reported to use as implant materials. Bait et al. (2011) demonstrated application of polystyrene NP−acrylamide and hydroxyethyl methacrylate hydrogel network as dermatological patch. Thevenot et al. (2007) have developed such composite with varied elastic moduli and physical deformation of such network produced electrical potential, which can be applied in developing soft tactile−sensing devices. By incorporating polypyrrole (PPy) NPs in agarose/alginate binetwork structure, Luo et al. (2014) have developed infrared-responsive releasing system. The recent progress has led to development of novel nanoparticle formulation for personalized drug delivery system (Gao et al., 2014; Kolishetti et al., 2010). To understand the pathological manifestation, highly responsive NPs have been developed, which can trigger the physic-chemical alterations in response to the external stimuli and enable preferential release of drug at the target site (Gao et al., 2010). The NPs that derived from natural biomaterials posses excellent biointerface and have successfully employed for drug delivery, vaccination, or detoxification. The viscoelasticity of hydrogel can be precisely controlled through varying the degree of cross-linkers, which subsequently modulate

the tunable release property. In case of release of two or more numbers of loaded drugs, the sequential release of drug can be achieved by modulating the size and surface properties of different NPs (Hu et al., 2011; Gao et al., 2016).

6.6 Conclusions and future perspectives

We have presented an overview of particle-loaded hydrogels as versatile materials platform for a wide gamut of biomedical applications. Unique synergism of property enhancements over any of the individual components is duly observed in composite systems. The stimuli-responsive and multifunctional render them as ideal "smart" or "intelligent" materials. Their main applications are in the areas of antimicrobial gels, soft material catalysts, drug delivery vehicles, sensors, and soft actuators. For better exploitation of their properties, control over particle−hydrogel interactions especially supramolecular interactions by in silico design and computational modeling is an important requirement. These studies should be performed at different length scales, including particle phase−polymer system and polymeric chains of the hydrogels and the nanophase and between loaded drug−polymer shell, etc. Another important area is incorporation of bioactive cues within the particle-loaded gels. These strategies are expected to provide more specific particle-loaded hydrogels for specific applications.

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Smart polymeric gels

7

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7.1 Introduction

Hydrogels are soft materials with a unique network structure. An important class of hydrogels is based on covalently cross-linked hydrophilic polymers enabling the uptake of significant amounts of water while maintaining the structural integrity, which makes them ideal as biomaterials for clinical use (Kopecek, 2009). Since the pioneering work of Wichterle and Lim reported in 1960 on copolymerization of 2-hydroxyethyl methacrylate (HEMA) with ethylene dimethacrylate, hydrogels have become the focus of intensive research due to their potential applications in diverse fields (Hoare and Kohane, 2008; Wichterle and Lim, 1960). The unique physical properties of the hydrogels can be tuned by controlling the cross-linking density (retractive force) and the affinity of the hydrogels for the aqueous environment (thermodynamic force of mixing) (Kopecek, 2009; Hoare and Kohane, 2008; Kopecek and Yang, 2007). Depending on the polymer properties and experimental conditions, the network structures of hydrogels are formed from a class of natural, synthetic, or hybrid (composed of synthetic and natural) polymers cross-linked by chemical or physical interactions (Kopecek, 2009; Zhu and Marchant, 2011). The chemical cross-linking approach is the most versatile route to create three-dimentional (3D) network structures in comparison to physical cross-linking due to the formation of permanent covalent bonds, which results in a strong hydrogel (Hennink and van Nostrum, 2002). Because they can absorb and retain a high amount of water, hydrogels exhibit various attractive properties, such as swelling, permeation, storage and immobilization capabilities, optical properties, and biocompatibility (Ahmed, 2015; Kawamura, 2017). All these properties made hydrogels a promising material for numerous applications, including superabsorbent materials (Chen et al., 1999; Zohuriaan-Mehr et al., 2010), drug carriers for drug delivery systems (DDSs) (Hoare and Kohane, 2008; Li and Mooney, 2016; Alvarez-Lorenzo and Concheiro, 2014; Arslan et al., 2017; Contreras-Garcia et al., 2011; Lopez-Barriguete and Bucio, 2017), contact lenses, and cell-culturing scaffolds for regenerative medicine. Utilization of conventional hydrogels as biomaterials especially in (DDSs) has been well reported. Moreover, it is well established that the release of drugs can be controlled by varying the chemical and physical properties of these materials (Hoare and Kohane, 2008; Arslan et al., 2017; Gong et al., 2013; Vashist et al., 2014).

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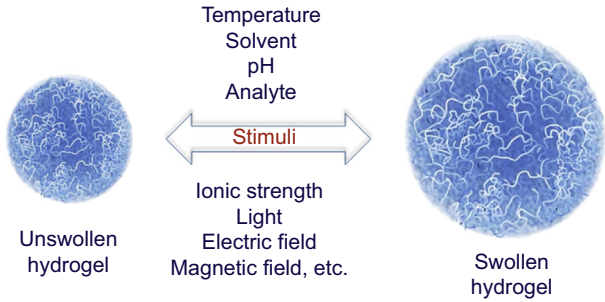

Figure 7.1 Schematic representation of the stimuli-responsive hydrogel.

In 1968, Dušek and Patterson theoretically predicted that the degree of swelling of a hydrogel can be by changes in the external conditions (Dušek and Patterson, 1968; Kopecek, 2007). This theoretical prediction was confirmed by experimental data 10 years later (Kopecek, 2009; Hrouz et al., 1981; Suzuki and Tanaka, 1990; Tanaka, 1978). Since the discovery of responsive hydrogels by Tanaka (1978), research in the field of stimuli-responsive hydrogels also known as smart/intelligent hydrogels has made rapid progress in the last 30 years. These smart hydrogels undergo reversible volume phase transitions or sol−gel transitions triggered by environmental stimuli such as pH, temperature, electrical field, light, pressure, ionic strength, solvent composition, etc. (Kopecek, 2009; Belal et al., 2016; de la Rosa et al., 2016; Dong et al., 2006; Grafe et al., 2017; Klouda and Mikos, 2008; Qiu and Park, 2012; Voorhaar and Hoogenboom, 2016; Zhao and Stoddart, 2009; Cao and Wang, 2016) (see Fig. 7.1).

Rapid response to environmental stimuli and the formation of viscoelastic gel are critical for the versatility of smart hydrogels. To surmount the unresponsive hydrogels, research efforts have been devoted to synthesizing smart hydrogels with improved response to the physiological conditions and elastic properties. Smart hydrogels are superior to unresponsive hydrogels by their dynamic response significantly broadening their application potential. The use of smart hydrogels in diverse applications such as actuators, sensors, tissue engineering, and drug delivery has been extensively reported (Doring et al., 2013; Fathi et al., 2015).

This chapter summarizes recent developments on smart polymeric hydrogels with focus on their synthesis, properties, and applications. At first, the most common methodologies for the synthesis of smart polymeric hydrogels based on synthetic polymers will be briefly discussed. Next, classification of smart polymeric hydrogels based on the stimuli and their controlling mechanism will be given. Finally, some potential applications and future perspectives will also be highlighted.

7.2 Different approaches for the synthesis of smart polymeric hydrogels

This section will discuss the different approaches that are used for the synthesis of polymeric hydrogels. Some important information on technologies adopted in

hydrogel preparation can be found in the review of Ahmed (2015). The properties of a hydrogel network can be tuned by changing the conditions in which the hydrogels are synthesized. For the preparation of hydrogels, hydrophilic monomers are used in most of the cases. However, to control the hydrogel properties sometimes hydrophobic monomers are used in combination with the hydrophilic monomers. Depending on the type of cross-linking of the network, hydrogels can be classified into (1) chemically cross-linked hydrogels obtained by polymerization, radiation, small-molecule cross-linking, or polymer−polymer cross-linking and (2) physically cross-linked hydrogels through ionic interactions, hydrogen bond interactions, hydrophobic interactions, stereo complexation, or supramolecular inclusion complexes (Kopecek, 2009). Free-radical polymerization (FRP) in the presence of multifunctional cross-linkers is the most versatile method that is commonly used to produce any type of hydrogels (Kopecek, 2009). However, it has been reported that the degree of swelling of the hydrogels depends on the polymer network structure (Kopecek, 2009; Yoshida and Okano, 2010). Therefore, it is desirable to use well-defined polymers for the synthesis of smart hydrogels resulting in a more homogeneously cross-linked network with improved properties.

Over the last two decades, controlled/living radical polymerization (CRP) has revolutionized polymer science by enabling the convenient synthesis of polymers with precise average molar masses, diverse compositions, and well-defined architectures (Anastasaki et al., 2016; Chen et al., 2016; Gao et al., 2015; Grubbs, 2011; Maji et al., 2014; Moad et al., 2005). CRPs, namely atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization, and reversible addition−fragmentation chain transfer (RAFT) polymerization, have been applied in the copolymerization of monomers and cross-linkers to synthesize either soluble branched polymers or insoluble polymer networks (Gao et al., 2015). Highly branched inhomogeneous polymer network structures are formed during most conventional FRP reactions because of the intrinsic features of the radical polymerization method, including slow initiation, fast chain propagation, and exclusive radical termination reactions. Whereas, CRP techniques possess several advantages when targeting the preparation of more homogeneous polymer networks and gel properties as result of higher structural homogeneity due to their fast initiation and rapid reversible deactivation reactions (Braunecker and Matyjaszewski, 2007; Goncalves et al., 2013).

ATRP as a single polymerization method or in combination with other methods has been applied for the development of advanced hydrogels, including thermoresponsive hydrogels, nanostructured hybrid hydrogels, and degradable hydrogels. More information on the design of hydrogels by ATRP can be found in the review of Matyjaszewski and coworkers (Gao et al., 2015). Costa et al. (Goncalves et al., 2013) reported the synthesis of several temperature and pH-responsive hydrogel particles by using water soluble monomers such as *N*-isopropylacrylamide (NIPAM) and acrylic acid (AA), as well as cross-linkers with different functionalities via inverse-suspension FRP and RAFT method. The anionic AA−based hydrogels synthesized using FRP or RAFT showed significant difference in equilibrium weight swelling ratio with pH. These results clearly indicate that primary chain length of the polymers and the cross-link density of the networks are strongly affected when FRP is replaced by RAFT, which

can eventually be used to tune the swelling properties of the hydrogels (Goncalves et al., 2013).

Stimuli-responsive hydrogels can be synthesized also by ionic polymerization. One important method is living cationic ring-opening polymerization (LCROP). This method is used to obtain poly(2-oxazoline)s (PAOx) a special class of synthetic polymers that currently receives great interest due to their versatile chemistry (Glassner et al., 2015; Sedlacek et al., 2012), tunable properties, biocompatibility, and stealth behavior (Bauer et al., 2012; Hoogenboom, 2009; Kelly and Wiesbrock, 2012). LCROP as a single polymerization or in combination with other chemical reactions has been used to develop responsive hydrogels (Hartlieb et al., 2015). Different strategies have been adopted for the design of poly(2-oxazoline)—based hydrogels (Legros et al., 2013; Obeid, 2017). Saegusa et al. synthesized redox-, light-, or temperature-responsive hydrogels from partially hydrolyzed poly(2-methyl-2-oxazoline), using different cross-linkers such as diisocyanate, dicarboxylic acid, etc. (Chujo et al., 1990a,b, 1993a,b). Recently, Lecommmandoux et al. (Legros et al., 2013) developed dual stimuli-responsive nanogels when partially hydrolyzed poly(2-ethyl-2-oxazoline) was reacted with a disulfide-containing bis-glycidyl ether cross-linker via inverse w/o emulsion.

Hydrogels can be synthesized by homogeneous (i.e., bulk and solution) or heterogeneous polymerization techniques (precipitation, suspension, and emulsion). A summary of the most commonly used techniques will be discussed in the following, while highlighting some of the advantages and disadvantages.

Bulk polymerization is the simplest method for the preparation of hydrogels/smart hydrogels. A bulk hydrogel can be prepared out of a wide variety of hydrophilic vinylic monomers using a small amount of cross-linking agent and suitable monomer soluble initiators (Ahmed, 2015; Shin et al., 2013). The polymerization reaction is usually initiated with radiation, ultraviolet (UV), or chemical catalysts. A high rate and degree of polymerization is observed due to the higher concentration of monomer in bulk polymerization. As the viscosity of the reaction increases with the conversion in combination with the strongly exothermic polymerization, a noticeable amount of heat is generated. These problems can be bypassed by controlling the reaction at low conversions, although this leads to a less efficient procedure (Ahmed, 2015). In general, hydrogels prepared by bulk polymerization have an inherently weak structure. Peppas et al. described the syntheses of pH-responsive hydrogels synthesized via bulk copolymerization (Brannonpeppas and Peppas, 1989) of 2-HEMA and methacrylic acid (MAA) using ethylene glycol dimethacrylate (EGDMA) as cross-linker. The rate of gelation increased linearly with increasing the MAA content in the copolymer. The copolymers showed pH-dependent swelling behavior owing to the presence of pendant carboxylic groups. Moreover, an increase in water uptake was observed above the pKa of the copolymers due to the ionization of the carboxylic group (Bettini et al., 1995; Soppimath et al., 2002).

Solution (co)polymerization/cross-linking between one or several monomers represents a very convenient method to obtain hydrogels that is most frequently used. The solution polymerization is a one-phase or homogeneous polymerization process. The polymerization is initiated thermally by UV irradiation or by a redox initiator system.

The presence of a solvent that serves as a heat sink is the major advantage of the solution polymerization over the bulk polymerization. Commonly used solvents for solution polymerization of hydrogels are water, ethanol, water—ethanol mixtures, and benzyl alcohol. In this method of polymerization, purification of the hydrogels can be done by washing with distilled water to remove the impurities such as monomers/oligomers, cross-linking agent, initiator, etc.

Suspension polymerization provides access to spherical hydrogel microparticles, with sizes between 1 μm and 1 mm. Inverse-suspension polymerization involves the dispersion of a solution of water-soluble monomer in a continuous organic phase. The shape and size of the particles depends on the viscosity of the monomer solution, stirring speed, rotor design, and dispersant type (Ahmed, 2015; Ogata et al., 2006). Costa et al. (Goncalves et al., 2013) reported acrylamide (AM)—AA-based pH-responsive hydrogel particles, synthesized using the inverse-suspension technique and *N,N′*-methylenebis(acrylamide) as cross-linker. Zhang et al. (2008) described the synthesis of graft-type microgels. First, a dual thermo- and pH-sensitive macromonomer was obtained by radical telomerization of NIPAM and AA monomers using 2-hydroxyethanethiol as a chain transfer agent. Then the macromonomer was copolymerized with NIPAM and AA using inverse-suspension FRP to yield the graft microgels.

Precipitation and dispersion polymerization starts with the initiation of polymerization in a homogenous solution of monomers and cross-linkers. During the reaction, the polymer precipitates forming particle nuclei. The cross-linkers present in the mixture prevent the polymer and solvent from freely mixing, even in good solvents for the polymer. The resulting nuclei aggregate into larger particles that continue to grow by capturing other particles, newly formed polymer chains, or by absorption and polymerization of monomer. The main difference between the two polymerization techniques is that in dispersion polymerization a stabilizer is used to control the size and to narrow the size distribution of the particles (Klinger and Landfester, 2012). The main drawback of these techniques is that incorporation of water-soluble comonomers (e.g., AA) is limited by their hydrophilicity. Therefore, *N*-isopropylacrylamide and *N*-vinyl caprolactam are the most used monomers (An et al., 2007; Pich et al., 2006).

Emulsion polymerization is a heterogeneous polymerization technique in which an organic monomer phase is dispersed in an immiscible aqueous continuous phase. Emulsion polymerization depends strongly on the interaction between various phases where the initiator is water soluble and the reaction occurs within the particle droplets (Mathur et al., 1996). Responsive polymeric hydrogel microspheres can be prepared by emulsion polymerization. Also, surfactant-free emulsion copolymerization was reported by Uragami et al. (Kawamura et al., 2012), as a method to synthesize glucose-responsive gel particles. The recent work on design, synthesis, and characterization of microgels has been summarized by Watanabe (Suzuki et al., 2017).

Chemically cross-linked smart polymeric hydrogels can be obtained using (1) high-energy radiation, such as gamma (γ) and electron beam radiation on preformed polymers, (2) small-molecule cross-linking, and (3) polymer—polymer cross-linking via condensation reaction. Earlier publications have highlighted some commonly

used cross-linking techniques for the preparation of smart hydrogels (Ebara et al., 2014). Hirasa et al. have reported fast-response, thermoresponsive poly(vinyl methyl ether) (PVME) (Suzuki and Hirasa, 1993; Kishi et al., 1993) and PNIPAM hydrogels prepared by γ-ray irradiation (Kishi et al., 1997). The structure of PVME hydrogels is dependent on the intensity of the γ-rays and the temperature during γ-ray irradiation. When the radiation intensity is lower than 1.5 kGy/h, the temperature of the PVME solution does not change at room temperature during irradiation. Same conditions were used for the cross-linking of PVME below lower critical solution temperature (LCST) that lead to a transparent gel with a homogeneous and dense structure. Aoyagi et al. (Kim et al., 2012; Matsukuma et al., 2006) synthesized photocrosslinkable temperature-responsive polymer hydrogels based on photocrosslinkable NIPAM copolymers with a UV-reactive benzophenone (BP)-conjugated comonomer. In presence of UV irradiation, BP ketyl radical and an on-chain polymer radical readily recombine to generate a new C−C bond, thus resulting in cross-linking within the polymer networks. The main advantage of this process for hydrogel formation is that it can be carried out in water under mild conditions without the use of a cross-linking agent. However, a major drawback of this method is that biomolecules must be loaded after gel formation, as UV irradiation can cause irreversible denaturation. Recently, the synthesis and cross-linking of water-soluble poly(2-oxazoline) copolymers containing vinyl groups in the side chains were reported (Dargaville et al., 2016). The polymers were obtained by copolymerizing 2-methyl-2-oxazoline with either 2-undecenyl-2-oxazoline or 2-(3-butenyl)-2-oxazoline. It was found that the time to cure and the cross-linking mechanism were greatly influenced by the hydrophobicity of the side chains. All the copolymers yielded hydrogels with good swelling properties and high optical transparency.

Smart polymeric hydrogels have also been prepared by *physical cross-linking* of polymers or small molecules using a wide variety of supramolecular motifs to obtain supramolecular polymeric hydrogels (Appel et al., 2012). The most important supramolecular motifs that result in supramolecular hydrogels include hydrophobic interactions, hydrogen bonding, electrostatic interactions, host−guest interactions, and metal−ligand coordination. Recent reviews (Voorhaar and Hoogenboom, 2016; Lu et al., 2017) highlight the state of the art and progress in the field of supramolecular hydrogels, including smart supramolecular hydrogels. Papadakis et al. reported thermoresponsive hydrogels prepared from triblock copolymers, consisted of long hydrophilic PNIPAM as thermoresponsive middle block and relatively small, polystyrene (PSt), poly(2-ethylhexyl acrylate), and poly(*n*-octadecyl acrylate) as hydrophobic outer blocks (Bivigou-Koumba et al., 2010). Triblock copolymers were synthesized via RAFT polymerization. All these copolymers formed small flower-like micelles in dilute and semiconcentrated aqueous solution, whereas at high concentrations, typically above 30−35 wt%, formed hydrogels. Insignificant variation of cloud points (T_{cp}'s) was observed (32−28°C) for the copolymers in comparison with the homopolymer (T_{cp} ∼ 32 C) with increasing concentration from 0.01 to 50 wt%. The minimal effect of the hydrophobic blocks onto the T_{cp} is attributed to minimize hydrophobic contacts due to micelles formation. Hydrogels were formed above the T_{cp} due to additional interpolymeric hydrophobic contacts of the corona-forming middle blocks.

Later, similar type of thermoresponsive hydrogels was reported based on triblock copolymer PSt-*b*-poly(di(ethylene glycol)methyl ether acrylate)-*b*-PSt (PSt-*b*-PmDEGA-*b*-PSt) synthesized via RAFT polymerization with different sizes of the thermoresponsive middle block (Miasnikova et al., 2012). The copolymers with longer thermoresponsive middle blocks formed hydrogels at low concentrations even below 4 wt% compared with the shorter polymers, together with an increase in T_{cp}. Lemmers et al. (2010) obtained supramolecular hydrogel based on the electrostatic interactions by mixing ABA-triblock copolymers containing neutral poly(ethylene glycol) (PEG) middle block and negatively charged poly(potassium 3-sulfopropylmethacrylate) outer block with poly(allylamine hydrochloride) as oppositely charged homopolymers. Flower-like micelles were formed at lower concentration where micelles are stabilized by a corona of looped solvophilic chains. At a high concentration, the copolymer forms bridges between two different micellar cores resulting in the formation of reversible supramolecular hydrogels that were responsive to temperature, ionic strength, and pH. One of the major advantages of physically cross-linked hydrogels is that hydrogels are formed without the need for chemical modification or the addition of cross-linking entities (Ebara et al., 2014).

7.3 Types of smart polymeric hydrogels and their governing mechanisms

In this section, we will describe the representative stimuli, namely temperature, pH, light, and different analytes used for modulating the volume transition in smart hydrogels. We will focus our attention on covalently cross-linked hydrogels due to space limitation. However, recent inspiring examples from physically cross-linked hydrogels will be briefly mentioned.

7.3.1 Temperature-responsive hydrogels

The first temperature-responsive hydrogel was reported 40 years ago in 1978 by Tanaka and since then the field has rapidly expanded and a myriad of examples on thermoresponsive hydrogels can be found in the literature and have been reviewed extensively (Klouda, 2015; Suntornnond et al., 2017). Thermoresponsive hydrogel materials with a transition around body temperature have been studied in detail for medical in vitro and in vivo applications. The balance between the hydrophobic and hydrophilic segments in the network is the key to control the swelling properties and transition temperature of the hydrogel. Temperature-responsive hydrogels can be classified into positive or negative systems. Positive temperature-responsive hydrogels have a higher degree of swelling by heating above the upper critical solution temperature (UCST), whereas negative thermoresponsive hydrogel expand by cooling below the LCST. Based on these subtle changes in their surrounding temperature, they exhibit a reversible volume-phase transition (VPT). Further on a noncomprehensive overview will be given of the most important types of synthetic thermoresponsive hydrogels reported in the last 10 years. In addition, the main synthetic procedures for

the synthesis of these hydrogels will briefly be addressed. More comprehensive information regarding thermoresponsive hydrogel systems can be found in the recent review of Klouda (2015).

7.3.1.1 *Poly(*N*-alkyl) substituted amides*

The most commonly studied thermoresponsive LCST hydrogels are based on poly(*N*-isopropylacrylamide) and its copolymers. PNIPAM has an LCST around 32°C, close to physiological temperature, with a robust phase transition behavior, making it an ideal target for biomedical applications. The polymerization of NIPAM can be achieved by FRP of the vinyl group using common radical initiators. The controlled CRP has also been developed, resulting in polymers with narrow molecular weight distribution and defined end groups. Hydrogels based on PNIPAM can be divided into chemically or physically cross-linked. In the first category, usually a cross-linker such as *N,N′*-methylenebis(acrylamide) or ethylene glycol dimethacrylate is used, while the physically cross-linked gels are based on the *sol−gel* transition of the polymer in water. In a series of papers, Miko et al. reported a systematic study of chemically cross-linked hydrogels based on NIPAM copolymers with glycidyl methacrylate (Ekenseair et al., 2012a,b; Tzouanas et al., 2014). In the first step, the copolymers were synthesized by radical polymerization. In situ cross-linking was done by reacting the glycidol groups with polyamidoamine having piperazine end groups. Network formation through the epoxy−amine reaction was rapid and facile, and the incorporation of hydrophilic polyamidoamine cross-linker led to materials without postformation gel syneresis (Ekenseair et al., 2012a). Using a similar approach, they functionalized a PNIPAM macromonomer having pendant phosphate groups with chemically cross-linkable methacrylate groups via degradable phosphate ester bonds yielding an injectable, degradable dual-gelling macromer (Watson et al., 2014) (see Fig. 7.2).

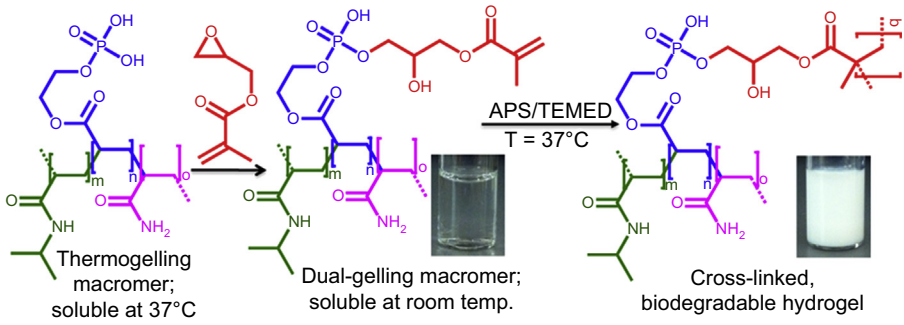

Figure 7.2 Synthesis of degradable dual-gelling macromonomer. *APS*, ammonium persulfate; *TEMED*, tetramethylethylenediamine.
Reproduced from Watson, B.M., Kasper, F.K., Engel, P.S., Mikos, A.G., et al., 2014. Synthesis and characterization of injectable, biodegradable, phosphate-containing, chemically cross-linkable, thermoresponsive macromers for bone tissue engineering. Biomacromolecules 15, 1788−1796, copyright 2014, with permission from American Chemical Society.

Simultaneously, physically and chemically cross-linking polymer systems were developed by Vernon et al. (Censi et al., 2010; Robb et al., 2007). In the first approach, they obtained PNIPAM ABA triblock copolymers using a PEG initiator and *N*-(2-hydroxypropyl) methacrylamide dilactate as comonomer (Censi et al., 2010). Subsequently the hydroxy group was reacted with acrylic anhydride to introduce reactive groups. Then Michael addition was performed with thiolated hyaluronic acid (HA) to give in situ gelling, biodegradable but structurally stable and biocompatible hydrogels. The second strategy involved the copolymerization of NIPAM with *N*-acryloxysuccinimide, followed by further modification with cysteamine hydrochloride to introduce a thiol group on the thermoresponsive precursor (Robb et al., 2007). The hydrogels were obtained by reacting the copolymer with a difunctional PEG acrylate in aqueous solutions. Matyjaszewski used RAFT and ATRP to obtain degradable thermoresponsive hydrogels based on poly(*N*-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane) copolymers (Siegwart et al., 2008). Chemically cross-linked dual stimuli-responsive hydrogels for drug delivery were obtained by copolymerization of NIPAM with MAA (Peng et al., 2013) or itaconic acid (Milasinovic et al., 2010). In addition to PNIPAM, other *N*-alkyl substituted acrylamides that display LCST behavior have been studied (Plate et al., 1999). Using an innovative strategy, Hoogenboom et al. (Belal et al., 2016) developed temperature-responsive hydrogels from nonthermoresponsive poly(*N*,*N*-dimethylacrylamide). The hydrogel was functionalized with dialkoxynaphthalene guest units that significantly swells on complexation with the tetracationic cyclophane host cyclobis(paraquat-*p*-phenylene) ($CBPQT^{4+}$). By introducing a PNIPAM end-functionalized polymer with tetrathiafulvalene (TTF) as a stronger binding competitive guest temperature-induced swelling of the naphthalene gel was obtained. In this three-component system, TTF−PNIPAM is complexed with $CBPQT^{4+}$ below the LCST phase transition, as TTF is a more strongly binding guest than naphthalene. Heating of the system above the phase transition temperature of the TTF−PNIPAM·$CBPQT^{4+}$ complex led to the collapse of TTF-PNIPAM and induced dethreading of the host−guest complex and release of the free cyclophane unit into the aqueous solution. Transfer of the host to the hydrogel induced the positive thermoresponsive behavior.

Recent work has been focused on injectable thermoresponsive hydrogels cross-linked by physical interactions (Nitschke et al., 2007; Overstreet et al., 2013). Incorporation of pH-responsive groups very useful in tuning the solidification, hydrogel swelling, and drug release in various regions in the human body was also investigated (Cui et al., 2011; Fujimoto et al., 2009). Usually controlled radical copolymerization was the method of choice (Garbern et al., 2010; Li et al., 2011). Because PNIPAM is not biodegradable, the introduction of enzyme cleavable bonds or hydrolytically unstable groups represented another major objective (Overstreet et al., 2010; Guan et al., 2008; Nelson et al., 2012). Hydrogels from comb-type grafted PNIPAM were reported for rapid detachment of cell sheet as cells adhere to the hydrophobic PNIPAM surface above the transition temperature and detach when the PNIPAM is hydrated on cooling (Tang et al., 2010). The graft chains have a free end that results in increased mobility of the polymer chains leading to faster hydration below the LCST. The cell detachment was shown to be accelerated with this type of polymer-coated surface.

7.3.1.2 Poly(ethyleneglycol)

PEG has a negative temperature response in aqueous environments, albeit with a transition temperature above 200°C (Dormidontova, 2002), and possesses stealthing properties (Waku et al., 2007). PEG is also noncytotoxic, nonimmunogenic and has protein repellent properties. PEG is typically prepared via ring-opening polymerization and thus it can be functionalized only at the chain ends. Hydroxyl-terminated di- and multifunctional PEG polymers are usually the precursor. Using postmodification reactions, a variety of functional groups can be introduced: azide (Deforest et al., 2010), thiol (Fu and Kao, 2011), and maleimide (Koehler et al., 2013). However, to obtain a tunable temperature response in the physiological relevant window poly(oligo ethylene glycol methacrylate) (POEGMA) was investigated as an alternative. Synthesis can be done by conventional or controlled/living FRP offering excellent control over polymer composition, functionality, and architecture in the presence of a cross-linker (Lutz et al., 2007b; Yoon et al., 2011; París and Quijada-Garrido, 2009; Lei et al., 2013). Also, different comonomers can be used to impart the desired functionalities and responsive properties (Meenach et al., 2010; Son and Lee, 2016; Lutz, 2008). Another advantage is that POEGMA has a minor hysteresis of the temperature transition as compared with PNIPAM due to the lack of hydrogen bonding (Lutz et al., 2006). Moreover, POEGMA was proven to be bioinert (Hyun et al., 2003) and to have noncytotoxic properties (Lutz et al., 2007a). Volume phase transition temperature (VPTT) of POEGMA hydrogels corresponds to the LCST reported for soluble polymers, being 26−90°C (Lutz, 2008). For a comprehensive review about soluble and cross-linked POEGMA-based materials, the reader should consult the recent review of Hoare (Bakaic et al., 2015). The main drawback of POEGMA hydrogels is the lack of biodegradability, which can limit their applications. Consequently, in a recent paper, Hoare et al. (Smeets et al., 2014) have tried to offer a solution by synthesizing POEGMA precursor, with hydrazide and aldehyde groups, respectively. Hydrazone cross-linked hydrogels were prepared by coextruding the two functionalized polymers. In vivo experiments showed that the POEGMA hydrogels could be fully degraded under physiological conditions without any obvious signs of local tissue toxicity. Varying the length of the oligo ethylene spacer allowed tuning of the thermoresponsive behavior (i.e., VPTT). The hydrogels with VPTTs close to and above physiological temperature exhibit biological properties similar to those typically observed for PEG hydrogels, whereas the ones with VPTTs lower than physiological temperature exhibit biological properties more analogous to PNIPAM.

7.3.1.3 Other synthetic polymers

Poly(vinyl caprolactam) possesses noteworthy properties for biomedical applications, e.g., solubility in water, high absorption ability, and a transition temperature similar to PNIPAM. Macro-, micro- and nanogels have been obtained and investigated for biomedical applications (Rao et al., 2016). The main drawback encountered in the synthesis of this polymer is the control over the molecular weight distribution. However, using xanthates as RAFT agents, well-defined polymers are obtained (Van Nieuwenhove et al., 2017).

PVME is another thermoresponsive polymer that has the LCST around body temperature (≈37°C) (Arndt et al., 2001). Microporous hydrogels were prepared by γ-ray irradiation and displayed a rapid volume transitions on a timescale of about a minute (Kishi et al., 2005). The degree of cross-linking and the overall swelling response could be controlled by the level of irradiation.

Hydrogels having a positive volume phase transition (i.e., swelling degree increases on raising temperature) have been reported too (Echeverria et al., 2009; Katono et al., 1991; Yang et al., 2010; Dai et al., 2006). The most investigated system is composed of acrylamide AA copolymers, which reveal UCST behavior. Increasing the AA content of the copolymer has led to a shift to higher values in the collapse temperature (Echeverria et al., 2009). These results can be interpreted by considering that the volume phase transition of this system is driven by hydrogen bonding. The number of hydrogen bonds is higher when the content of AA is increased and, therefore, chain mobility is decreased leading to a delay in temperature of the collapse.

7.3.2 pH-responsive hydrogels

pH-responsive hydrogels can be defined as polyelectrolytes that include weak acidic or basic groups in their structure. Their swelling/deswelling behavior can be controlled by the environmental pH. Depending on the nature of the polyelectrolyte, the pH-responsive hydrogels can be divided into three classes: acid-containing anionic, base-containing cationic, and amphoteric microgels (acid and base). The parameter determining the swelling behavior of pH-responsive microgels is the critical pH value (pH_c) at which the phase transition occurs. Because ionization (protonation or deprotonation) determines the swelling degree of the gel, it is mandatory to understand the factors influencing this process. The most important ones are (1) ion osmotic pressure, (2) free energy of mixing of the polymer network with the solvent, and (3) elastic retractile response of the expanding gel network.

7.3.2.1 Anionic hydrogels

Anionic hydrogels incorporate weak acidic polymers (e.g., poly(acrylic acid) [PAA]). Below the pKa, the gel is found in the collapsed state, whereas above the pKa the acidic groups are deprotonated causing an increase in the hydrophilicity of the polymer. In addition, the swelling degree is increased also due to the osmotic pressure generated by the repulsion of the anionic groups present in the network. The factors influencing the swelling degree are the pKa of the polyelectrolyte, the amount of acid groups present on the polymer, the ionic strength of the solution, and obviously the cross-linking density. Correlations between polyelectrolyte structure, pKa, cross-linking degree, and ionic strength have been thoroughly investigated in the literature and are covered in other reviews (Singhal and Gupta, 2016; Gupta et al., 2002; Kocak et al., 2017; Sood et al., 2016). The main research on anionic hydrogels has focused on two polymers, namely PAA and poly(methacrylic acid). Various macromolecular architectures with different topological structures were obtained by copolymerization with different comonomers. Moreover, hydrogels responding to a combination of pH

and other stimuli, such as light, temperature, and analyte, have been reported. In the following, we will highlight the recent state-of-the-art examples regarding anionic hydrogels with focus on biomedical applications.

The group of Peppas et al. has focused on developing anionic hydrogels for the delivery of different therapeutic drugs (Carr et al., 2010; Kamei et al., 2009; Morishita et al., 2006; Carr and Peppas, 2010; Koetting and Peppas, 2014; Schoener et al., 2013). They managed to successfully obtain a nanogel carrier for oral vaccine delivery. The nanogel was synthesized via surfactant-free emulsion copolymerization of MAA and 2-hydroxyethyl methylmethacrylate (Durán-Lobato et al., 2014). As cross-linker ethylene glycol dimethacrylate was used. The surface of these carriers was modified by the covalent linkage of mannan to mimic carbohydrate moieties found on the surface of pathogens. The pH-sensitive nanogels were loaded with ovalbumin and afterward the release behavior was studied. The hydrogel efficiently entrapped and protected the cargo at low pH values and triggered protein release only after pH switching to intestinal pH values. Interestingly, the unmodified poly(HEMA-*co*-MAA) nanogels were efficiently internalized by macrophages and induced the expression of costimulatory molecules, although at a lower level then the mannan modified ones. In a recent paper, Guan et al. (Li et al., 2016b) developed stem cell carriers for cardiac therapy. A copolymer composed of *N*-isopropylacrylamide, propylacrylic acid, HEMA-co-oligo(trimethylene carbonate), and methacrylate poly(ethylene oxide) methoxy ester was synthesized by free radical copolymerization. At pH 8.0, the LCST of the hydrogel solutions was well above 37°C, whereas lowering the pH at 6.5 resulted in a substantial decrease in the LCST. Furthermore, the authors proved that the hydrogels could be injected through 0.2 mm catheters at 37°C when the solution pH is adjusted to 8.0 and were able to form solid gels at pH 6.5 environment just like that of the infarcted heart tissue. Encapsulation of cardiosphere-derived cells (CDCs) into hydrogels proved that CDCs could survive for 7 days. Also, the surviving cells differentiated into cardiac lineage.

pH-responsive hydrogels have also found applications as artificial muscles materials. Using a biomimetic molecular-level approach triggered by light, Weaver et al. (Dicker et al., 2017) were able to actuate soft, pH-responsive hydrogel artificial muscles (see Fig. 7.3(a)). The pH-responsive hydrogels used in this work were tough semiinterpenetrating polymer networks of PAA and an ether-based hydrophilic polyurethane. The actuation process is based on a combination of nonlinear chemical reactions and responsive hydrogels. By using a mixture of photoacid, sodium bisulphite, and potassium iodate an amplifying reaction system is obtained that generates acid (see Fig. 7.3(b)). The generated acidic solution then actuates (contracts) the PAA hydrogel. Even though this reaction is triggered by light, the actual "fuel" comes from excitation and runaway chemical reaction of a light-sensitive acid autocatalytic solution in which the actuator is immersed.

Other inspiring examples of anionic pH−sensitive hydrogels include salecan-g-PAA hydrogel for controlled release of doxorubicin (DOX) (Hu et al., 2015), injectable hollow particle for soft tissue regeneration (Halacheva et al., 2014), enzymatically responsive hydrogel microparticles for the oral delivery of therapeutic proteins (Koetting et al., 2016), composite hydrogels for sustained delivery of anticonvulsants

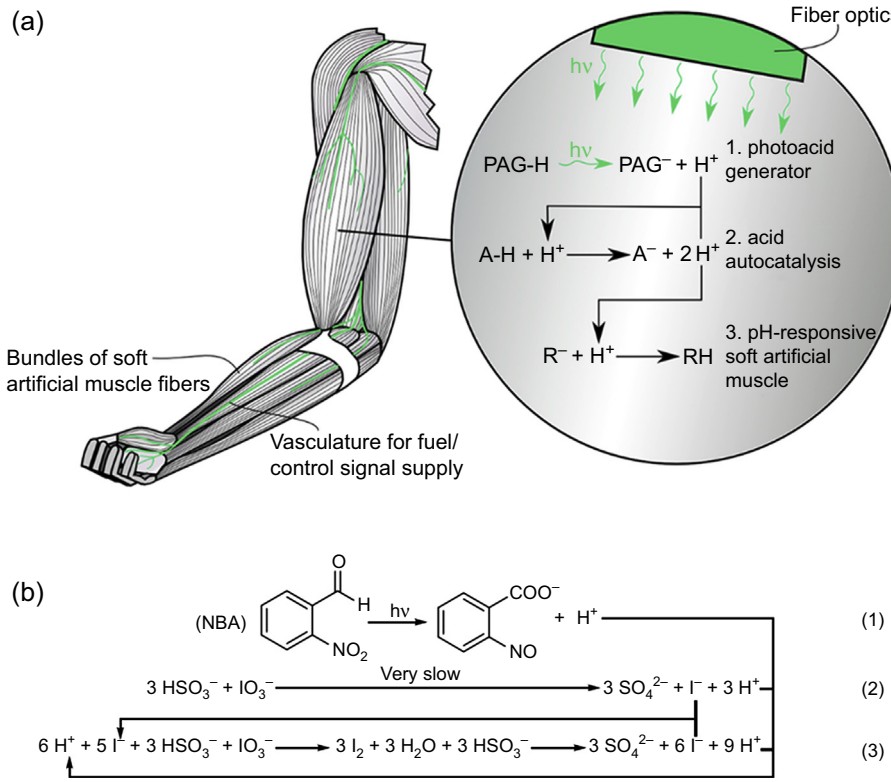

Figure 7.3 Molecular level—controlled robotic system. (a) Detail of investigated system: (1) light generates an acid, (2) which triggers an acid autocatalytic reaction, (3) resulting in the contraction of a pH-responsive soft artificial muscle; (b) chemical reactions taking place in the system triggered by light.
Reproduced from Dicker, M.P.M., Baker, A.B., Iredale, R.J., Naficy, S., Bond, I.P., Faul, C.F.J., Rossiter, J.M., Spinks, G.M., Weaver, P.M., et al., 2017. Light-triggered soft artificial muscles: molecular-level amplification of actuation control signals. Scientific Reports 7, 9197, copyright 2017, with permission from Nature Publishing Group.

drugs (Cevik et al., 2015), and sulfamethazine hydrogels for transcatheter arterial chemoembolization therapy (Lym et al., 2016).

7.3.2.2 Cationic hydrogels

Cationic hydrogels have opposite swelling behavior to anionic hydrogels polymers and they contain weak basic groups such as, e.g., amino moieties. The hydrogel is in the swollen state at low pH (pH < pKa) and collapses on exposure to a higher pH environment (pH > pKa). The most common synthetic hydrogels are based on polymers such as poly(ethylene imine) (PEI) (Vinogradov et al., 2002) and poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA). PEI is considered to be

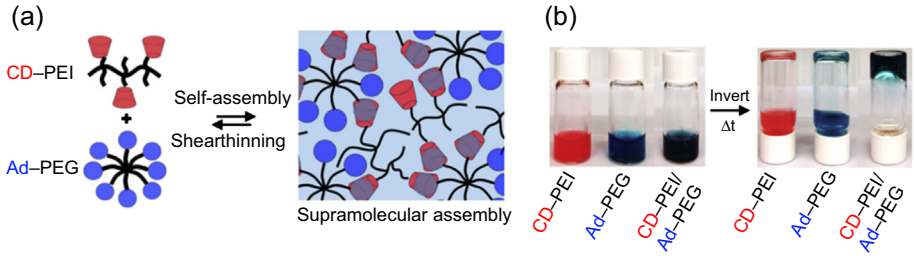

Figure 7.4 Guest−host cyclodextrin (CD)−poly(ethylene imine) (PEI)/adamantane (Ad)−poly(ethylene glycol) (PEG) assemblies. (a) Schematic of CD−PEI and Ad−PEG assembly through guest−host complex formation. The reversible guest−host bond permits shear thinning and rapid reassembly. (b) Qualitative inversion after 1 min of CD−PEI, Ad−PEG, and CD−PEI/Ad−PEG.
Reproduced from Wang, L.L., Sloand, J.N., Gaffey, A.C., Venkataraman, C.M., Wang, Z., Trubelja, A., Hammer, D.A., Atluri, P., Burdick, J.A., 2017. Injectable, guest−host assembled polyethylenimine hydrogel for siRNA delivery. Biomacromolecules 18, 77−86, copyright 2016, with permission from American Chemical Society.

a "gold standard" in gene delivery systems (Godbey et al., 1999) and has been extensively studied. An injectable polymer assembly from PEI and PEG to promote local and sustained siRNA delivery has been developed by Burdick et al. (Wang et al., 2017). The authors utilized host−guest complexation of β-cyclodextrin (CD) and adamantane (Ad) by attaching them to branched PEI and PEG, respectively (Fig. 7.4). Afterward by mixing equimolecular amounts of the modified polymers, a hydrogel was obtained by host−guest supramolecular association. Rheological measurements suggested that host−guest bonds can reverse under shear to permit flow with rapid bond reformation and reassembly on cessation of shear. The hydrogel was proven to promote local and sustained siRNA delivery. Also, the hydrogel had improved transfection and decreased cytotoxicity compared with PEI alone.

Other interesting applications of PEI hydrogels include controlled nitric oxide release and cell proliferation modulation (Kim et al., 2011), healing of pressure sores (Lee et al., 2012), glucose detection (Yang and Kim, 2011), and copper ion removal from wastewater (Liu et al., 2017).

Hydrogels based on PDMAEMA, which is a weak polycation, can simultaneously respond to three external stimuli, such as temperature, pH, and ionic strength. Dragan et al. (Dragan and Cocarta, 2016) recently reported the synthesis of macroporous interpenetrating polymer network (IPN) cryogels containing PDMAEMA segments. In the first step, PDMAEMA was obtained by cross-linking polymerization of DMAEMA below the freezing point of the water. Then the hydrogel was soaked in an aqueous solution of acrylamide and *N,N′*-methylenebisacrylamide with polymerization taking place also at 0°C, thus obtaining full-IPN cryogels. The ability of these IPN cryogels to adsorb and release drugs in a controlled manner on changing the pH and/or temperature was investigated, and the potential of the cryogels in DDSs was demonstrated. Tasciotti et al. (Khaled et al., 2016) developed pH-responsive hybrid nanogel based on DMAEMA. The novelty of the study was the use of a one-pot method to produce

a cross-linked PDEAEMA hydrogel shell around the silica nanoparticle. Moreover, the use of nanogels as nonviral carriers for siRNA therapeutics was demonstrated. Another example of tough but transparent hydrogels with outstanding fatigue resistance was reported by copolymerization of Pluronic F127 diacrylate triblock copolymer with acrylamide and methyl chloride quaternized DMAEMA (Sun et al., 2015).

Other cationic hydrogel systems based on poly(allylamine) have generated breakthrough research. For example, Birkedal et al. (Krogsgaard et al., 2013) reported a self-healing multiresponsive hydrogel system by incorporating mussel adhesive proteins. Also, gellike coacervates that adhere to both hydrophilic and hydrophobic substrates under water have recently been prepared by ionically cross-linking poly(allylamine) with pyrophosphate and tripolyphosphate (Lawrence and Lapitsky, 2015). Moreover, different other weak basic groups were incorporated in pH-responsive hydrogels such as hexamethylene guanidine hydrochloride, (Du et al., 2016) piperazine (Deen and Mah, 2016), acetylthio (Montero-Rama et al., 2015) or imidazolinium (Tamesue et al., 2016) groups.

7.3.2.3 Amphoteric hydrogels

Polyampholyte hydrogels possess both cationic and anionic charges; therefore, they usually exhibit two-phase transitions in both acidic and basic environments, rather than in neutral medium. Synthetic amphoteric hydrogels have been studied extensively and recently reviewed (Kudaibergenov et al., 2012). Patrickios et al. (Pafiti et al., 2014; Constantinou et al., 2016) recently reported an interesting study on regular and inverse polyampholyte. Inverse polyampholytes are polymers where the roles of the acidic and the basic groups are reversed, namely the pK value of the positively ionizable groups becomes lower than that of the negatively ionizable ones or vice versa. To obtain such hydrogels, acidic units with uncommonly high pK values or basic units with uncommonly low pK values, or the combination of both units have to be used. Thus, hydrogels were synthesized by RAFT copolymerization of (pyridin-2-yl)methyl methacrylate (basic monomer) and tetrahydro-2H-pyran-2-yl methacrylate (THPMA), while as cross-linker EGDMA was used. Generation of the carboxylic groups has been realized by the hydrolysis of the THPMA units. These types of hydrogels were proven to be less sensitive to the isoelectric point and their degrees of swelling were found to be generally lower than the regular ones.

Amphoteric hydrogels cross-linked via hydrazine chemistry for mimicking native tissues and/or regulate the release kinetics of biological therapeutics have been developed by Hoare et al. (Bakaic et al., 2017). The charged poly(oligo ethylene glycol methacrylate) precursor polymers were synthesized by copolymerizing OEGMA475 with a functional monomer to enable covalent cross-linking via hydrazide/aldehyde chemistry and a functional monomer to impart charge in the polymer (AA for anionic polymers, DMAEMA for cationic polymers) (see Fig. 7.5). Using this strategy cationic, anionic, and amphoteric gels were obtained. Amphoteric hydrogels showed improved mechanical properties and slower degradation times at physiological pH than the corresponding neutral gel, due to secondary network formation via charge complexation.

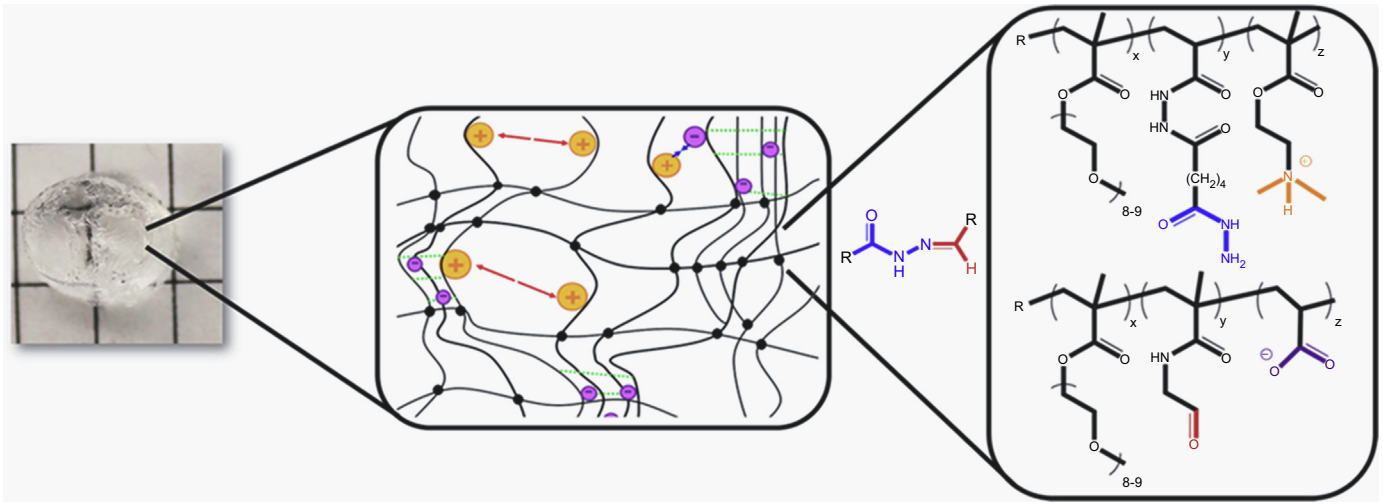

Figure 7.5 Synthesis of amphoteric hydrogels based on poly(oligo ethylene glycol methacrylate).
Reproduced from Bakaic, E., Smeets, N.M.B., Barrigar, O., Alsop, R., Rheinstädter, M.C., Hoare, T., 2017. pH-ionizable in situ gelling poly(oligo ethylene glycol methacrylate)-based hydrogels: the role of internal network structures in controlling macroscopic properties. Macromolecules 50, 7687−7698, copyright 2017, with permission from American Chemical Society.

pH-sensitive hydrogels inevitably suffer strength deterioration, whereas the responsive weak acid or base groups are in the ionized state (Gong, 2010). Using an interesting approach Liu et al. synthesized pH-sensitive high-strength hydrogels by copolymerization of hydrogen-bonding motif-containing monomers, namely 3-acrylamidophenylboronic acid and 2-vinyl-4,6-diamino-1,3,5-triazine (Gao et al., 2013). The two monomers can form hydrogen bonds in both acid and alkaline media providing supplementary physical cross-link points to the chemically cross-linked network. Consequently, the hydrogel exhibited high strengths even when the pH responsive groups were in their ionized state.

Other ionic comonomers pairs used for the synthesis of amphoteric gels include AA and DMAEMA (Li et al., 2016a; Liu et al., 2015), AA and *N*-3-(dimethylamino)propyl methacrylamide (Du et al., 2015), AA and 4-vinylpyridine (Su and Okay, 2017), DMAEMA and MAA (Pafiti et al., 2011), sodium acrylate and 2-(methacryloyloxy) ethyltrimethylammonium chloride (Shukla et al., 2012), allylamine, and MAA (Tatykhanova et al., 2012).

7.3.3 Light-responsive hydrogels

Photoactive molecules play a pivotal role within photoresponsive systems, being able to capture an optical signal and convert it via a photoreaction, to a useful property change (Spiridon et al., 2012, 2013, 2015; Jerca et al., 2011, 2013, 2015). The photoresponsive hydrogels typically are composed of a polymeric network and a photoactive moiety, usually a photochromic chromophore, as the functional part. The optical signal is first captured by the photoactive molecules. Then, it is converted to a chemical signal through a photoreaction such as isomerization, cleavage, or dimerization, and this processed signal is transferred to the functional part of network to alter its properties.

Photoactive molecules used in hydrogels include, but are not limited to, azobenzenes, spiropyrans, coumarins, and *o*-nitrobenzylesters. These photoresponsive groups can be incorporated in the 3D network as cross-linkers and/or as pendant groups. A wide variety of photoresponsive hydrogels (Tomatsu et al., 2011) have been developed to control material properties and a selection of most notable examples will be discussed in this section. Photoresponsive hydrogels can exhibit multiple changes on photoirradiation in their physical and/or chemical properties such as elasticity, viscosity, shape, and degree of swelling, properties that are of interest to control, e.g., drug delivery. To design a photoresponsive system, the choice of the photoreactive group and the fundamental structure of the gels are decisive. Both physically (noncovalently) cross-linked and chemically (covalently) cross-linked networks have been used to incorporate the photoactive group into the hydrogel structure. Photoresponsive hydrogel systems can be divided into four categories: (1) chemically irreversible cross-linked hydrogels that incorporate photoresponsive molecules; (2) chemically reversible network-forming hydrogels based on photodimerization; (3) physically reversible network-forming hydrogels containing intermolecular interacting side groups that respond to photoisomerization; (4) physically irreversible network-forming hydrogels based on the interactions of photocleavable side groups.

7.3.3.1 *Chemically irreversible cross-linked hydrogels that incorporate photoresponsive molecules*

Azobenzene containing hydrogels are the most investigated ones because of the reversible *cis*−*trans* isomerization, good photostability, and relatively easy synthesis of these photoactive compounds (Tomatsu et al., 2011; Rosales et al., 2015). The main disadvantage of azobenzene is that their properties are regulated using UV light that is not usable for in vivo applications. Light-responsive hydrogels are part of a large field and have been reviewed extensively (Tomatsu et al., 2011; Lim et al., 2014; Wells et al., 2010), therefore only significant recent examples will be further highlighted here. Light-responsive hydrogels were prepared by cross-linking four-armed, amine-terminated PEG with di-*N*-hydroxysuccinimide azobenzene derivatives (Rastogi et al., 2017). By changing the temperature of the curing reaction from room temperature to 37°C, the morphology of the hydrogels could be changed from homogeneous to porous. On UV irradiation, the gels were found to decrease in size to an average of 77% of their initial area. The authors speculate that the response is due to the compact *cis* conformation of the azobenzene cross-linkers and to the generation of dipole−dipole interactions between the polar *cis* azobenzene and the polymer network (see Fig. 7.6).

Photoresponsive hydrogels based on acrylamide, 2-vinyl-4,6-diamino-1,3,5-triazine, a spiropyran containing monomer (2-[1-acrylate-3′,3′-dimethyl-6-nitrospiro (indoline-2′,2-[2H-1]-benzopyran)] acrylamide) and a PEG diacrylate cross-linker

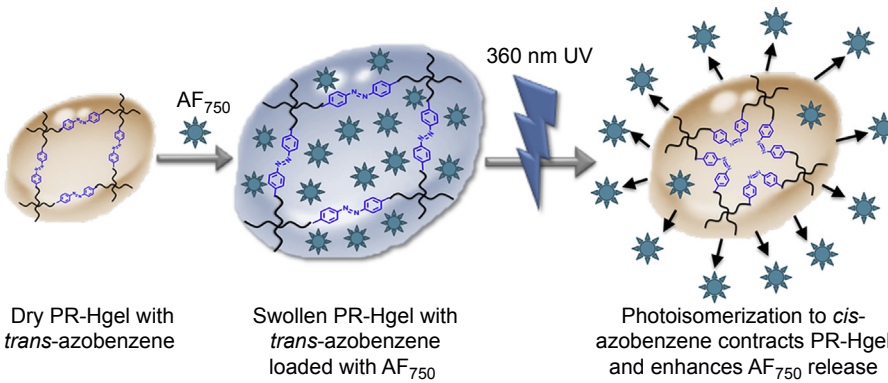

Figure 7.6 The ultraviolet (UV)-triggered reduction in hydrogel size accompanied by enhanced release of the near-infrared fluorescent dye Alexa Fluor 750.
Reproduced from Rastogi, S.K., Anderson, H.E., Lamas, J., Barret, S., Cantu, T., Zauscher, S., Brittain, W.J., Betancourt, T., 2017. Enhanced release of molecules upon ultraviolet (UV) light irradiation from photoresponsive hydrogels prepared from bifunctional azobenzene and four-arm poly(ethylene glycol). ACS Applied Materials and Interfaces. https://doi.org./10.1021/acsami.6b16183, copyright 2017, with permission from American Chemical Society.

were reported by Liu et al. (Wang et al., 2014a). By irradiating the gel with UV light cell attachment and detachment could be controlled due to the isomerization of the spiropyran that led to reversible hydrophobic−hydrophilic changes. Following the same synthetic strategy but replacing acrylamide with PEG acrylate the authors also investigated the cell attachment/detachment and gene transfection on the surface of this hydrogel (Wang et al., 2014b). Spiropyran-based hydrogels, which are sensitive to more than one stimulus and display a cooperative function in response to the applied stimuli, were synthesized by radical copolymerization of NIPAM with acrylated spirobenzopyran in the presence of a cross-linker (Filipcsei et al., 2014). The hydrogels exhibited both temperature- and light-induced shrinkage. The highly cross-linked cylindrical gel showed rapid bending deformation due to the asymmetric light irradiation.

Molecularly imprinted hydrogels fabricated from tailor-made conformation-switchable functional monomers are another category of chemically cross-linked photoresponsive hydrogels. An eloquent example of such photoresponsive molecular imprinted hydrogels has been reported by the Lam et al. (Gong et al., 2008). The preparation of several hydrogel compositions was carried out from a water-soluble azobenzene-containing functional monomer cross-linked with various bisacrylamide and bismethacrylamide bifunctional monomers. The hydrogels were prepared in the presence of paracetamol, chosen as the molecular template for the imprinting. Because photoisomerization of the azobenzene moieties induces structural changes of the recognition sites, the release and uptake of paracetamol could be controlled by light. This study demonstrated that such photoregulated release and uptake processes are repeatable and can be utilized as a controlled DDS. The same authors reported molecular imprinted hydrogels using caffeine as a molecular template (Gong et al., 2006). The photoresponsive molecularly imprinted polymeric material was obtained from an azobenzene-based functional monomer and trimethylolpropane trimethacrylate as the cross-linker.

7.3.3.2 *Chemically reversible network-forming hydrogels based on photodimerization*

In addition to isomerization, studies have employed light-activated dimerization to create responsive hydrogels (Kaur et al., 2014). The photoinduced gelation provides several advantages over conventional chemical hydrogel synthesis: mild to moderate reaction conditions, absence of toxic catalysts and initiators, and easily controllable rates of cross-linking (by switching the light source on and off). Hydrolyzed poly(2-oxazoline)s having a coumarin moiety in the side chain were the first reported material for the synthesis of hydrogels based on photodimerization cross-linking (Chujo et al., 1990c). The polymer was irradiated with UV light to produce a gel, which was swollen in water and demonstrated characteristic hydrogel properties. PEG (Nagata and Yamamoto, 2008), poly(vinyl alcohol) (Lee and Kim, 2012), and pluronics (Yoon and Kim, 2017) based hydrogels have also been reported. Photocrosslinkable nanogels

were developed starting from diblock copolymers composed of poly(ethylene oxide) and poly[2-(2-methoxyethoxy) ethyl methacrylate-*co*-4-methyl-[7-(methacryloyl)oxy-ethyloxy]coumarin] (He et al., 2009). The nanogels were easily prepared by photocrosslinking of the micellar aggregates that were formed above the LCST followed by cooling to room temperature. The volume of the nanogel particles could be increased by the gradual photocleavage of the coumarin dimers.

Cinnamate derivates represent another viable alternative for the synthesis of hydrogels by [2 + 2] cycloaddition (Micic et al., 2003). Photocrosslinkable biodegradable elastomers were synthesized by reacting hyperbranched polyesters, namely poly(glycerol-*co*-sebacate) with cinnamoyl acid chloride (Zhu et al., 2013). The gels had a projected in vitro degradation half-life time between 90 and 140 days were cell adherent and support rapid proliferation of fibroblasts. Synthesis of PEG hydrogels modified with anthracene groups and their photoresponsive properties have also been demonstrated (Zheng et al., 2002; Wells et al., 2011).

Recently thymine was used as a photoresponsive group to generated cross-linked structures (Yang and Zeng, 2013). Polyacrylamide was functionalized with thymine groups and photoresponsive gel−sol transition was achieved by alternating irradiation with 365 and 240 nm UV light. The hydrogel was also formed in the presence of Hg^{2+} ions because of the formation of thymine−Hg^{2+} complex.

7.3.3.3 *Physically reversible network-forming hydrogels containing intermolecular interacting side groups that respond to photoisomerization*

An alternative type of photoresponsive hydrogels that have attracted a great deal of interest are the so-called supramolecular hydrogels. These hydrogels can undergo reversible sol−gel transitions in response to the applied photostimulus due to their reversible host−guest interactions. Different systems based on inclusions complexes between CD and azobenzenes have been reported (Zhao and Stoddart, 2009; Liao et al., 2010; Tamesue et al., 2010; Zhou et al., 2013; Wang et al., 2016). The polymers used in these systems are either PAA (Zhao and Stoddart, 2009) or PEG (Liao et al., 2010). The materials are attractive candidates for potential applications in drug delivery, optical sensors, and cell culture. Using an innovative approach, Harada et al. (Tamesue et al., 2010) prepared PAA bearing azobenzene side groups and glucan curdlan modified with α-CD side groups. In this study, the morphology of the supramolecular hydrogels was successfully photoregulated with an appropriate wavelength to control the formation of inclusion complexes. Azobenzene supramolecular hydrogels responding to red light were reported recently (Wang et al., 2015). The gelator precursors were synthesized by grafting PAA with tetra-ortho-methoxy-substituted azobenzene and β-CD (see Fig. 7.7). Supramolecular hydrogels were prepared by mixing the two modified polymers in PBS buffer in the dark. Red light irradiation triggered the disassembly of the supramolecular complexes by isomerization of the azobenzene. The sol-to-gel transition was reversible and could be induced either by blue light irradiation or heating.

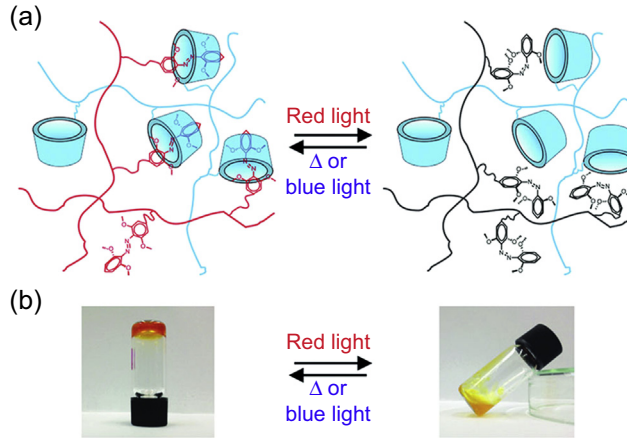

Figure 7.7 Schematic model (a) and photographs (b) of the reversible sol−gel transition of the poly(acrylic acid) (PAA)-Azo/PAA-β-cyclodextrin (CD) mixture.
Reproduced from Wang, D., Wagner, M., Butt, H.-J., Wu, S., 2015. Supramolecular hydrogels constructed by red-light-responsive host-guest interactions for photo-controlled protein release in deep tissue. Soft Matter 11, 7656−7662, copyright 2015, with permission from Royal Society of Chemistry.

7.3.3.4 Physically irreversible network-forming hydrogels based on the interactions of photocleavable side groups

Hydrolysis and enzymolysis are the two most common mechanisms employed for hydrogel degradation but neither allows time nor spatially controlled release of cells. In contrast, photodegradation allows external real-time spatial and temporal control over hydrogel degradation and allows for staged and sequential release of cells. The most common group utilized for photodegradable moieties is the o-methoxy nitrobenzene family of monomers. The ease of functionalization of the benzene ring has been used to generate a considerable number of monomers and cross-linkers that have been utilized for photolabile hydrogels (Jiang et al., 2015; Tibbitt et al., 2013a,b; Wong et al., 2010).

Photodegradable cross-linked polymer networks composed of poly(o-nitrobenzyl methacrylate) and PEG segments of variable molecular weights were synthesized using ATRP (Zhu and Bettinger, 2013). A library of amphiphilic linear ABA triblock copolymers with poly(o-nitrobenzyl methacrylate) A blocks and PEG B blocks was synthesized by Bettinger et al. (Zhu and Bettinger, 2015) and used in the preparation of photoreconfigurable physically cross-linked hydrogel networks. A quantitative model to correlate molecular-scale photolysis of NBMA groups with macroscopic mechanical properties was also proposed.

Macromonomers incorporating *o*-nitrobenzyl groups that have different photolysis rates were investigated by Kasko et al. (Griffin and Kasko, 2012a). Hydrogels were obtained by redox polymerization of the macromonomers, and the apparent rate constants of degradation were quantified by photorheology. Preferential release of one

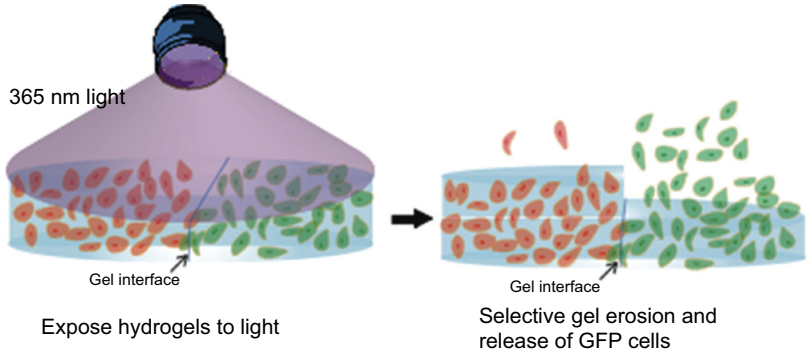

Figure 7.8 Wavelength-biased release of encapsulated cells. Macromers containing *o*-nitrobenzyl derivative with slower (left of interface) or faster degradation rate, respectively, (right of interface) were used to encapsulate red fluorescent protein−expressing human mesenchymal stem cells (hMSCs) or green fluorescent protein (GFP)−expressing hMSCs.
Reproduced from Griffin, D.R., Kasko, A.M., 2012a. Photodegradable macromers and hydrogels for live cell encapsulation and release. Journal of the American Chemical Society 134, 13103−13107, copyright 2012, with permission from American Chemical Society.

stem cell population (green fluorescent protein expressing human mesenchymal stem cells [hMSCs]) over another (red fluorescent protein expressing hMSCs) could be achieved very easily by exploiting the differences in photolysis reactivity of the two different *o*-nitrobenzyl linkers (see Fig. 7.8).

An eloquent example of photodegradable hydrogels was provided by Anseth. A photodegradable macromonomer cross-linker was synthesized by reacting PEG-*bis*-amine with a carboxy nitrobenzyl ester derivative (Kloxin et al., 2009). Hydrogels were obtained by redox polymerization of PEG acrylate in the presence of the cross-linker in PBS. On irradiation with UV light, the hydrogels degrade into modified carboxy-PEG and *o*-nitroso benzaldehyde modified PEG, without generation of low-molecular weight toxic compounds. 3D photopatterning by locally degrading the network of the hydrogels with two-photon laser scanning microscope was realized on a timescale of second to minutes generating controlled 3D cell culture microenvironments.

7.3.4 Analyte-responsive hydrogels

Analyte-sensitive hydrogels designed to exhibit swelling changes in response to specific (bio)molecules represent another class of smart hydrogels. Further on we will focus on giving representative examples for this class of materials, whereas for detailed information the review of Peppas et al. should be consulted (Culver et al., 2017). The sensing properties of such hydrogels are based on two mechanisms: the first one uses the activity of the target molecule itself (i.e., peptide hydrogels that degrade in response to the target enzyme), whereas the second one, more common, is the use

of a transduction pathway to convert the recognition of the target molecule into a pH, temperature, or electrical charge change that drives swelling, collapse, or degradation of the hydrogel (Koetting et al., 2015).

7.3.4.1 Glucose-responsive hydrogels

The measurement of glucose is very important in the treatment of diabetes and in monitoring cell growth (Bratlie et al., 2012). Therefore, precisely engineered glucose-sensitive hydrogels represent an important area of research. They could represent a more convenient and viable alternative to frequent insulin injections by acting as long-term insulin reservoirs that respond to increased glucose levels in the blood. Detection of glucose can be done by three methods: (1) incorporating glucose oxidase in the hydrogel and use it as a detecting enzyme, (2) using lectin-loaded hydrogels, and (3) using phenylboronic acid (PBA)−functionalized hydrogels. Each of these three strategies has advantages and disadvantages that have been highlighted and discussed in detail by Peppas (Peppas and Bures, 2008). A limited number of publications dealing with synthetic polymer hydrogels and glucose oxidase or lectins can be found in the literature (Gordijo et al., 2010). For this type of recognition, natural polymers and proteins are preferred. Therefore, we will focus our discussion on synthetic molecular recognition with molecules that can bind glucose such as PBAs. PBAs are Lewis acids known to reversibly bind *cis* diols, such as glucose. PBA hydrogels are very promising candidates for clinical use because they offer high detection (sensitivity) and they do not have the degradability and stability issues observed in glucose oxidase or lectin-based gels. Therefore, multiple and different chemical strategies have been used for the synthesis of glucose sensors based on PBAs (Dong et al., 2016; De Geest et al., 2006; Yang et al., 2014; Zhang et al., 2013; Horgan et al., 2006). For example, Pelton et al. (Hoare and Pelton, 2008) synthesized an amphoteric microhydrogel by copolymerization of AA as the anionic monomer and *N,N*-dimethylamino ethylacrylate as the cationic monomer. Afterward, the PBA functional groups were incorporated into the microgel via the "graft to" approach by reacting the carboxylic groups with 3-aminophenylboronic acid. The amphoteric PBA microgels had a high capacity for insulin uptake and could selectively release more insulin at higher glucose concentrations under physiological conditions. Another interesting approach is the fabrication of multifunctional ratiometric probes for glucose and temperature (Wang et al., 2011). The covalently cross-linked microgels were synthesized by emulsion copolymerization of *N*-isopropylacrylamide with *N*-acryloyl-3-aminophenylboronic acid and fluorescent dye functionalized monomers (see Fig. 7.9). The dyes were especially chosen to form a Fröster resonance energy transfer (FRET) pair. The spatial proximity of FRET donors and acceptors within microgels could be tuned via thermoinduced microgel collapse or glucose-induced microgel swelling at appropriate pH and temperatures. The MTT assays revealed that thermoresponsive microgels were noncytotoxic up to a concentration of 1.6 g/L, making them suitable for use in in vivo applications.

Using an elegant approach, Lowe et al. (Yetisen et al., 2014a) managed to develop a robust and accurate photonic holographic nanosensor that allows diagnosis of

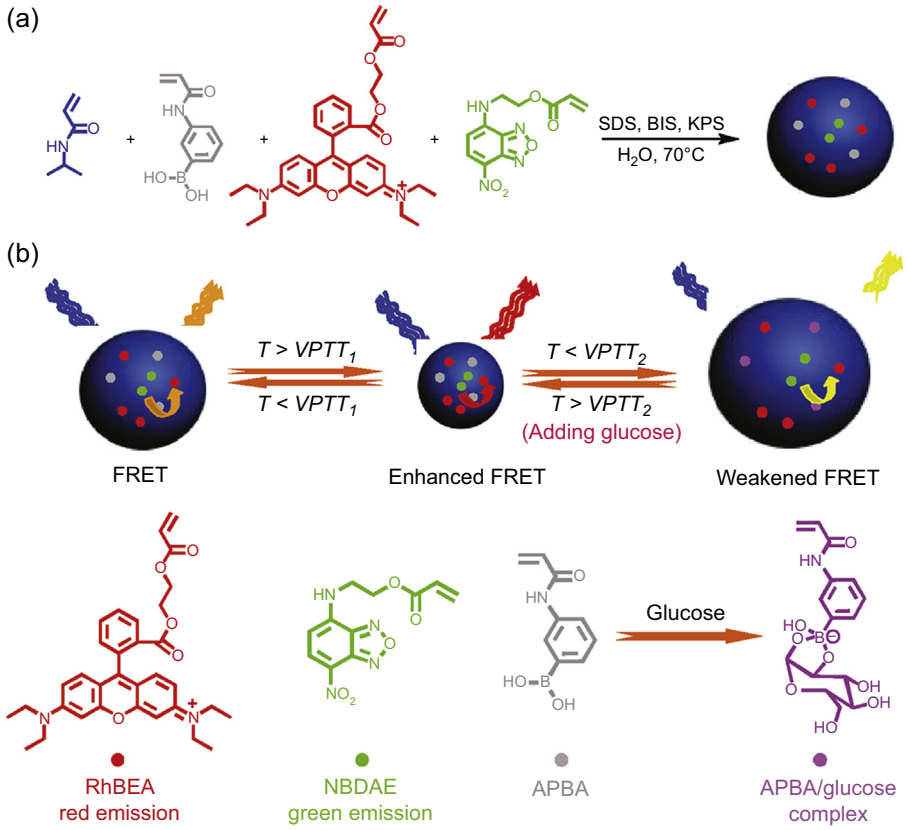

Figure 7.9 (a) Synthetic schemes employed for the preparation of thermo- and glucose-responsive fluorescent microgels via emulsion polymerization. *BIS,* methylenebis(acrylamide); *KPS,* potassium persulfate; *SDS,* sodium dodecyl sulfate. (b) schematic illustration for the modulation of Fröster resonance energy transfer (FRET) efficiencies within microgels by temperature variations and the addition of glucose. *VPTT*, volume phase transition temperature.
Reproduced from Wang, D., Liu, T., Yin, J., Liu, S., 2011. Stimuli-responsive fluorescent poly(N-isopropylacrylamide) microgels labeled with phenylboronic acid moieties as multifunctional ratiometric probes for glucose and temperatures. Macromolecules 44, 2282−2290, copyright 2011, with permission from American Chemical Society.

glucosuria in the urine samples of diabetic patients. Laser ablation in Denisyuk reflection mode was used to fabricate the diffraction gratings in polyacrylamide-based holograms. The diffracted light by the holographic sensor was visible with the naked eye and it shifted from green to yellow to orange to red before moving into near-infrared as a function of glucose concentration. Another notable aspect is that the performance of the sensor showed improved accuracy as compared with commercial colorimetric dipsticks read by automated readers and comparable accuracy with fully automated clinical chemistry systems.

7.3.4.2 Hydrogels responsive to other analytes

Other classes of analyte sensing hydrogels include but are not limited to glutathione (GSH) (Quinn et al., 2017), antigen (Miyata, 2010), and enzymes (Abul-Haija and Ulijn, 2014). The most employed method for the synthesis of enzyme-responsive hydrogels is to use peptides as cross-links in the hydrogels or as spacers between the polymer backbone and drug molecules (Glangchai et al., 2008). From synthetic polymers, polyethyleneglycol is the most investigated due to its biocompatibility, tunable properties, availability of end group modification and because it is bioinert (Secret et al., 2014). As an eloquent example, Phelps et al. (2012) developed bioactive PEG hydrogels using thiol−maleimide cross-linking chemistry. Four-arm maleimide-functionalized PEG macromers were first conjugated with thiol-containing RGD adhesive peptides, followed by cross-linking reaction in the presence of dithiol protease-cleavable peptide to form hydrogels. The protease-cleavable peptide cross-linker also played a significant role in cell encapsulation as demonstrated by the authors. Another noteworthy aspect is that the reaction did not require UV light and gelation was achieved in 1−5 min at slightly basic pH.

7.4 Applications

7.4.1 Applications in biomedical field

7.4.1.1 Drug delivery

Drug delivery applications require a precise control of the cargo release over time. Therefore, smart hydrogels are important candidates because the rate control of the drug can be controlled "on-demand" by external and/or internal stimuli. Moreover, smart hydrogels with specific, tunable, and even reversible responses have been developed. Epithelial barriers that have been exploited for drug delivery from hydrogels include skin, intestinal epithelium, and mucosa.

Temperature-responsive hydrogels have most extensively been studied for drug delivery applications. Applications of bulk hydrogels are limited to surgical implantation and transepithelial drug delivery (Li and Mooney, 2016). Consequently, epithelial barriers that have been exploited for drug delivery include skin, intestinal epithelium, and mucosa. Although PNIPAM is frequently used for drug delivery applications, its potential utility for in vivo application is limited due to unwanted cytotoxic effects at physiological temperature (Vihola et al., 2005). To overcome this problem, Wang (Wang et al., 2013) synthesized a poly(vinylcaprolactam) biodegradable hydrogel by precipitation polymerization (see Fig. 7.10). *N,N*-bis(acryloyl) cystamine was used as cross-linker to introduce biodegradable linkages in the structure, whereas AA was used as comonomer to provide pH-responsive properties. Erosion of the hydrogel structure in the presence of GSH or dithiothreitol led to degradation into soluble polymers due to the cleavage of the disulfide linkage. Cytotoxicity assays demonstrated that the blank microgels were nontoxic to normal cells. The hydrogels

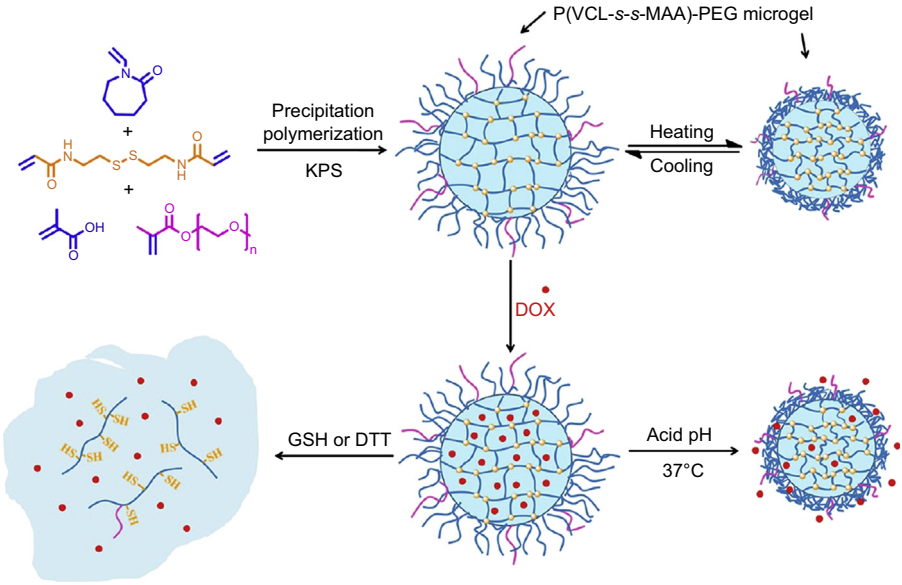

Figure 7.10 Schematic representation of the preparation, biodegradable behavior and stimuli-responsive drug release of poly(vinylcaprolactam) microgels. *DTT,* dithiothreitol; *GSH,* glutathione; *KPS,* potassium persulfate; *MAA*, methacrylic acid; *PEG*, poly(ethylene glycol). Reproduced from Wang, Y., Nie, J., Chang, B., Sun, Y., Yang, W., 2013. Poly(vinylcaprolactam)-based biodegradable multiresponsive microgels for drug delivery. Biomacromolecules 14, 3034−3046, copyright 2013, with permission from American Chemical Society.

could effectively encapsulate DOX and presented stimuli-triggered drug release in acidic or reducing environment.

In situ-gelling hydrogels that can be injected in liquid form and undergo a sol−gel transition inside represent a promising alternative (Boustta et al., 2014). Versatile UCST thermoresponsive hydrogels based on poly(*N*-acryloyl glycinamide) for regional sustained drug delivery were developed by Vert et al. By adjusting the molecular weight and the concentration of the polymer, the UCST of the solution could be easily controlled. Proteins, neutral, and ionic drugs soluble in water were loaded into the polymer solution and injected into mice. Release experiments proved that burst was minimal, and the release was progressive.

Hydrogels for oral drug delivery were extensively investigated by Peppas et al. (Caldorera-Moore et al., 2015; Kim and Peppas, 2003; Torres-Lugo and Peppas, 1999; Serra et al., 2006). Their focus was mainly on pH-responsive systems based on PAA (Sharpe et al., 2014; Vela Ramirez et al., 2017). pH-responsive hydrogels with enzymatic degradable oligopeptide cross-links were successfully used for insulin delivery to the small intestine. The hydrogels were stable to gastric enzymes but were degraded by trypsin (prevalent in the small intestine) due to the rich content of arginine and lysine groups in the oligopeptide used (Knipe et al., 2015). The scaffold of the

hydrogel was a pH-responsive poly(methacrylic acid-*co*-*N*-vinylpyrrolidone) copolymer, which was responsible for "regulating" the enzyme activity by allowing diffusion of enzymes into the polymer network due to pH-responsive complexation.

In photoresponsive hydrogels, on-demand drug delivery can be accomplished in two ways depending on the utilized chromophores: (1) by photoinduced swelling−deswelling or (2) by erosion of the hydrogel network due to the cleavage of photolabile groups. Although several examples of photoactuated drug delivery are reported in the literature (Tomatsu et al., 2011; Rastogi et al., 2017; Ninh et al., 2014; Bisht et al., 2016), they have a major drawback due to the limited penetration of visible and UV light. However, light-controlled degradation offers an interesting option due to the use of NIR-absorbing chromophores and to the possibilities of "burst release." Using an innovative strategy, Kasko et al. managed to photoselectively release three separate therapeutics from a hydrogel, allowing real-time spatial and temporal control over multiple chemical signals in a cell microenvironment in 2D and 3D (Griffin and Kasko, 2012b). By covalently linking the therapeutics through different *o*-nitrobenzyl linkers and by exploiting differences in reactivity and absorption of the *o*-NB groups, they could obtain complex, multistage release profiles of the model therapeutics from hydrogels.

Zhao et al. (Yan et al., 2012) managed to overcome the issues related to the UV absorption by combining the characteristic NIR-absorbing properties of upconversion nanoparticles (UCNPs). They used core−shell lanthanide−doped upconverting nanoparticles to convert NIR light into UV light, enabling UV light−induced photoreactions that would ultimately alter the structure of the hydrogel (see Fig. 7.11). Using this strategy, they could release "on-demand" large biomacromolecules. The biomacromolecules were inactive in the hydrogels but on NIR irradiation and subsequent release their bioactivity was recovered.

A transdermal caffeine−delivery system for preterm neonates was recently reported by covalently incorporating spiropyran and spirooxazine photoactive dyes into an amphiphilic polymeric conetwork membrane (Schöller et al., 2014). The permeability of the membrane could be easily adjusted by copolymer composition and by UV light. The authors developed an in vitro skin model to test the permeation of an aqueous caffeine solution through this membrane under UV and Vis light. When the spirooxazine was in the closed form, minimum release was detected, while switching to merocyanine state (e.g., open form) with UV light triggered the release.

7.4.1.2 Tissue engineering

Tissue engineering has emerged as a promising technology for the design of artificial tissues and/or organs using engineered (synthetic) materials. Hydrogel applications in this field are related to space filling agents, structures that organize cells, and present stimuli to ensure the development of a required tissue. The incorporation of hydrogels into the body greatly depends on the cellular adhesion onto the surface of the materials (Griffith, 2000). The process can be affected by several factors such as protein absorption, generation of fibrous tissue, which will compartmentalize the implant from the rest of the body, and other (Griffith, 2000). Although protein absorption can be

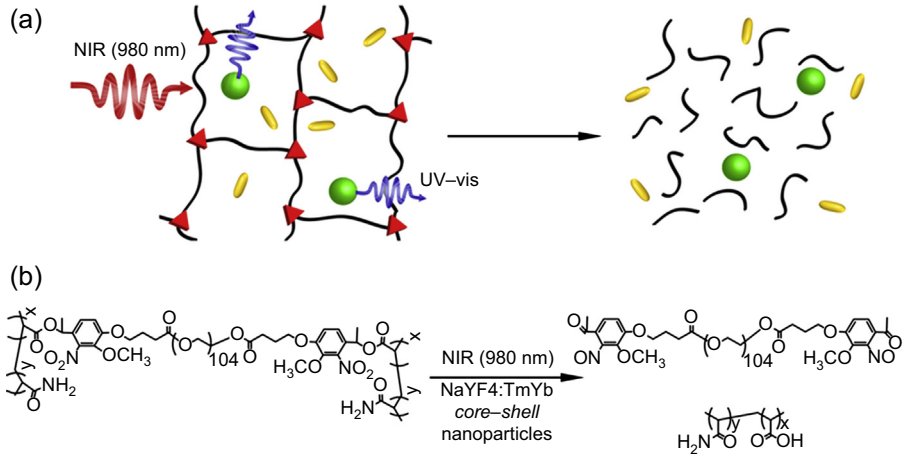

Figure 7.11 (a) Schematic illustration of the near-infrared (NIR) light−triggered degradation of a photosensitive hydrogel using the ultraviolet light generated by encapsulated upconversion nanoparticles. (b) Chemical structure of the hydrogel containing photocleavable *o*-nitrobenzyl moieties in the cross-linker.
Reproduced from Yan, B., Boyer, J.-C., Habault, D., Branda, N.R., Zhao, Y., 2012. Near infrared light triggered release of biomacromolecules from hydrogels loaded with upconversion nanoparticles. Journal of the American Chemical Society 134, 16558−16561, copyright 2012, with permission from American Chemical Society.

minimized by coating the surface with synthetic protein repellent polymers, it does not represent a viable solution because the material will remain biologically inert. Therefore, the need of new materials leads to the investigations of smart responsive hydrogels, due to their tunable properties and functionalization to promote organized cell growth and architecture (Knipe and Peppas, 2014). The synthetic hydrogels used as scaffolds include polyurethanes, pluronics, poly(vinyl alcohol), PAA, and PNIPAM.

Biodegradable thermosensitive PNIPAM-based scaffolds with controlled porosity were successfully synthesized by copolymerization with 2-methylene-1,3-dioxepane and polycaprolactone dimethacrylate (Galperin et al., 2010). The porous structure was realized by sphere-templating technique with poly(methylmethacrylate) particles, allowing facile access to controllable pore size (from 30 to 204 μm) and a highly interconnected porous structure. To demonstrate the potential for tissue engineering, the scaffold was loaded with NIH3T3 cells as a model system for many cell types. Scanning electron microscope images demonstrated cell attachment and infiltration throughout the scaffold, supporting the histological data. PNIPAM nanocomposite gels have also been used for cartilage repair due to their promising mechanical properties (Wang et al., 2012), whereas PEG hydrogels have been extensively used for engineering functional cardiac tissues (Young and Engler, 2011). Thiolated HA hydrogels were cross-linked with PEG diacrylate, and their dynamics were modulated by changing cross-linker molecular weight. The hydrogel stiffening could be tuned to mimic the mechanics of heart muscle during development (Young and Engler, 2011;

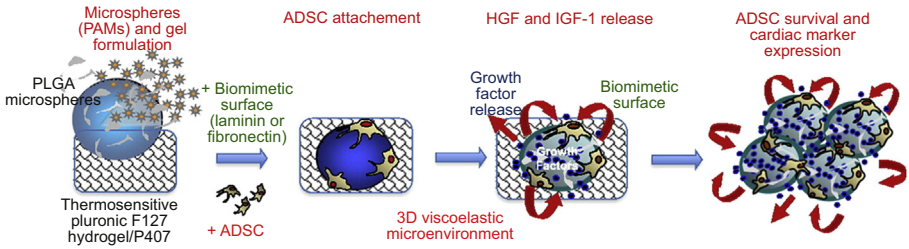

Figure 7.12 Synthesis and mechanism for myocardial repair of pharmacologically active microcarrier integrated in thermosensitive hydrogels. *ADSC*, adipose-derived stem cell; *PAM*, pharmacologically active microcarrier; *PLGA*, poly(lactic-co-glycolic acid).
Reproduced from Karam, J.-P., Muscari, C., Sindji, L., Bastiat, G., Bonafè, F., Venier-Julienne, M.-C., Montero-Menei, N.C., 2014. Pharmacologically active microcarriers associated with thermosensitive hydrogel as a growth factor releasing biomimetic 3D scaffold for cardiac tissue-engineering. Journal of Controlled Release 192, 82−94, copyright 2014, with permission from Elsevier.

Jongpaiboonkit et al., 2008). Potential in vivo applications were envisioned due to the possibility of slow degradation of the ester linkages present into the cross-linker. Temperature-responsive hydrogels were also used to create "cell sheets" for tissue engineering applications, offering noninvasive control of cell attachment and detachment, and preserving complete cell−cell junctions (Haraguchi et al., 2012).

In a recent report, Karam et al. (2014) developed pharmacologically active microcarriers (PAMs) with a biomimetic coating of laminin for cardiac tissue-engineering (Fig. 7.12). Poly(lactic-*co*-glycolic acid) microspheres were used as PAMs. The microcarriers induced adipose-derived stem cells commitment into cardiac lineage, while stimulating their proliferation. Taking one step further for in vivo applications the authors used an injectable thermoresponsive hydrogel (poloxamer P407) to integrate the PAMs. The hydrogel would mechanically support the beating myocardium without interfering with the electric signal conduction. However, further in-depth studies are needed to investigate how this system influences the recovery of cardiac function.

Dual thermal and chemically responsive composite hydrogels for bone tissue engineering have been reported (Vo et al., 2016). The polymeric scaffold was a PNIPAM macromonomer with a hydrolyzable lactone ring and epoxy pendant groups, which was cross-linked in situ with diamine-functionalized polyamidoamine. Gelatin microparticles were also incorporated into the hydrogel structure to provide sites for cellular attachment and to act as an enzymatically digestible porogen. Mesenchymal stem cells (MSCs) were successfully and homogenously encapsulated without detriment to cell viability or hydrogel gelation. By using microcomputed tomography, the authors proved that gelatin incorporation led to enhanced bone bridging and mineralization within the defect at each timepoint. Only the hydrogels that incorporated both MSCs and gelatin microparticles facilitated the osteoconductivity and osteoinductivity of synthetic scaffolds.

Smart hydrogels based on amphiphilic poloxamers labeled with RGD-containing peptides for skeletal tissue engineering were successfully obtained (Garty et al., 2010).

The materials allow a minimally invasive approach and could carry and support cell survival and viability. The sturdy structure of the hydrogel provided mechanical properties needed for cell attachment and its bulk matrix facilitated cell proliferation and tissue regeneration.

7.4.2 Sensors

Smart hydrogels can be very useful for detecting various kinds of analytes. Consequently, they can be used in a variety of sensing applications. In the following, the most important sensing applications will be briefly discussed.

Glucose detection is one of the most commonly studied sensing applications of smart hydrogels as quick and easy detection of glucose is desirable. The detection mechanism is based on the analyte-induced volume change of the hydrogel. A promising sensor for continuous monitoring of glucose in blood was developed by Stokke et al. A hydrogel containing PBA derivatives was covalently connected at the end of an optical fiber, and the readout was measured by an interferometric technique. Bulk hydrogels change their volume, but their response time is quite slow; therefore, micro- or nanogels have been developed for this application. Zhou et al. synthesized a hybrid nanogel made of Ag nanoparticle cores covered by a hydrogel shell of poly(4-vinylphenylboronic acid-co-2-(dimethylamino)ethyl acrylate) (Wu et al., 2010). The hydrogel shell is responsible for the detection of glucose, whereas the Ag nanoparticles convert the variation in glucose level to optical signals (i.e., fluorescence).

DNA sensors play a key role in genomics, disease diagnostics, and forensic science. Accordingly, easy and label-free DNA detection is highly desired. Radioisotopes, redox probes, or luminescent tags are usually employed to facilitate the detection of the DNA hybridization process. Hydrogels based on PNIPAM and PAA with or without Au nanoparticles have been developed for DNA detection at femto molar level (Kowalczyk et al., 2014, 2015). A simple biosensing platform, which involves the application of thermoresponsive hydrogels poly(NIPAM-*co*-AA), was reported recently (Kaniewska et al., 2016). The detection of target DNA sequences was achieved successfully by monitoring the VPTT. The main advantage is that no labeling with tags of DNA is required, but the detection limit is only 1 pM. Tan et al. reported colorimetric agent−caging hydrogels for visual detection of cocaine that relies on DNA base-pair recognition and aptamer−target interactions (Zhu et al., 2010). The hydrogel was suitable for micro- or nanopatterning opening an avenue for lab-on-a-chip devices for forensic analysis.

pH sensors have been developed for many years (Thong Trinh et al., 2006). pH-responsive polymer networks consist of a backbone polymer carrying weak acidic (i.e., MAA or basic (i.e., *N,N′*-dimethylaminoethyl methacrylate) groups. The backbone polymer provides a mechanical stability of the gel, whereas the ionizable group contributes to the pH sensitivity. pH-sensors employing free swelling hydrogels must be able to note the changes in one or more hydrogel properties. The most important sensing principles are optical, conductimetric, and oscillator resonant frequency (Richter et al., 2008). The key features of a pH sensor are sensitivity, response time,

and signal reproducibility. The volume change of the hydrogel within the phase transition results in a sensitivity per pH unit, which is in the order of 10^{-3}–10^{-5}. pH measurements outside of the hydrogel phase transition range are not recommendable. However, the overall measurement accuracy of most sensors is in the order of $\pm 10^{-2}$ pH units (Richter et al., 2008). The response time of sensors depends on the characteristic dimensions. In the nm range a sensor response within the upper millisecond range is obtained, whereas dimensions in the order of 100 μm result in response times of several minutes. Continuous pH monitoring by incorporating pH sensors in wound dressing is desirable for chronic venous leg ulcers and in pressure ulcers, where an increase in pH (i.e., alkaline or neutral pH) is a sign of infection, if compared with the normal surrounding skin (Schneider et al., 2007). Takahata et al. (Sridhar and Takahata, 2009) developed a hydrogel-based passive wireless sensor with a micromachined inductive transducer for such applications. The sensor is made from a poly(vinyl alcohol)–PAA hydrogel, which is placed in the gap between two-folded planar spiral coils that form the transducer. The volume variation of the hydrogel as a response to pH changes modifies the frequency response of the transducer. A spectrum impedance analyzer is used to detect the frequency variations. pH holographic sensing is a newly developed technique. Ionizable monomers are incorporated into a hydrogel films and converted into a volume hologram using a Nd:YaG laser (Marshall et al., 2003; Khan et al., 2017). Volume modifications due to change in pH are measured by the diffraction wavelength of the holograms. Holographic sensors can have milli-pH resolution sensitivity and are reversible in nature. This type of sensors was applied in ex vivo measurement of blood plasma and real-time continuous monitoring applications. More details about this topic can be found in the recent review of Yetisen et al. (2016).

Metal ion detection is very important because highly toxic and bioaccumulative heavy metal can cause serious health problems. Most studies focused on detecting Hg^{2+} and Pb^{2+} ions. Cyclic ligands functionalized with polymerizable groups are introduced in the structure of the thermoresponsive hydrogels by copolymerization with monomers such as NIPAM (Kuckling and Pareek, 2003; Petrovic et al., 2000; Luo et al., 2010). The ligand plays the role of an ion-signal sensor to selectively capture metal ions, and the PNIPAM serves as actuator to induce a sudden volume phase transition. However, the limitations of the existing sensors have motivated the investigation of photonic structures embedded in stimuli-responsive hydrogels that change their water content and volume on interacting with the metal ion. The volumetric change in the hydrogel is reported through modulations of reflection, diffraction, surface plasmon resonance, or emission. These optical changes act as transducers, allowing various light properties to be analyzed spectroscopically and correlated with the concentration of the analyte (i.e., metal ion) (Yetisen et al., 2016). Based on this principle, a series of holographic sensors for K^+, Cu^{2+}, Fe^{2+}, and Pb^{2+} have been developed (Mayes et al., 2002; Yetisen et al., 2014b; Hong et al., 2011). Yetisen et al. (2015) reported a photonic nanosensor consisting of nanocrystals (NCs) produced in situ within an ionically charged and functional hydrogel using laser writing. The principle of operation was simple, efficient, and elegant relying on the dynamic volume modulation of a poly(acrylamide-*co*-carboxylic acid) hydrogel,

which incorporates an AgBr NC Bragg grating. Quantification of Pb^{2+} and Cu^{2+} was possible over the range of 0.1−100 mM. Although the detection limit is quite high, optimization of the ratio between the hydrogel constituents is expected to increase the sensitivity of the sensor.

7.4.3 Actuators

One of the key features of stimuli-responsive hydrogels is their ability to undergo reversible, discontinuous, and large volume changes when subjected to external or internal stimuli. This ability of smart hydrogels to exhibit reversible "on−off" swelling behavior has been used to develop models mimicking various attributes of living systems such as motility and actuating functions. A large number of studies is devoted to the use of smart hydrogels as actuators in different research areas such as microfluidics, biotechnology, medicine, and so on (Ahn et al., 2008; Ionov, 2014). Chemically actuated hydrogels convert chemical energy into a mechanical response similar to living systems. The main drawback of the macroscopic hydrogels is the slow response rate; therefore, the use of micro- or nanogels is a prerequisite condition in applications requiring a very fast response. The possibilities and limitations of hydrogel-based actuators were briefly discussed in a recent review of Ionov (2014).

Thermally actuated hydrogels are mainly based on PNIPAM and pluronics, although some examples using poly(*N*-vinyl caprolactone) can also be found in the literature (Ahn et al., 2008). Chemically cross-linked hydrogels exhibit better mechanical strength and tunability of properties in comparison with the corresponding physical hydrogels. The main disadvantage is that the cross-linkers are not biocompatible or degradable, even though some recent steps into this direction have been made by using degradable cross-linkers (Ionov, 2013; Lee and Konst, 2014). In a recent paper, Aida et al. obtained an electrostatically anisotropic hydrogel actuator consisting of cofacially oriented unilamellar electrolyte titanate (IV) nanosheets embedded in a PNIPAM matrix (Kim et al., 2015). Because of the lower water uptake and release, the distance between the nanosheets rapidly expands and contracts on heating and cooling, respectively, so that the hydrogel lengthens and shortens significantly, even in air. The hydrogel could be programmed to undergo unidirectional mechanical motion through internal conversion of isotropic energy. Furthermore, the hydrogel was photohealable and suitable for photomicropatterning applications.

The change in shape of polyelectrolyte gels in response to external stimuli resembles the biological motions related to muscle and ciliary movement at the molecular level, resulting in biomimetic materials. Based on this ability of stimuli-responsive hydrogels to oscillate between swollen and collapsed states, Dong et al. (2006) managed to create a prototype of an artificial cornea. The microlens developed with the integration of pH-responsive hydrogels allowed for autonomous focusing and had a response time of 10 to a few tens of seconds. Reversible patterning and actuation of hydrogels by the ionoprinting technique was reported by Velev et al. (Palleau et al., 2013). This technique is facile, rapid, and can reversibly pattern ions in hydrogels in 2D and 3D by directionally controlled injection and binding of ions. The ionoprinting has been done with Cu^{2+} but it can be extended to biocompatible ions such as Ca^{2+} or

Zn^{2+}. The ionoprinted gel regions were stiffer than the unprinted ones, therefore allowing access to configurable shape memory soft materials and actuators such as grippers. The authors proved that organic solvent−induced swelling and contraction offer a rapid and facile actuation without affecting the binding of the complexed cupric ions. By controlling the orientation and spacing of the ionopatterned lines, a variety of geometric shapes could be created.

Photoactuated hydrogels convert light into mechanical motion, and consequently, they are important materials that can be implemented in a wide range of microdevices with future applications in medicine, robotics, and energy. By using two-photon and interference photolithography, one can have access to very complex repetitive 2D and 3D patterns (Han et al., 2016; Kasko and Wong, 2010), which are not accessible by other methods. However, they have some disadvantages such as low fatigue resistance, synchronizing the movement of the aligned chromophores with the movement of the polymer network and some biocompatibility issues. Recent efforts were focused on developing hydrogels based on spiropyran (Francis et al., 2017; Dunne et al., 2016). Copolymerization of AA with NIPAM and an acrylated spiropyran is used to effectively control the actuating properties of the hydrogel (Ziolkowski et al., 2013). Due to the relative pKa values of AA and the spiropyran and merocyanine isomers, the protonation and deprotonation occurs internally within the gel and there is no need for an external source of protons. The hydrogels reversibly shrink and swell in aqueous environments when exposed to different light wavelengths. These photoresponsive hydrogels can also change their surface topography upon exposure with visible light in a neutral environment (Stumpel et al., 2014). Depending on the cross-link density of the hydrogel, it is possible to switch between a ratchet and flat surface topography or even an inverse ratchet surface by using UV light. Schenning et al. developed reversible light-responsive hydrogel valves for microfluidics based on the above reported system (Ter Schiphorst et al., 2015). They study the effect of various substituents on the photoisomerization speed of spiropyran derivatives and the corresponding macroscopic impact on photoactuating properties (hydration−dehydration).

7.4.4 Self-healing

Nature has been a source of inspiration for scientist, but mimicking it is a challenging task. Therefore, self-healing hydrogels have attracted a lot of attention in the scientific community. Despite the considerable number of studies (Lim et al., 2014; Gyarmati et al., 2017; Wei et al., 2014), self-healing in permanently cross-linked networks remains a challenge due to the irreversible cross-links and presence of water. However, several strategies have been developed to overcome these drawbacks. Interactions used in self-healing hydrogels can be divided into two categories: dynamic covalent (Diels−Alder reactions, phenylboronate ester complexation, disulphide bonds, acylhydrazone bonds) and noncovalent (hydrophobic associations, electrostatic, guest−host, and metal−ligand coordination). Self-healing through *electrostatic interactions* is usually based on pH-responsive hydrogels. Wei et al. reported a facile method to obtained self-healable hydrogels based on the interaction

between PAA chemically cross-linked hydrogel and free Fe^{3+} ions (Wei et al., 2013). Higher self-healing efficiency could be obtained by increasing the concentration of Fe^{3+} ions. The self-healing of the hydrogel was repeatable and reproducible after many cycles of cutting and healing. Hydrogels that self-healed by electrostatic interactions between zwitterionic units, process called "zwitterionic fusion" was also reported (Bai et al., 2014). Moreover, the healing process enabled the connection of different cell−hydrogel constructs without compromising cell viability. *Host−guest interactions* have been used also to obtain self-healable hydrogels. CD is the most used host molecule, although crown ethers (Yan et al., 2014) and cucurbit[8] uril (Mckee et al., 2014) have been tested too. Harada et al. reported a redox-responsive self-healing hydrogel based on 6-amino-β-CD as the main host and ferrocene (Fc) as guest group (Nakahata et al., 2011). The groups were covalently attached to PAA and the hydrogel was formed by mixing the PAA-modified polymers. The redox-responsive Fc derivative mediated a reversible sol−gel phase transition by redox stimulus. The healing efficiency was 84%, but the storage modulus was 176 Pa, which indicates that they are very soft hydrogels. Consequently, their applications could be limited. The *self-healing of phenylboronate* hydrogels is based on the complexation between diols and boronic acid. Reversible boronate ester is formed in aqueous solution, and their stability is pH dependent. Messersmith et al. published a detailed study regarding self-healing of hydrogels derived from 4-arm PEG modified with catechol groups and cross-linked with 1,3-benzenediboronic acid (He et al., 2011). The hydrogel had self-healing properties at pH 9, whereas at neutral pH the gel turned into sol state. The hydrogel almost fully recovers its G' in 100 s when subjected to a large amplitude deformation (1000% strain). Similar results were reported on PNIPAM hydrogels modified with dopamine and cross-linked with boric acid (Vatankhah-Varnoosfaderani et al., 2014). The *disulfide and acylhydrazone* cross-linking had been applied more to self-healing of organogels, which is outside of the topic of this chapter. Still, a dynamic 3-arm PEG polymer hydrogel containing both acylhydrazone and disulfide bonds was synthesized by Chen et al. (Deng et al., 2012). The hydrogel was self-healable under a wide range of pH conditions; acylhydrazone repairs the damage in a mild acidic (pH = 3 and 6) environment, whereas the disulfide is responsible for self-healing at pH = 9. Moreover, all the self-healing processes were reversible and effective without an external stimulus at room temperature in air.

7.5 Conclusions and future perspectives

This chapter summarizes recent advances in stimuli-responsive hydrogels. We have highlighted different synthetic approaches, properties that can be switched and major applications of smart hydrogels. However, it should be noted that the research on this field is not limited to the examples presented in this chapter and the reader is always encouraged to consult the recommend reviews. Exciting progress has been achieved in this field from the fundamental perspective and from the applications point of view. Owing to the development of molecular design and synthetic technology

(i.e., controlled polymerization techniques), a variety of smart hydrogels have been developed. Moreover, advances in physics especially laser optics have generated a myriad of applications based on stimuli-responsive hydrogels. While most of these advancements remain proof of concept, some of these functional aspects have been harnessed toward biomedical applications such as drug delivery devices, cell sheet engineering, and sensing. Thermo- and pH-responsive materials have almost reached their practical realm, whereas light-responsive hydrogels are still far away from reaching their full potential. A noteworthy feature of light-responsive hydrogel is that they can be cleaved photochemically with near-IR light by two-photon absorption processes to alter polymeric system's properties on demand. The versatility in synthesis is also a trait that favors wide architectural compositions. However, they lack the chemical reversibility of the photoisomerization processes, and some of the photocleaved small molecules that are released in the process are often toxic. Analyte-sensitive hydrogels combined with photonics represent viable solutions in the field of sensor.

In the field of drug release, exciting progress has been made at the proof of concept level but translation to clinical applications is slow. Although inspired by nature, hydrogels with self-healing capabilities are not a viable solution yet for implants in long-term use. Self-healing should be autonomous, while shape memory is triggered by an external stimulus, which is apparently contradictory. Moreover, the majority of self-healing gels have poor mechanical properties, which limits their applications severely. Consequently, the need for strong self-healing hydrogels is stringent.

The desire to have a hydrogel that is sensitive to all important stimuli, such as temperature, light, pH, and analyte, while preserving good mechanical properties has become the "Philosopher's stone" for many scientists. The question arising from this quest is: "should scientist focus their efforts on finding such "panacea" material or should they focus their efforts on optimizing the chemical structure and architecture in response to the desired applications."

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Oleogels

8

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8.1 Introduction

Gels or semisolid formulations can be found in a wide range of industries and applications such as biomedical, biotechnology, food, petrochemical, and coating. Their utilization in such a wide range of applications arises from the diverse physiochemical properties achieved using different formulations.

Generally, gels can be referred as systems with high concentration of solvent confined with low concentration of structurant agent, which form the gel skeleton. The gel skeleton can be formed by small molecules that self-assemble in different structures (Buerkle and Rowan, 2012; Skilling et al., 2014) or by polymer network (Suzuki and Hanabusa, 2010; Rubinstein and Colby, 2003). The structural elements building the three-dimensional (3D) network are formed by physical or chemical interactions. Physical interactions involve hydrogen bonds, electrostatic interactions, van der Waals forces, hydrophobic forces, etc. Such interactions are strongly affected by environmental conditions such as pH, temperature, ionic strength, etc., thus can be altered for specific application (Buerkle and Rowan, 2012; Skilling et al., 2014). Chemical interactions, on the other hand, involve the formation of permanent covalent bond. Such bonds cannot be manipulated thus leading to highly stabilized network (Rubinstein and Colby, 2003).

Gel formulations can be divided into two major classes based on the solvent used for their production; water-based gels, termed *hydrogels*, and organic liquid–based gels, termed *organogels*. The formation of these gels is achieved using low-molecular weight gelators such as peptides and fatty acids (FAs) or high-molecular weight gelators such as polymers and proteins. The aqueous environment presence in hydrogels provides an easy platform for physiological application thus hydrogel systems are intensively explored (Ahmed, 2015; Hoare and Kohane, 2008). Organogel systems, on the other hand, offer a different spectrum of adjustable physical properties together with additional unique chemical properties, such as hydrophobicity and antibacterial characteristics, resulting from a lack of aquatic media (Skilling et al., 2014; Vintiloiu and Leroux, 2008). Many studies focused on organogel systems, however, only few were aimed for pharmaceutical or biomedical applications. This is mostly due to the toxicity and biocompatibility issues related to the organic solvents and the structurant type used (Vintiloiu and Leroux, 2008; Murdan, 2005). Most pharmaceutical formulations studied to date involve the use of edible oil as their organic solvent. Edible oil is a renewable resource with a generally recognized as safe status thus can be used in physiological-based applications.

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The current manuscript will review various edible oil-based gel formulations and their potential use in different applications.

8.2 Oil structuring

Oil structuring, or oleogelation, is the process of giving solid properties to liquid edible oil. Liquid edible oils are unique solvents due to the variety of molecular structures comprising them. The building blocks that make up fats and oils constitute, for the most part, a variety of triacylglycerol (TAG) molecules. TAGs molecules comprise from a triple-alcohol glycerol backbone attached to three FAs via an ester bond (Marangoni, 2012). Two major categories of FAs can be found: *saturated* verses *unsaturated*. Saturated FAs typically exhibit higher melting temperatures compared with unsaturated FAs thus producing solid structure at room temperature (O'Brien et al., 2000). Such structure is a result of molecular self-assembly of TAGs into lamella crystal structures termed *nanocrystal platelets* (Acevedo and Marangoni, 2015). The platelets further associate through one-dimensional stacking to create larger scale crystal clusters responsible for the final fat structure and properties (Peyronel et al., 2014). This is a temperature-induced process that results in the crystallization of the solid TAG molecules into a crystal network that physically binds the liquid TAG molecules into it (Marangoni, 2012). Unsaturated FAs, on the other hand, consist double bonds along the FA backbone thus can be divided into two group; *cis* and *trans*. Cis-FAs have truncated backbone therefore have low-melting temperature. Trans-FAs have a relatively linear backbone thus demonstrate higher melting temperature similar to saturated FAs (Akoh and Min, 2008).

Oleogel formation is based on the mixture of a structuring agent—a *structurant*—together with liquid oil, which self-organize further to create a stable 3D network. Oleogels can be classified into two major groups depending on the molecular architecture used; low-molecular weight oleogelators (LMOGs) and high-molecular weight oleogelators (HMOGs), Fig. 8.1. LMOGs mimic the natural ability of TAGs to self-assemble and crystallize to form an organized fat structure (Marangoni, 2012). These structures are formed by hierarchical assembly of gelator molecules governed by weak physical molecular interactions such as hydrogen bonding, $\pi-\pi$ stacking, van der Waals forces, electrostatic interactions, dipole forces, and hydrophobic forces. Those assemblies form 3D architectures such as tapes, rods, fibers, and sheets (Vintiloiu and Leroux, 2008). The gelation mechanism is based on aggregation processes initiated by external forces such as temperature and shear. The molecular self-assembly and aggregation processes strongly depend on the gelator's solubility/insolubility equilibrium, which is highly affected by environmental conditions such as solvent characteristics, temperature, etc. (Vintiloiu and Leroux, 2008). The 3D LMOG network acts as a skeleton and confers strength and resilience to the oleogel network (Murdan, 2005). Hence, its physical properties are strongly affected by the primary structurant geometric characteristics such as

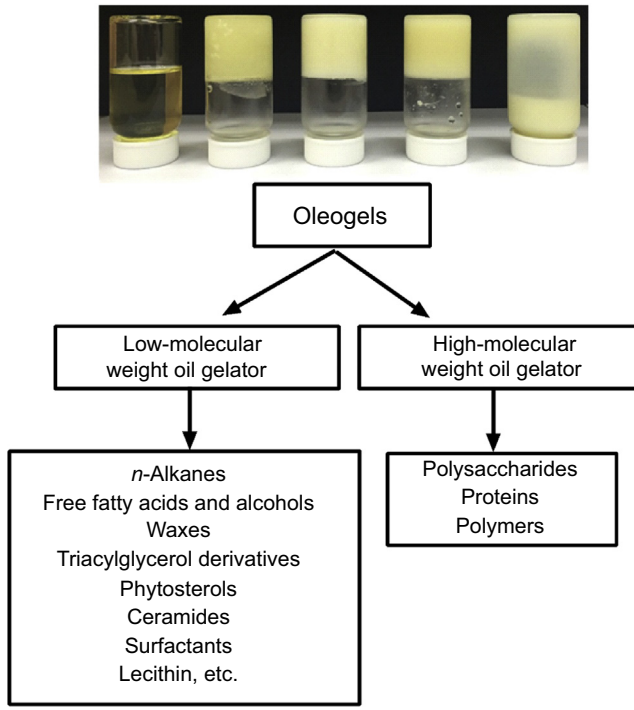

Figure 8.1 Various oil structuring approaches used up to date.

surface area and volume, parameters that are directly affected by cooling rate and shearing during gelation (Skilling et al., 2014). A variety of LMOGs can be found in the literature based on: *n*-alkanes, FAs and fatty alcohol (FO), waxes, mono- and diacylglycerols, phytosterols, ceramides, surfactants, lecithin, and others, all of which able to self-assemble and create crystalline 3D networks, which maintain the liquid oil within (Co and Marangoni, 2012; Marangoni and Garti, 2011).

Less common are oleogel systems based on HMOGs such as ethyl-cellulose (EC), water-soluble polysaccharides, and proteins, which do not form gel network using crystallization processes (Marangoni and Garti, 2011; Patel et al., 2014b, 2015a; Mazzenga, 2011; Vries et al., 2015). In comparison with LMOG, HMOGs demonstrate different properties arising from the extended polymeric chain characteristics and their gelation mechanisms (Suzuki and Hanabusa, 2010; Sagiri et al., 2014). The polymer chains are usually organized through physical interactions, which facilitate the supramolecular network (Suzuki and Hanabusa, 2010). Such interactions lead to reversible dynamic network strongly affected by the polymer molecular weight and concentration (Suzuki and Hanabusa, 2010; Sagiri et al., 2014).

The differences in molecular architecture, which assemble the network skeleton, lead to different gel properties. Therefore, recent studies have used combination of

different gelator molecules to broaden the gel characteristics using different gelation mechanisms (Gandolfo et al., 2003; Gravelle et al., 2017; Lopez-Martínez et al., 2015). Such approach can potentially enhance the use of oleogels in various applications.

8.3 Types of oleogelators

8.3.1 Low-molecular weight oil gelators

8.3.1.1 n-*alkanes*

n-alkanes, also termed paraffins, are a saturated hydrocarbon chains composed of single carbon−carbon bonds with hydrogen atoms attached to the carbon free spaces. Such simple organic structures are capable to organize in organic solvent and form lamellar crystal structures. These lamellas further aggregate into crystal platelets, which form 3D crystal network in organic solvents (Abdallah et al., 1999; Abdallah and Weiss, 2000). Self-assembly process is driven by relatively weak physical interactions such as van der Waals forces, electrostatic forces, π-π stacking, or even London dispersion forces. Due to the lack of functional groups necessary for these molecular interactions, London dispersion forces are assumed to be the dominant force involved in *n*-alkanes self-assembly (Mallia et al., 2009). Different *n*-alkanes length between 24 and 36 carbons in silicon oil demonstrated gelling ability depending on the molecular length scale. Decrease in the minimum molecular concentration for gel formation was observed with increase in molecular length. Moreover, increase in gel stability was observed while increasing the hydrocarbon length. This behavior was attributed to the relative heats of sublimation and solubilities of the *n*-alkane gelators (Abdallah and Weiss, 2000). Further examination of the molecular conformation and packing of *n*-alkane C36 verified the formation of lamellar platelets structures, which further organized into 3D crystal network (Abdallah et al., 2000). This type of systems is extensively used in petrochemical applications specifically for oil spillage treatments (Ohsedo, 2016). Modification of alkane chains using functionalized groups along the primary alkane chain was also suggested as a way to improve network stability and functionality (Mallia et al., 2009; Mallia and Weiss, 2014). Functional groups, such as hydroxyl end group, can be inserted at various positions along the carbon chain, i.e., at the end or in the middle. Such modification could lead to additional potential interaction between molecules based on other physical interaction such as dipole−dipole and H-bonding (Rogers and Weiss, 2015). For example, 12-hydroxylated stearic acid (12HSA) is an 18-carbon alkane with carboxylic acid at the end and hydroxyl side group at the 12th carbon. This molecule has a chiral carbon at the 12th position, which demonstrated a good oil structuring ability. Different self-assembled structures and gel properties were obtained using pure D-12HSA or racemic mixture of DL-12HSA (Mallia and Weiss, 2014; Rogers and Weiss, 2015). The structuring ability of DL-12HSA in vegetable oil was affected by the storage temperature

(Rogers et al., 2009a), gelator concentration (Rogers et al., 2009a), and oil type (Gao et al., 2013; Wu et al., 2013). Further examination of the gelation process and the prediction of the oil structuring ability of DL-12HSA were performed using the Hansen Solubility Parameters (HSP) where a direct correlation between critical gelator concentration (CGC) and polar HSP formed was obtained (Liu et al., 2015). Ricinelaidic acid (REA), a derivative of HSA, was found to gel vegetable oils, depending on concentration, temperature, and the presence of hydrogen-bonding moieties in the oils (Wright and Marangoni, 2006, 2007). The addition of hydroxyl or carboxylic end group at the end of alkane chain produces FA or FO, which will be discussed in detail in the next section.

8.3.1.2 Free fatty acids and fatty alcohols

The oil structuring ability of FAs, FOs, and their mixtures was suggested due to the fatty acids and alcohols biocompatibility with physiological environment. Homologous series of FA and FO with chain lengths ranging between 16 and 22 carbons was studied. Linear relationship between the gel hardness and oleogelator concentration was detected for both FA and FO. Decrease in hardness values with length chain was observed during concentration increase while using FA (Gandolfo et al., 2004). FA and FO demonstrated varied mechanical properties depending on the acid/alcohol ratio (Gandolfo et al., 2004; Schaink et al., 2007). A distinct maximum in mechanical properties was observed at specific acid/alcohol ratio depending on the FA and FO chain length (Gandolfo et al., 2004). For example, in stearic acid (SA)/stearyl alcohol (SO) mixture, the maximum appears at 3:7 acid:alcohol ratio. This behavior was attributed to crystal size and morphology (Schaink et al., 2007). Recent study on the SA:SO crystallization behavior concluded that the mechanical strength enhancement cannot be solely related to the crystal size and morphology. Additional significant effects can be related to the scaling exponent of the hardness to the mass fraction of the crystalline material, i.e., the spatial distribution of the mass (Blach et al., 2016).

8.3.1.3 Waxes

Waxes are commercially available as a mixture of organic components with high content of wax esters and other components such as hydrocarbons, FAs, and FOs. Due to the large component variety, waxes display a broad melting profile with different temperature range depending on the wax source. The differences in chemical composition of different waxes also lead to different oil structuring ability (Patel, 2015a). Various studies have demonstrated the effect of wax composition on the nanostructure and mesoscale characteristics (Blake et al., 2014; Hwang et al., 2012; Patel et al., 2015b). For example, sunflower wax with high content of wax esters produced gel network in high oleic sunflower oil at relatively low concentration compared with beeswax, which required higher concentration and has high content of FAs. In addition, these samples demonstrated different morphologies where sunflower wax forms a rodlike crystals and beeswax forms spherical crystals. Moreover, the resulted

gel formulations demonstrated very different rheological and mechanical properties based on the wax used (Patel et al., 2015b). Other study examined wax samples from different sources and manufactures with soybean oil and concluded that wax ester with longer alkyl chains produces gel network more effectively than short alkyl wax ester. In addition, they have concluded that the minor component presence in the wax sample has a major effect on the gelation ability and gel properties (Hwang et al., 2012).

8.3.1.4 Triacylglycerol derivative

The natural ability of TAGs to self-assemble and form crystal network known as fat can be exploited to produce oil structuring system. Mixtures of low-melting point and high-melting point TAGs were examined for their oleogelation ability. It was concluded that gel properties such as melting/crystallization temperatures, morphology, and solid fat content varied in the mixture state compare the original component characteristics, i.e., high- and low-melting TAGs thus suggesting a possible interaction between the components leading to different behavior (Higaki et al., 2003). The use of monoglycerides (MAGs) as oil structuring agent was studied extensively using various MAG types and oils (Pieve et al., 2010; López-Martínez et al., 2014; Chen and Terentjev, 2011). MAGs are able to structure oil at relatively low concentration thus can potentially reduce the level of saturation in food while maintaining texture attributes (Chen and Terentjev, 2011). The effect of diglycerides (DAGs) on TAG crystallization was previously discussed (Wright and Marangoni, 2002; Oliveira et al., 2014); however, the use of DAG as oil structuring agents is less common. Oleogel formulations with stearin fraction of palm-based DAGs and various vegetable oils were examined as shortening blend. High DAG concentrations in the range of 50%−60% were required to produce acceptable shortening blend (Latip et al., 2013). Their ability to structure oil was found to be limited compared with other structuring agents such as TAG and MAG (Pernetti et al., 2007b).

Different TAG derivatives based on SA were examined for their oil structuring ability in canola oil, Fig. 8.2. The oil structuring ability was evaluated using texture profile analysis. According to the results, it is clear that each derivative demonstrates different oil structuring ability depending on the molecular architecture. According to these results, it appears that MAG demonstrating the higher structuring ability compared with other TAG derivatives. This observation may explain the extensive study done on MAG oleogelation compared with the other derivatives.

8.3.1.5 Phytosterols

Mixture of phytosterol with phytosterol ester produces stable oleogel system (Bot and Floter, 2011). More specifically, mixtures of γ-oryzanol and β-sitosterol produced transparent oleogel using sunflower oil. The gel network was attributed to the formation of tubular structure with diameter varies between 6.7 and 8.0 nm

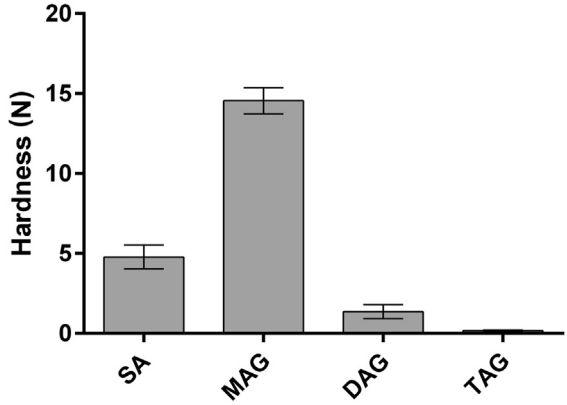

Figure 8.2 Hardness values of 10 wt% triacylglycerol (TAG) derivatives based on stearic acid (SA) in canola oil. *DAG*, diglyceride; *MAG*, monoglyceride.

and wall thickness between 0.8 and 1.2 nm, formed by the two phytosterols (Bot et al., 2009). This structure was also seen using various sitosterol molecules such as ergosterol, stigmasterol, cholesterol, and cholestenol (Bot et al., 2008, 2009; Bot and Agterof, 2006). The molecular self-assembly was strongly affected by the oil viscosity and polarity leading to longer gelling time in high viscous oil such as castor oil compared with low viscous oil such as sunflower oil. Moreover, gels prepared using low viscous oils demonstrated better mechanical properties compared with gel prepared using high viscous oil. It was suggested that the molecular self-assembly and packing of β-sitosterol and γ-oryzanol was hindered due to the oil viscosity and polarity (Calligaris et al., 2014). Microstructural analysis of γ-oryzanol and β-sitosterol oleogel formulation with sunflower oil reveals a 3D bundle-like structure suggesting the ribbons created by the self-assembly process aggregate in bundles in 3D (Matheson et al., 2017).

8.3.1.6 Ceramides

Ceramides are the simplest sphingolipids derived by the amidation of FA onto the amine group of sphingosine. They can be produced synthetically using chemical catalysis or by using enzymatic routes. The final product can potentially exhibit a structural diversity in terms of chain length and the sugar side group. Ceramides play a role in the signal transduction pathway responsible for cell division and death thus play a role in cancer development. Evidents regarding its involvement in cholesterol level reduction were also demonstrated (Rogers et al., 2011).

Ceramides from natural and synthetic sources exhibited oil structuring ability using canola oil. This ability was attributed to the formation of ceramide bilayers forming lamellar structure. Concentration as low as 2 wt% was used to form stable gel (Rogers et al., 2009b).

8.3.1.7 Surfactants

Surfactants tend to self-assemble under various conditions such as concentration, pH, temperature, solvent type, ionic strength, etc. In oil phase surfactants tend to organize in lamellar structures thus forming crystal platelets. These platelets further aggregate and form 3D, which stabilizes the oil phase (Murdan et al., 1999; Peyronel and Marangoni, 2014). Several formulations have been studied in the literature, including sorbitan monostearate (SMS) (Murdan et al., 1999; Peyronel and Marangoni, 2014; Sánchez et al., 2008), glycerol monostearate (GMS) (Sánchez et al., 2011b), and sorbitan monopalmitate (SMP) (Shah et al., 2013). It was demonstrated that oleogel mechanical properties were strongly influenced by the surfactant type, concentration, and oil type used (Sánchez et al., 2011b). Generally, GMS produced stronger gels than SMS (Sánchez et al., 2011b), whereas SMS demonstrated higher thermal and physical strength compared with SMP (Shah et al., 2013). Recently, a comprehensive study on the gelation ability of four different food-grade surfactants, namely, glyceryl tristearate (GTS), sorbitan tristearate (STS), SMS, and GMS, in medium-chain triglyceride and high oleic sunflower, was conducted. In general, it was concluded that both oil type and surfactant head group affect the oleogel physical properties. More specifically, structural analysis showed that all surfactants organized in lamellar structure with different d-spacing values, which allude to differences in the rheological behavior (Cerqueira et al., 2017).

8.3.1.8 Lecithin

Lecithin is a major phospholipid component, which plays a vital role in cellular function and in transport of lipids. Solutions of lecithin in organic solvents can be transformed into transparent gels by addition of a critical amount of water (Scartazzini and Luisi, 1988). The formation of these gels is based on the ability of lecithin to self-assemble and form reverse hexagonal tubules micelles. The gel network forms due to branching and overlapping of these bundles at specific junction zones along the reverse micellar chain (Bodennec et al., 2016). Spherical micellar aggregates were also detected during lecithin gelation. The nature of the apolar solvent was found to be the source of each structural architecture, i.e., spherical or tubular micelles (Shchipunov, 1996; Angelico et al., 2005).

8.3.1.9 Others

Other LMOGs that can be found in the literature include shellac waxes, sugar-based gelators, amides, etc. These oleogel systems represent the attempts to develop new gelator materials.

Shellac is a natural material composed of a complex mixture of polar and nonpolar components consisting of polyhydroxy polycarboxylic esters, acids, and alkanes, which tends to self-assemble into colloidal structures based on the solvent used. The ability of this material to gel liquid oil at a concentration as low as 2 wt% was related to the ability of the molecule to form crystal network (Patel et al., 2013b,c).

Medium-chain sugar alcohol−based gelators, namely, mannitol and sorbitol dioctanoate, have been suggested as structuring agent. This type of molecules offers added nutritional values arising from the fact that sugar alcohol are nonreducing and have low calorific/glycemic indexes. Oil structuring ability with nanoscale multilayered structures was demonstrated in various edible vegetable oils, i.e., canola oil, olive oil, soybean oil, and grape-seed oil, using critical gelation concentration as low as 1−3 wt% (Jadhav et al., 2013).

Amides can form hydrogen bonds using both the oxygen and nitrogen atoms. Therefore, addition of primary or secondary amide functional group to hydroxylated SA can increase the number of hydrogen-bonding sites thus can potentially stabilize molecular aggregation. This approach was taken to produce novel gel systems with improved structuring ability and mechanical properties. These gel thermal parameters, solid content, microstructure, and rheology properties were not affected by the cooling rate due to the additional binding sites. In addition, these gelators seem to crystallize as fibrillar spherulites (Toro-Vazquez et al., 2013).

Novel supramolecular gels were prepared using amino acid derivatives with paraffin oil. 1-hexadecyl amino acid ester demonstrated oil structuring ability with minimum CGC in the range of 0.7−1.5 wt% depending on the specific gelator side chain (Yu et al., 2016). L-alanine derivatives also demonstrated oil gelation ability using vegetable and synthetic oils approved for parenteral administration, however, higher concentration above 2.5 wt% was required. Hydrogen bonds and van der Waals interactions were shown to be the main forces implicated in the network formation (Couffin-Hoarau et al., 2004; Motulskya et al., 2005). Cinnamic acid (CA) was also studied as oil structuring agent using rice barn oil. Stable crystal−based gel network was obtained using CA concentration as low as 4 wt% (Li et al., 2017).

8.3.2 High-molecular oil gelators

8.3.2.1 Cellulose derivatives

EC is a linear polysaccharide derived from cellulose. Its production involves the replacement of hydrogen in the hydroxyl end group with ethylene molecule (Davidovich-Pinhas and Marangoni, 2016). This process leads to the formation of hydrophobic molecules compatible with various organic solvents (Koch, 1937). Gel formation in liquid oil is based on polymer dissolution above the ECs glass transition, i.e., $\approx 140\ ^{\circ}C$, and cooling to room temperature (Davidovich-Pinhas et al., 2015). Due to the role of hydrogen bonds in the network formation, cooling conditions and storage temperature were found to affect the gel mechanical properties, Fig. 8.3 (Davidovich-Pinhas et al., 2015). EC is the only polymer known to date capable of structuring edible oil directly. Other cellulosic derivatives demonstrating oil structuring ability in castor oil are methylcellulose (32% methoxy), ethyl acetate (40% acetyl content), and α-cellulose (Sánchez et al., 2009).

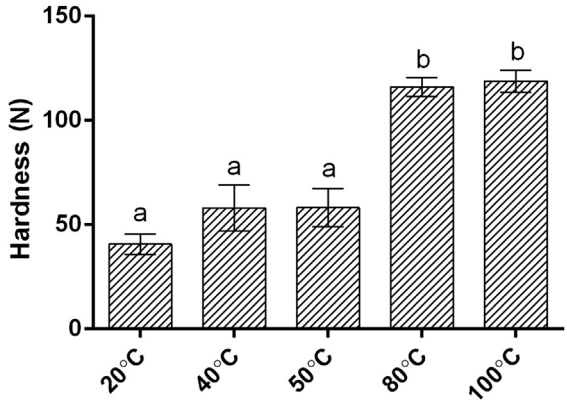

Figure 8.3 The effect of annealing temperature on the gel hardness of 15 wt% ethyl-cellulose 20 cP in canola oil. Values followed by the same letter are not significantly different ($P > 0.05$).

8.3.2.2 Proteins

The use of proteins as oil structuring agents is a great challenge due to the chemical nature of its building blocks, i.e., amino acids, which usually do not dissolve in oil phase. The first work done in this field was based on self-assembled monodisperse oil-in-water emulsion, where the oil droplets were stabilized by a cross-linked protein monolayer adsorbed at their interface. The water phase was then evaporated thus producing a structure resembling a dry foam matrix where the protein network constitute the walls of the foam and the air is replaced by oil forming closely packed droplet array. The elastic gel physical properties can be tuned by changing either the average diameter size of the emulsion template or the cross-linking process of the protein film (Romoscanu and Mezzenga, 2006).

More recently, oleogel system was developed based on whey protein isolate (WPI) through solvent exchange procedure. WPI hydrogels were prepared by thermal treatment and then solvent exchange procedure using intermediate solvent was performed in order to obtain oil-based gel (Vries et al., 2015, 2017a). The gel properties were found to be affected by the intermediate solvent used (Vries et al., 2015), oil type (Vries et al., 2017b), temperature, and water content (Vries, 2017).

8.3.2.3 Water-soluble polysaccharides

The formation of hydrogel using polysaccharides is well studied; however, the use of these polymers for oil structuring is less common. Hydroxy propyl methylcellulose (HPMC) was used to form an aqueous foam, which was subjected to freeze-drying process to remove water content, resulting in a formation of porous cryogel. Oil was then absorbed into the porous cryogel using shearing. Surprisingly, this porous material showed excellent oil sorption property, absorbing close to 100 times its weight by oil (Patel et al., 2013a). Additional strategy to form water soluble−based oleogel system uses oil-in-water emulsion stabilized by polysaccharides such as

HPMC, methylcellulose, and xanthan gum. In this approach, the polysaccharide was dissolved in the water phase followed by oil-in-water emulsion preparation finalized by water dehydration to form the oleogel system (Patel et al., 2014b,c). Hybrid protein−polysaccharide systems were also suggested using the same water-continuous emulsion procedure (Patel et al., 2015a). The use of chitin, chitosan, and acylated derivatives as thickener agents of vegetable oils was also suggested (Sánchez et al., 2011d).

8.3.3 Hybrid oleogels

Hybrid oleogel formulations composed of two types of gelator molecules exploit synergistic effects, which can potentially contribute to the oleogel performance and functionality. In addition, gelator concentration is sometimes restricted due to regulation issues and can be overcome using combination of gelators.

In the literature, one can find hybrid oleogels composed of two different types of LMOG, which together exhibit different and improved properties. For example, combination of SO and SA using various ratios demonstrated very different mechanical properties (Blach et al., 2016). Combination of α-tocopherol and phosphatidylcholine presence in lecithin in 1:1 ratio produced strong and stable oleogel matrix in sunflower oil (Nikiforidis and Scholten, 2014). The combination of lecithin with surfactant, STS, was also studied (Pernetti et al., 2007a). Three-component mixture of SA, sucrose erucate, and tea polyphenol in peanut oil was used to form oleogel system with natural antioxidant activity (Shi et al., 2014). Crystallization behavior can be modulated using combined crystal entities, i.e., crystalline molecules. The addition of surfactants, i.e., SMS and polyethylene glycol sorbitan monolaurate, to SA oleogel produced different crystal structure and properties (Uvanesh et al., 2016a,b).

Fig. 8.4 demonstrates the sol−gel transition obtained for lauric acid (LA), behenic acid (BA), and their 50:50 mixture during cooling using total FA concentration of 30 wt%. According to these results, the sol−gel transition of LA and BA occurred as a sharp step of approximately 4 order of magnitude in G′ values at approximately 20 and 55 °C, respectively. However, their 50:50 ratio mixture demonstrated a more moderated sol−gel transition at 42 °C. These results suggest that combination of the two oleogelators produces different crystal network with various gel properties.

Combination of two HMOGs, i.e., gelatin and xanthan gum, was suggested using emulsion template approach. The protein−polysaccharide interactions at the oil−water interfaces were exploited to entrap liquid oil to form oleogel system (Patel et al., 2015a).

Hybrid oleogel formulated using combination of HMOG and LMOG exploits the differences in physical properties of these gelation mechanisms. Gelation of EC with SOSA (Gravelle et al., 2017) and MAG (Lopez-Martínez et al., 2015) have been studied. The presence of EC shifted the SOSA crystallization temperature to higher values and produced different crystal morphology. It was suggested that EC backbone acted as a platform for SOSA crystallization (Gravelle et al., 2017).

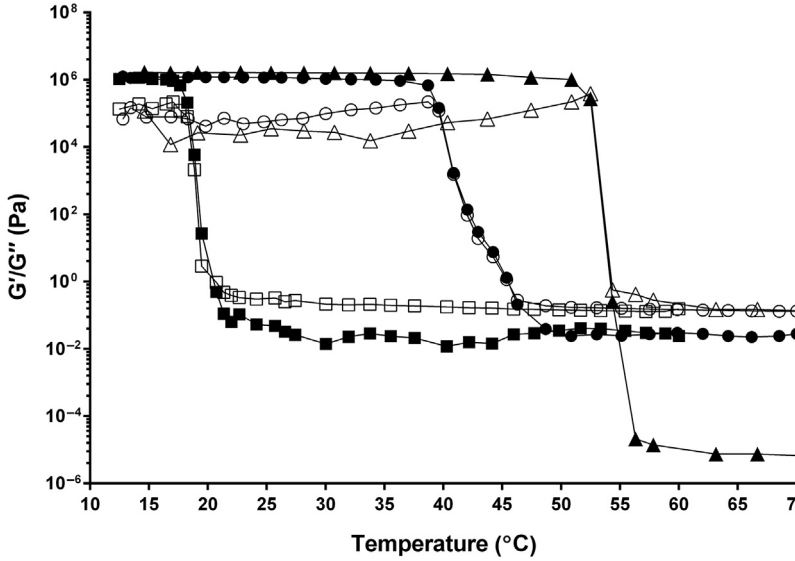

Figure 8.4 Temperature dependent modulus obtained for 30 wt% lauric acid (□), behenic acid (Δ), and their 1:1 mixture (○) with canola oil using 20° min^{-1} cooling/heating rate. G′ (closed symbol) and G″ (open symbol).

8.4 Utilization of oleogel systems in various applications

Oleogel formulation can be utilized in a wide range of applications from physiologically oriented applications in pharmaceutical and food industries to plastics and coating applications.

Utilization of oleogels in foods is mainly aimed at structuring liquid oil with an emphasis to replace solid fats or to prevent oil migration. A wide range of applications in foods can be found in the literature (Patel, 2015b). EC-based oleogels with surfactants such as SMS were used as fat replacement in meat products (Jimenez-Colmenero et al., 2015) such as breakfast sausages (Barbut et al., 2016b) and frankfurters (Barbut et al., 2016a). Formulation of heat resistance chocolate by the addition of EC dissolved in ethanol during the tempering process was also suggested (Stortz and Marangoni, 2011). Other examples for the use EC include the formulation of fat-reduced chocolate (Do et al., 2010), preventing oil migration in cookies and cream filling with partial replacement of fat using EC oleogel (Stortz et al., 2012) and replacement of fat in cream cheese (Bemer et al., 2016). Carnauba wax (Mert and Demirkesen, 2016a,b; Kim et al., 2017), candelilla wax (Jang et al., 2015), sunflower wax, rice bran wax (Hwang et al., 2013), shellac wax (Patel et al., 2014a), and water-soluble polysaccharide (Patel et al., 2014b) were used for the manufacturing of shortening blend. Waxes were also used as oil phase stabilizers in chocolate spread to prevent phase separation or "oiling out" (Patel et al., 2014a) and as fat replacers in ice cream manufacturing

Figure 8.5 Chocolate spread stabilized using 0, 4, 10, 15 wt% oleogelator (of the oil phase) after 21 days in 40 °C incubator.

(Botega et al., 2013). Fig. 8.5 demonstrates the effect of structuring agent addition on oil migration in chocolate spread. Other examples for the use of oleogels in foods include replacement of fat with oleogels in praline fillings to control oil migration, a process that known to induce fat bloom (Patel and Dewettinck, 2016; Si et al., 2016).

Oleogel systems offer a unique hydrophobic environment that can be exploited for the delivery of hydrophobic bioactive molecules. This characteristic offers a natural way to improve the bioavailability and bioaccessibility of hydrophobic bioactive molecules during oral administration (Davidovich-Pinhas, 2016; O'Sullivan et al., 2017). These attributes can be used in various applications such as pharmaceutical therapeutic treatments, dietary supplements, and bioactive delivery in cosmetics (Davidovich-Pinhas, 2016). Several reviews were written based on many studies demonstrating the use of oleogels in drug delivery (Vintiloiu and Leroux, 2008; Sagiri et al., 2014; Balasubramanian et al., 2012; Jha and Maurya, 2013) and tissue engineering (Skilling et al., 2014). The use of oil structuring approach to produce free petroleum cosmetic products was also suggested using oleogel formulations based on EC (Stortz and Marangoni, 2014; Aiache et al., 1992), MAGs (Wang and Marangoni, 2016), and others (Balasubramanian et al., 2012; Lebok et al., 1999).

Other application of oil structuring includes the formation of green lubricants using EC as thickener agent (Quinchia et al., 2014; Sánchez et al., 2011a,c), paint (Sprogar and Pinzer, 2006), coating (Wang et al., 2015), and oil spillage treatments (Ohsedo, 2016).

8.5 Summary and conclusions

Oleogel systems offer a unique chemical composition arising from the solvent nature, i.e., oil, with a controllable set of mechanical properties arising from the gelator molecule used for their production. This manuscript covered the wide range of gelator molecules used up to date to form such gel network. Each gelator type produces a unique physical gel with specific set of properties providing a wide range of possibilities. Combination of two or more types of gelator molecules broadens this range even further thus providing an excellent platform for the development of new applications

for oleogels. Up to date, there are many studies on the use of oleogels in various applications from pharmaceutical and biomedical to foods. The ongoing research and interest in this field will further broaden the gelator types used, combination, and applications of oleogels.

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Emulgels

9

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9.1 Introduction

Emulsion-based formulations have long been used not only in the food (Galus and Kadzińska, 2015; Lett et al., 2016) and the cosmetics industries (Herman et al., 2013; Terescenco et al., 2018) but also in the pharmaceutical industry (Deng et al., 2014; Akhtar et al., 2016). The main advantage of an emulsion-based formulation is its ability to improve the bioavailability of the poorly soluble bioactive agents (Ting et al., 2015; Raikos and Ranawana, 2017). These formulations consist of an aqueous phase and a nonaqueous phase.

One of these phases forms an internal phase, which is dispersed in the other phase. Usually, such a dispersion is thermodynamically unstable in nature and undergoes phase separation when allowed to stand for a certain duration (Jauregui, 2016). The thermodynamic stability of the emulsion is increased using a surfactant/emulsifier (Owusu et al., 2017). The emulsifier reduces the interfacial energy among both the phases, thereby, improving the thermodynamic stability of the emulsions (Pan et al., 2017). If the composition is chosen in the proper way, the emulsion remains stable for a prolonged duration. As discussed previously, emulsion consists of a dispersed phase. If the bioactive molecules are solubilized in the dispersed phase, it acts as a reservoir for the drug.

Due to this reason, the emulsion has been reported as a controlled delivery system (Singh et al., 2014). In such a system, the release of the drug is governed by the partitioning of the drug in both the phases. Hence, it is possible to control the release of the drug just by altering the composition of the apolar phase. In general, vegetable oils are used in food and pharmaceutical grade formulations (Galus and Kadzińska, 2015; Lakkis, 2016). The vegetable oils are, in general, a mixture of various fatty acids, which are liquid at room temperature (Knothe et al., 2015). This results in altered hydrophobic characteristics of each of the vegetable oils. Hence, it is possible to control the release of the drug just by altering the vegetable oil phase.

The emulsions have also been proposed for encapsulation applications (Lu et al., 2016). The bioactive agents incorporated within the dispersed phase are protected (to a greater extent) from the atmospheric environment. Furthermore, when consumed by an oral route, the emulsions may allow safe passage of bioactive agent through the acidic environment of the stomach. This is of utmost importance for delivery of drugs via an oral route (Wais et al., 2017). This is because the acidic environment of the

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stomach is decrement to many of the bioactive agents (e.g., probiotics) and significantly reduces their bioactivity.

The emulsions can be broadly classified into two categories, namely, oil-in-water (o/w) and water-in-oil (w/o) emulsions (Ahtikari et al., 2014). In o/w emulsions, the dispersed phase is oil phase, and continuous phase consists of water phase, whereas in w/o emulsions the dispersed phase is water phase, and continuous phase consists of the oil phase. Even though emulsifiers have been reported to improve the thermodynamic stability, the stability of those emulsions cannot be increased for a long duration. This significantly lowers the shelf life of the finished product.

Recently, many scientists have proposed to immobilize the external phase of the emulsion using suitable gelators (Patel et al., 2013; Singh et al., 2015). A gelator is defined as a solid material, which forms an interconnecting network structure (where the external phase liquid is immobilized). This results in a formation of a gelled structure in the continuous phase. A gel is defined as a jelly-like material, which can immobilize a liquid phase (O'brien et al., 2015). The conversion of external liquid phase into a jelly-like structure increases the viscosity of external phase. This, in turn, restricts the movement of the internal phase and hence prevents the coagulation of dispersed phase droplets. The process of coagulation is the major phenomenon associated with the destabilization of the emulsion. This results in the improvement of the thermodynamic stability of the emulgel. Emulgels combine the advantages of an emulsion and a gel (Eswaraiah et al., 2014). Like emulsions, the emulgels can provide an alteration of the release process of the bioactive agents from the external environment. Like gels, the thermodynamic stability of the emulgel is also very high (Pant et al., 2015). In this manuscript, we will discuss the different methodology of emulgel preparation, their characterization techniques, and its biomedical applications.

9.2 Considerations for emulgel formulation development

In the previous section, we have seen that emulgels are a special class of emulsions, where the emulsions are gellified using a gelling agent. Hence, many of the emulgel formulation considerations are similar to that of the emulsions. The primary requirement for the development of an emulgel formulation includes the selection of an oil phase and an emulsifier. Additionally, because emulgels are semisolid formulations, there is a need to select a gelling agent to meet the requirement of a specific application. In general, lipids of both natural and synthetic origin have been explored for preparing formulations for pharmaceuticals, cosmetics, and food industry (Lee et al., 2016). The choice of the lipid phase is mainly governed by the estimated physical properties of the final product. Depending on the source of the oil, the oil used may have medicinal benefits. For example, oil extracted from garlic has been reported to promote wound healing associated with burns (Londhe, 2014). Similarly, geranium oil has been proposed not only for wound healing properties but also insecticidal, antimicrobial (especially antibacterial) properties (Nadjib Boukhatem et al., 2013).

Like the oil phase, the selection of an emulsifying agent is also an important criterion. Previously, we have mentioned that the emulgel may either be o/w or w/o type.

The formulations of either of these of emulsions are mainly dependent on the type of emulsifying agent used. The emulsifying agents are bipolar in nature, i.e., it has both hydrophilic and lipophilic groups. Depending on the nature of the hydrophilic and the lipophilic groups, the type of emulsion formed by an emulsifying agent is governed. Usually, the balance between the hydrophilic and lipophilic group is quantified by "Hydrophilic−Lipophilic Balance" (HLB) value (Yeon et al., 2014). An emulsifying agent having HLB value greater than 8 promotes the formation of an o/w emulsion. On the contrary, emulsifying agents having HLB value less than 8 are used for making w/o emulsions. In general, nonionic emulsifying agents are preferred for biomedical applications (Issa and Assaad, 2017). This is because the nonionic emulsifying agents are inherently nontoxic (or biocompatible) in nature. Table 9.1 enumerates the list of oils and identifies various emulsifying agents, which have been used for the development of emulgels in the last 5 years for drug delivery applications.

Immobilization of the external phase of the emulsion helps in converting the emulsion into an emulgel. The substances that are used to induce gelation are regarded as gelators. If the gelators are capable of mobilizing polar phase, they are characterized as hydrogelators. On the other hand, organogelators are responsible for inducing gelation of the apolar phase. The occurrence of the gelation is tested by a simple method, namely, inverted test tube method. In this test, gelators are added in the test tube. Thereafter, the flowability of the emulsions is checked by inverting the test tube. Under this condition, if the emulsion shows flow, then it is considered that the gelator has not been able to induce gelation. On the contrary, if the emulsion does not show any flow, the gelator is regarded to have successfully induced gelation.

As discussed above, the gelation process converts a free-flowing liquid emulsion into a nonflowing formulation. Usually, such nonflowing formulations are semisolid in nature having viscoelastic properties. Depending on the nature of gelators and mechanism of induction of gelation, the emulgel may either be physical (showing sol-to-gel and gel-to-sol transition) or permanent (emulgel). Furthermore, the gelators that are used may either be of natural origin or synthetic origin. Table 9.2 summarizes the list of gelators, which have been explored for the preparation of emulgels.

The nature and properties of gelators have been reported to alter the drug release profile of the drug, incorporated within the emulgel in a concentration-dependent manner. As a matter of fact, an increase in the concentration of the gelators has been found to reduce the rate of drug release. When a combination of gelators is used, the rate of release of the drug is dependent on the interaction among the gelator molecules and also the gelator and the drug molecule. Usually, the stability of the emulgel is far superior to the emulsion due to increased viscosity of the external phase.

9.3 Preparation of emulgel

Various methods of emulgel preparation have been proposed. Alexander et al. (2013) have reported that the properties of the structure dispersing phase play a significant role in tailoring the microarchitecture of the emulgels. This, in turn, results in the significant variation in the properties of the emulgels (Alexander et al., 2013). Accordingly, many

Table 9.1 **List of oils and various emulsifying agents used in the development of emulgels for drug delivery applications in the last 5 years**

Oil used	Formulations developed	Emulsifier/ surfactant	Bioactive agent used	Applications	References
Emu oil	Nanoemulgel	Cremophor RH 40 and Labrafil M2125CS	Curcumin	Amelioration rheumatoid arthritis	Jeengar et al. (2016)
Soybean oil	Emulgel (oil-in-glycerol)	Glycerol	β-carotene	Retardation of oil oxidation process, as margarine alternative	Chen et al. (2016)
Olive oil	Emulgel	Sorbitan monopalmitate	Ciprofloxacin and metronidazole	Potential applications in sexually transmitted diseases (STDs)	Singh et al. (2014)
Castor oil	Emulgel	Poloxamer 188	Cyclosporine A	Topical ocular drug delivery system	Shen et al. (2015)
Paraffin oil	Emulgel	Kollicream and Kolliphor CS20	Calcipotriol	Local treatment of psoriasis	Varma et al. (2014)
Light paraffin oil	Emulgel	Span 20	Mefenamic acid	Control of inflammatory and analgesic activity	Khullar et al. (2012)
Sunflower oil, castor oil	Emulgel	Polyglycerol polyricinoleate	Metronidazole	Pharmaceutical industries and food industry	Behera et al. (2015)

Oleic acid	Nanoemulgel	Tween 80 and ethanol	Piroxicam	Enhancing the transdermal and oral delivery systems.	Dhawan et al. (2014)
Soy oil, sunflower oil extract	Emulgel	Sodium caseinate	β-carotene	Sustained drug delivery for lipophilic components, food and pharmaceutical industry	Liu and Tang (2016)
Mineral oil	Emulgel	Tween 80 and span 60	Pravastatin	Pharmaceutical industry	Burger et al. (2015)
Sesame oil, soybean oil	Emulgel	Tween 80	Ciprofloxacin	Topical drug delivery applications	Sagiri et al. (2015)
Olive oil	Emulgel	Tween 60	Pectin	Cosmetic product design and pharmaceutical industries	Lupi et al. (2015)
Sesame oil	Emulgel	Span 60	Ciprofloxacin	Antimicrobial drug delivery system	Satapathy et al. (2015)
Emu oil	Emulgel	Polysorbate 80	Insulin	Alternative method for insulin delivery system	Akram et al. (2013)

Table 9.2 List of gelators used for the preparation of emulgels

Type of gelators (synthetic or natural)	Name of gelators	References
Synthetic	Sorbitan monopalmitate	Singh et al. (2014)
	Polycarbophil	Shen et al. (2015)
	Polyethylene glycol	Varma et al. (2014)
	Propylene glycol	Varma et al. (2014)
	Span 40	Behera et al. (2015)
	Carbopol 934	Dhawan et al. (2014)
	Carbomer	Akram et al. (2013)
	Hydroxypropyl methylcellulose	Akram et al. (2013)
Natural	Soy glycerin	Liu and Tang (2016)
	Pectin	Lupi et al. (2015)

authors have reported the development of emulgels using the different types of structuring agents. Furthermore, the processing parameters for the preparation of the emulgels can also alter the physical properties. Keeping a note of this, this section reports the different methods of preparation of the emulgels, reported by the various researchers, in brief.

Lupi et al. (2015) reported the development of polysaccharide-based emulgels. The method is applicable for the preparation of emulgels using polysaccharides, which undergo ionic cross-linking. In this method, o/w type of emulgels may be conveniently prepared. In this study, the authors reported that Tween 60 (nonionic emulsifier) was dissolved in virgin olive oil at a temperature of 70°C. This mixture was cooled down to 25°C and was subsequently used for the preparation of the emulsion. Because the apolar phase is heated at 70°C, thermolabile lipophilic drugs cannot be incorporated into the apolar phase. The polar phase consists of polysaccharides. The emulsion is prepared by dropwise addition of Tween 60 dissolved virgin olive oil to the aqueous polysaccharide dispersion, which is being homogenized continuously. This step allows the formation of an emulsion, which consists of an inner oil phase, homogenously dispersed within an aqueous polysaccharide phase. Thereafter, calcium chloride solution is added with the vigorous homogenization of the solution. This allows proper mixing of calcium chloride solution within the polysaccharide phase. The addition of calcium ions within the polysaccharide phase induces gelation of the polysaccharide phase, resulting in the formation of o/w emulgels. Emulgels with different properties may be prepared either by varying the composition of emulgels or using different polysaccharides. The main advantage of developing emulgels by this method is that the gel structure is not damaged by the homogenization process. Fig. 9.1 shows the schematic representation of the formation of emulgels by this method.

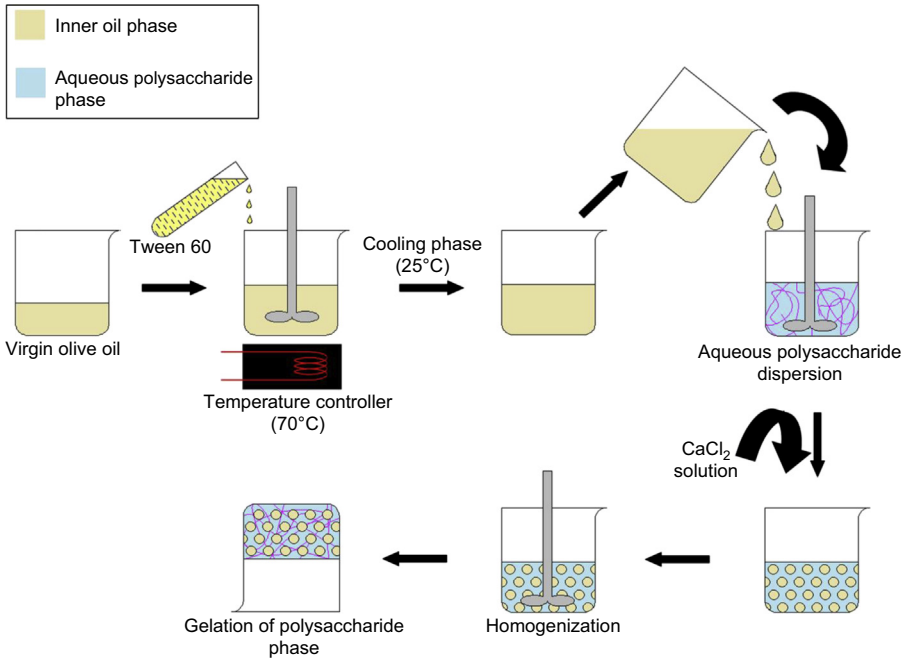

Figure 9.1 Schematic diagram for the preparation of polysaccharide-based emulgels by ionic cross-linking.

Shen et al. (2015) reported the formation of emulgels using zein, a protein commonly found in maize. The authors reported that the protein was dissolved in food-grade glycerol solution maintained at 150°C with continuous homogenization (Fig. 9.2). Soybean oil, maintained at 95°C, was slowly added to the zein solution and homogenized thoroughly at 135°C. When the mixture was cooled down to the ambient temperature, the gelation of the emulsion by the zein protein was achieved. The authors used the emulgels for the delivery of β-carotene. The emulgels were found to improve the UV photostability of β-carotene to a significant level. In a similar study, Sagiri et al. (2015) have reported the development of gelatin-based emulgels. However, in the study, the aqueous phase was cross-linked using glutaraldehyde. This resulted in the formation of chemically cross-linked emulgels unlike the zein-based emulgels, which were a physical emulgel. Satapathy et al. (2015) have also reported a similar method of emulgel preparation as has been reported by Sagiri et al. (2015). In the study, the authors have compared the properties of emulgels with that of hydrogels and bigels.

Dong et al. (2015) reported the development of emulgels using synthetic hydrophilic polymers Sepigel 305, Simulgel INS 100, Sepiplus 400, and Simulgel EG. The authors reported the preparation of the preemulsion by adding sufficient water (at 70°C) to the oil phase. The preemulsion, so formed, was thoroughly homogenized to form an emulsion (Fig. 9.3). Subsequently, the aqueous polymeric dispersion in required amount of water was added to the previously formed emulsion and

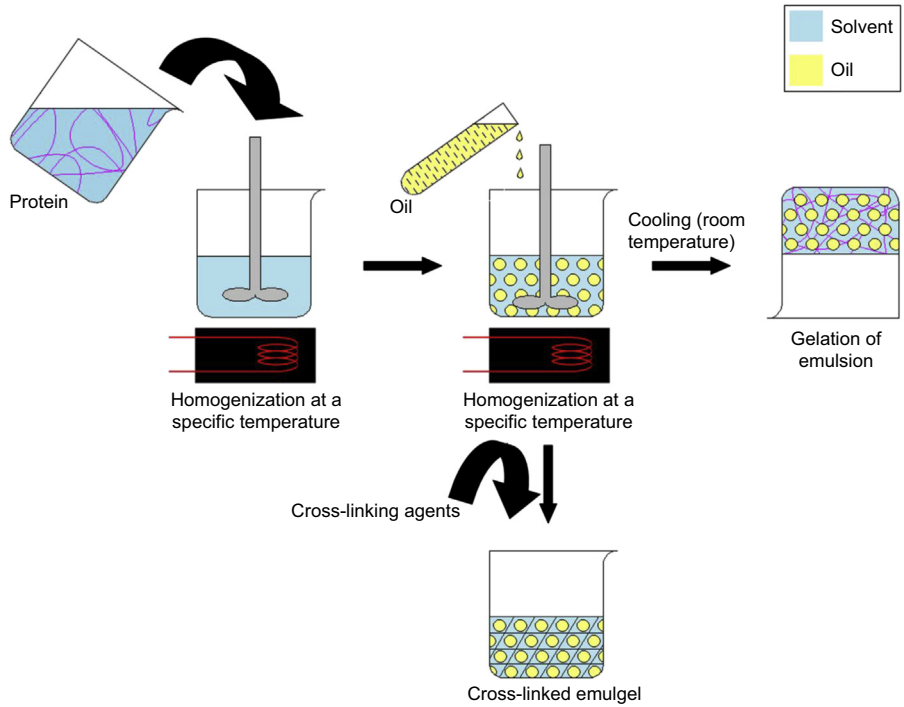

Figure 9.2 Schematic diagram of the formation of emulgels using proteins.

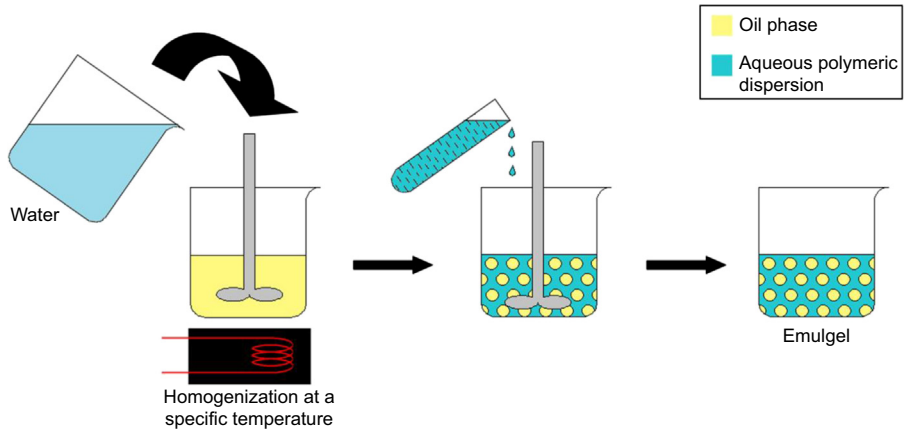

Figure 9.3 Schematic diagram for the preparation of emulgels using synthetic hydrophilic polymers.

homogenized thoroughly. The addition of the hydrophilic polymeric dispersion resulted in the increase in the viscosity in the aqueous phase. The analysis of the microstructures of the emulgels suggested that the aqueous phase was the external medium. The oil phase was immobilized within the aqueous media. A similar methodology of emulgel preparation was reported by Shen et al. (2015). In the study, the authors have used polycarbophil polymer for gelling the aqueous phase (Shen et al., 2015). The pH of the emulgel was adjusted to 6.8 so that the formation can be used for ocular drug delivery. Varma et al. (2014) reported the development of emulgels using carbopol as the synchronous hydrophilic polymer. To the aqueous carbopol dispersion drug-containing propylene, glycol, and PEG was added and homogenous thoroughly. Thereafter, a mixture of Kollicream 3C, Kolliphor CS20, and liquid paraffin (oil phase) was added to the aqueous carbopol−based mixture with continuous homogenization. This resulted in the formation of an emulgel. Recently, Jeengar et al. (2016) have reported the preparation nanoemulgels using carbopol as the synthetic hydrophilic polymer. In the study, they have reported that nanoemulsions were initially synthesized. The synthesized nanoemulgels were added to the carbopol gel, based at a weight ratio of 1:1. In the study, propylene glycol was used as a plasticizer.

Like Shen et al. (2015), a recent manuscript by Liu and Tang (2016) prepared physical emulgels using soy glycerin protein molecules as the physical gelling agent. Singh et al. (2014) have reported the development of olive oil−based thermoreversible emulgels (emulsion hydrogel) (Singh et al., 2014). The authors reported that the aqueous phase (distilled water at 60°C) was slowly added to a mixture of sorbitan monopalmitate and olive oil, which was being stirred at 500 rpm (60°C). After the addition of required amount of water, the hot emulsion was further homogenized for 30 min. Thereafter, the hot emulsion was allowed to attain room temperature. Depending on the composition of the formulations, liquid emulsion or emulsion hydrogel was formed. The authors reported that the emulgels so formed were thermoreversible in nature.

9.4 Applications

Previously, we have discussed that the structured emulsion (also known as emulgels) has been used for food, cosmetic, and pharmaceutical application. Some authors have also reported these types of emulsions as emulsion filled gels. In this chapter, we will be restricting our discussion to the application of emulgels in pharmaceutical and food industries. Because many of the emulgels are semisolid in nature and can be easily spreadable, such formulations are explored of topical drug delivery. Topical drug delivery provides an advantage of better patient compliance and tolerance as compared with other conventional drug delivery systems. The main advantage of such emulgels is its ability to accommodate hydrophobic drugs in formulations having an aqueous base. This type of formulation has been reported stable for a longer period. Khullar et al. (2012) reported the development of carbopol 940−based emulgels formulations for mefenamic acid. Mefenamic acid is a nonsteroidal antiinflammatory drug and is

used in medicine for its antiinflammatory and analgesic effect. Unfortunately, the skin penetration of the aforementioned drug is poor. To overcome this disadvantage, the authors used menthe oil and clove oil as penetration enhancer. It was found in vivo that the antiinflammatory effect of the specific formulations prepared by the authors was far superior to the commercially available diclofenac gel. From the results, the authors concluded that the optimized emulgel formulations could be tried as topical drug delivery system for the delivery of the antiinflammatory analgesic agent.

Shen et al. (2015) have developed Cyclosporin A−based emulgels using polycarbophil as the gelling agent. The formulation was developed to deliver the drug in the eye. Cyclosporin A is an immunosuppressive drug, which can specifically inhibit the T-cell immune reaction. The drug has been commonly used in various contingent eye diseases. The development of emulgel-based Cyclosporin A system resulted in the increased retention time on the ocular surface. This resulted in the improved bioavailability of the drug. Furthermore, the developed emulgel did not initiate any irritation in the eye. The authors concluded that the emulgels might be used for topical ocular delivery.

Emulgels have been proposed as a delivery vehicle for calcipotriol for the treatment of psoriasis (Varma et al., 2014). The said disease is an immunological disorder of the skin. The persons suffering from psoriasis have recurrent inflammation and hyperkeratosis. The main problem associated with the treatment of this disease is the delivery of the drug to the diseased skin tissues. The drug calcipotriol is a hydrophobic drug and hence is a suitable candidate to be delivered through emulgel formulations. The authors added isopropyl alcohol and polyethylene glycol within the emulgel formulations so as to improve the permeation of the drug within the specific layer of the drug within the skin tissues. The emulgel formulations were found to have a higher rate of calcipotriol release and diffusion across the rat skin, as compared with the commercially available ointment of the drug, and can be improved significantly when delivered using emulgel formulations.

Singh et al. (2014) reported the development of a novel thermoreversible hydrogel for controlled release of metronidazole and ciprofloxacin. The authors reported that the developed emulgels could be explored for treatment of STD (for example, bacterial vaginosis and HIV infection). The drug-containing emulgels were challenged against *Bacillus subtilis*, a model gram-positive bacterium. The authors reported that the emulgels could be prepared in an easy and economical manner, which can be commercially viable for marketing purpose. The authors concluded that the developed emulgels could be used for vaginal delivery of antimicrobial drugs. In the year 2015, Behera et al. reported the development of sunflower oil−based novel emulgels, which used span 40 as structuring agent. The effect of polyglycerol polyricinoleate (PGPR) on the properties of the span 40−based emulgels was explored. The authors reported that when PGPR was added to the span 40−based emulgels, there was a significant alteration in the microarchitecture. The emulgels were loaded with metronidazole, and its antimicrobial activity against *Escherichia coli* was tested. Furthermore, the emulgels were found to be biocompatible in the presence of keratinocyte. From the results, the authors concluded that the prepared emulgels could be used as a controlled delivery vehicle for topical administration of an antimicrobial drug.

Satapathy et al. (2015) have reported gelatin-based cross-linked emulgels. The authors reported that the emulgels combine the advantage of emulsion and gels. Because gelatin is a mucoadhesive polymer, it was expected that the cross-linked emulgels would exhibit mucoadhesive properties. The mucoadhesive study carried out using goat intestinal mucosa confirmed the mucoadhesive nature of the prepared emulgels. The emulgels were loaded with ciprofloxacin, a commonly used antimicrobial drug. The drug-loaded emulgels were found to be acting against the *E. coli*. This suggests that the developed formulations may be used as a mucoadhesive drug delivery system for improved bioavailability of the drug.

A similar study was reported by Sagiri et al. (2015). In the study, the author prepared sesame oil and soybean oil−based emulgels. The prepared emulgels were found to be cytocompatible with human epidermal keratinocyte cells. In this study, the authors reported the mucoadhesive property of the gelatin-based cross-linked emulgels. Similar to the study reported by Satapathy et al. (2015) and Sagiri et al. (2015) also loaded ciprofloxacin within the emulgels and found to be active against *E. coli.*

β-carotene is a pigment, which is usually obtained from many fresh fruits and vegetables. Additionally, it is a very good oxidant and has a very good ability to scavenge free radicals. Due to this reason, it has been proposed that regular consumption of β-carotene can not only make the immune system stronger but also can help in reducing the chances of chronic diseases such as cancer and heart disease. Unfortunately, β-carotene is highly susceptible to oxidation. This significantly reduces the beneficial effect of β-carotene (Boon et al., 2010). In this regard, Liu and Tang (2016) and Chen et al. (2016) have reported the use of emulgels as a carrier for β-carotene. In both the studies, emulgels were prepared either using soy glycerin (major globulin in soy storage proteins) or zein (a water insoluble protein obtained from maize). In both the studies, the author reported that when β-carotene was incorporated within the emulgel, the photostability of β-carotene improved significantly.

Terpinen-4-ol is one of the main components of Australian tea tree oil. Due to its excellent antimicrobial and permeation enhancing capabilities, it has been considerably used to formulate pharmaceutical formulations. Dong et al. (2015) have reported the development of Sepigel 305, Simulgel INS 100, Sepiplus 400, and Simulgel EG as the synthetic polymer to induce gelation. Terpinen-4-ol was loaded within the prepared emulgels. Though the increase in the viscosity of the emulgels inversely affected the release profile of terpinen-4-ol, it did not affect the permeation process. Jeengar et al. (2016) developed curcumin-containing nanoemulgel. Curcumin is an organic compound, which is obtained from turmeric. It is a natural antiinflammatory drug and has been reported to not only help ease symptoms of osteoarthritis and rheumatoid arthritis but has also been found to stabilize colorectal cancer patient. But its low solubility and poor permeation are detrimental in formulating a formulation of pharmaceutical use. Keeping this in mind, Jeengar et al. (2016) developed nanoemulgels. The emulgels were prepared using emu oil obtained from emu bird. The authors reported that the prepared nanoemulgels containing curcumin as a bioactive agent for the treatment of rheumatoid arthritis. They supported their claim by conducting in vivo studies on a rat model.

9.5 Conclusion

From the thorough literature review, it can be concluded that the advancement in the field of pharmaceutical technology can help in formulating emulgels in an easy and convenient manner. It was observed that emulgels, for pharmaceutical applications, were mainly of the o/w type of emulgels. This is because emulgels were mostly used to develop formulations of hydrophobic bioactive agents. Usually, the hydrophobic bioactive agents have low bioavailability due to poor permeation behavior. The use of emulgels as a carrier for these bioactive agents has allowed the researchers to improve the penetration of such drugs by accommodating penetration enhancer within the same formulation. Furthermore, the stability of the bioactive agents (e.g., β-carotene) was significantly increased when they were incorporated within the emulgels. Due to the o/w nature of these emulgels, these formulations were nongreasy in nature and hence could be easily washed off from the skin. From the discussion, it is quite evident that the emulgels may serve as wonder formulations for the hydrophobic bioactive agents (which usually have poor bioavailability and unstable in nature). The application of emulgels in pharmaceutical industry is in its nascent stage with only a handful of topical emulgel preparation available commercially (e.g., voveran emulgel).

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Bigels

10

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10.1 Introduction

The notion of bigels is quite new as compared to the other gel formulations. Bigels are uniform semisolid dispersion systems in which two gel phases are mixed together by applying a high shear rate and appear as a single gel when seen visually (Varrato, 2012). The two gels in each bigel are prepared individually using a specific gelator. The bigels possess advantages of both the phases and are very stable (Andonova et al., 2017). The two gels, used in preparing the bigel, comprise colloidal gels either identical (water-in-water bigels, which are phase-separated systems) (Rehman and Zulfakar, 2017) or different in nature or can be an amalgam of a hydrogel and an oleogel (oleogel-in-hydrogel bigels or hydrogel-in-oleogel bigels) (Ibrahim et al., 2013; Wakhet et al., 2015; Sagiri et al., 2015; Kodela et al., 2017). Hu et al. (1995) used the term "bigel" for a combined system of two different gels interpenetrating into each other as in the so-called bigel strips. Rhee et al. (1999) reported a biphasic formulation named as "oleo-hydrogel" containing Ketoprofen for improved skin absorption and drug permeation. Kodela et al. (2017) reported the preparation of oleogel-in-hydrogel type bigels by mixing agar hydrogel and stearyl alcohol oleogel in different proportions. Bigels have been studied extensively for their applications in the food and pharmaceutical industries (Ibrahim et al., 2013). Currently, bigels are being widely explored as potential matrices for controlled drug delivery (Singh et al., 2014c,e).

10.2 Advantages of bigels over organogels and hydrogels

The major advantages of the bigel are its improved stability as compared to the emulsions (water-in-oil and oil-in-water), creams, emulgels, hydrogels, and oleogels, which makes it a potent carrier for pharmaceutical or cosmetic applications. This enhanced physicochemical stability of the bigels can be attributed to the formation of extrafine colloidal dispersions, which results in due to the immobilization of the mobile phases in a three-dimensional gel network (Almeida et al., 2008). On storage at the room temperature, the two components do not get separated and remain stable (Di Michele et al., 2014). Emulsions get destabilized with time and show phase separation and creaming. Therefore, they are stabilized by the incorporation of "emulsifier" during their preparation (Hu et al., 2015). The emulsion gels can be

Polymeric Gels. https://doi.org/10.1016/B978-0-08-102179-8.00010-7

stabilized by immobilizing the internal phase (Jadhav et al., 2014). Hydrogels possess high patient compliance due to their nongreasy, easy washability with water and cooling nature but they can carry only hydrophilic drugs and have limited access to penetration across the skin. Oleogels are the best carriers for the lipophilic drugs, but being oleagenous in nature, they possess greasy feeling, resulting in the low patient compliance (Lupi et al., 2016). Converting oleogels, emulgels, and hydrogels into bigels imparts easy washability and good patient compliance without compromising the beneficial effects of the oil. They are easy to prepare and do not contain huge quantities of surfactants, which are sometimes toxic (Lupi et al., 2015; Rehman and Zulfakar, 2014). They can accommodate both hydrophilic and lipophilic drugs due to the presence of both the components. The amalgamation of two gels may possess synergistic effect, resulting in the improved drug permeation due to the presence of both hydrophilic and lipophilic proportions (Lupi et al., 2016). They can easily penetrate through the skin, and hence can be a choice for topical or transdermal drug delivery. The bigels are capable of regulating the delivery of active substances (Sagiri et al., 2015; Singh et al., 2014c,e). They possess electrical conductivity, which makes them a suitable carrier for iontophoretic drug delivery. The electrical conductivity is accounted for the presence of water phase in the bigels (Almeida et al., 2008).

Apart from the abovementioned advantages, bigels also possess a few drawbacks such as their phase separation, which may be associated with the absence of emulsifiers. At higher temperatures, they get destabilized. Therefore, they might not be thermo-reversible (Singh et al., 2014c).

10.3 Types of bigels

Bigels can be classified into four categories, namely, oleogel dispersed in hydrogel system (O/W), hydrogel dispersed in oleogel system (W/O), bicontinuous bigel, and complex bigel on the basis of the structural organization and the disposition of organogels and hydrogels.

10.3.1 Oleogel dispersed in hydrogel system

This type of bigel is formed by dispersing the oleogel within the hydrogel system (Fig. 10.1(a)). So far, these types of bigels have been explored by many researchers. Behera et al. (2015b) prepared bigels by amalgamation of Span 40—sunflower oil oleogel with aqueous gels (plural/singular) of water-soluble synthetic polymers (e.g., polyvinyl alcohol and polyvinyl pyrrolidone). The micrographs obtained revealed that the aqueous gel was the dispersion medium with the oleogel behaving as the dispersed phase (Behera et al., 2015a). Singh et al. (2014e) reported the development of oil-in-water type of emulsion bigels by mixing Carbopol 934—based hydrogel and Span 60—sesame oil based organogel.

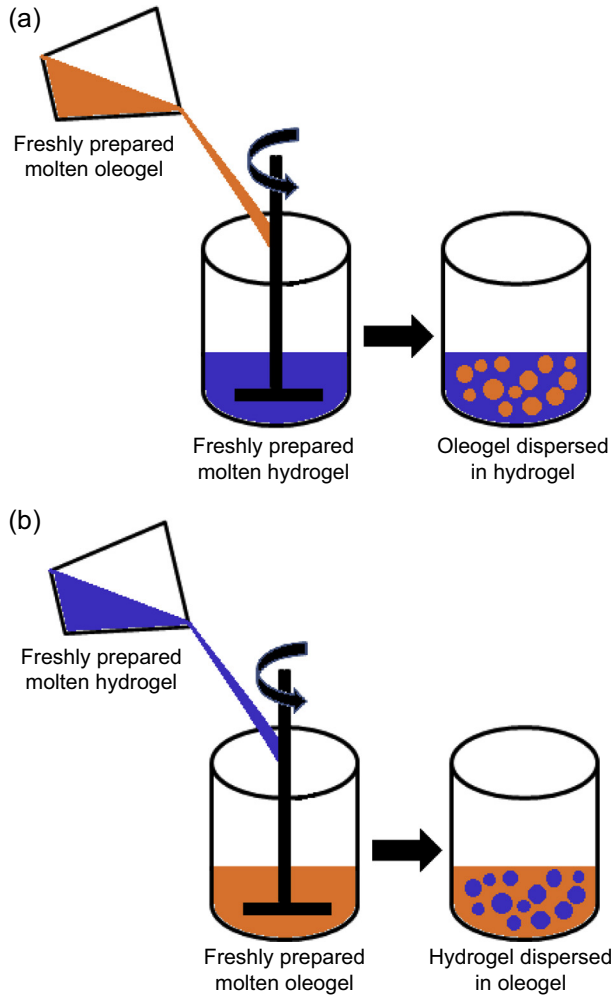

Figure 10.1 Schematic diagram representing the formation of different types of bigels (a) oleogel dispersed in the hydrogel and (b) hydrogel dispersed in oleogel.

10.3.2 Hydrogel dispersed in oleogel system

These types of bigels are formed by dispersing the hydrogel within the oleogel system (Fig. 10.1(b)) (Lupi et al., 2016). Patel et al. (2015) prepared bigels from the combination of hydrophilic-fumed silica and sunflower oil−based organogel with polysaccharides (1 wt% of locust bean gum: carrageenan, 1:1 ratio)-based weak water gel (Singh et al., 2013).

10.3.3 Bicontinuous bigel

Bicontinuous bigels are formed when the gel formation is carried out at higher proportions of hydrogel/oleogel dispersed in lower proportions of oleogel/hydrogel phase, respectively. Singh et al. (2014c) developed bicontinuous bigels in which guar gum based hydrogel coexisted with Span 60—sesame oil based organogel. Kodela et al. (2017) also reported the formation of bicontinuous bigel at higher proportions of oleogel while preparing oleogel-in-hydrogel type bigels.

10.3.4 Complex bigels

Complex bigels are produced by adding organogel/hydrogel to an oil-in-water/water-in-oil structured emulsion. Lupi et al. (2015) demonstrated the formation of a complex matrix-in-matrix system. The structural organization was confirmed by microscopy. The authors reported the formation of a bicontinuous bigel when the organogel fraction was the highest (Lupi et al., 2015).

10.4 Methods of preparation

The aqueous phase and the oleaginous phase are prepared separately by mixing the components at a defined speed and temperature.

10.4.1 Preparation of aqueous phase (hydrogel)

Hydrogels are aqueous dispersion systems, formed by mixing the necessary amount of hydrophilic gelling agent in water. The process parameters (e.g., speed and temperature) are optimized based on the gelling behavior of the system. Hydrogels are formed by physical or chemical cross-linking (Rehman and Zulfakar, 2014). The physical hydrogels are reversible in nature and the gelation is attributed to some interactions such as Van der Waals forces and hydrogen bonding (Otto and Drahoslav, 1960). Chemical hydrogels, which are also called as "permanent gels," are formed via covalent bonding resulting in the formation of a cross-linked network (Hoffman, 2012).

10.4.2 Preparation of oleaginous phase (oleogel)

Oleogel is prepared by dissolving the accurately weighed quantity of organogelator in a predefined oil phase at a defined homogenization condition and a temperature higher than the melting point of the organogelator. The gel formation occurs when the temperature is brought down to room temperature ($\sim 25^{\circ}C$). The oil phase can be an organic solvent (e.g., benzene, hexane) or vegetable oils such as castor oil (Singh et al., 2013), groundnut oil (Singh et al., 2016), olive oil (Yılmaz and Öğütcü, 2014; Lupi et al., 2012), and sesame oil (Singh et al., 2015). Similar to hydrogels, these are also formed by weak Van der Waals forces or hydrogen bonding (Rehman and Zulfakar, 2014).

10.4.3 Preparation of bigel

Bigels are prepared by combining hydrogel and oleogel at high shear rate, retaining the characteristic properties of both the components. The homogenous mixture forms a smooth gel by applying a definite shear speed and temperature. The mixture either forms a gel or shows phase separation on cooling to room temperature. The formation of a stable bigel is dependent on the composition of both the phases. The gel formation is checked by tube inversion test (Singh et al., 2014c).

10.5 Characterization techniques

10.5.1 Organoleptic evaluation

The bigels are cooled down to room temperature after formation, and analyzed for appearance, color, homogeneity, consistency, pH, and phase separation, if any. The bigels are white to off-white in color and viscous in nature. The formulations should appear homogenous on visualization. Kodela et al. (2017) reported an increase in the intensity of white color with an increased proportion of oleogel. The bigels with higher proportion of oleogel possessed a higher degree of scattering as compared to aqueous gel due to the occurrence of interfaces formed by the immiscible phases. The increased oleogel proportion also resulted in reduced firmness and increased brittleness in the bigels (Kodela et al., 2017).

10.5.2 Stability studies

The stability of the bigels is evaluated by accelerated stability study (freeze−thaw thermocycling method) and long-term stability study. Because bigels are a combination of two phases, which are semisolid in nature, their thermodynamic stability increases to several folds (Kodela et al., 2017). The formulations are evaluated by conducting studies on pH, color, homogeneity, consistency, phase separation, and overall appearance. Any other signs of destabilization after each cycle of freeze−thaw thermocycling and at predefined time intervals (0, 3, 6, 12, 18, and 24 months or up to 36 months based on the active substance present in the bigel) are also studied during long-term stability study.

10.5.3 Accelerated stability study

Accelerated stability study of the bigels is performed by performing five cycles of freeze−thaw thermocycling (15 min of freezing at −20°C followed by 15 min of thawing at 70°C). The bigels are evaluated for pH, color, homogeneity, consistency, phase separation, and overall appearance or any other sign of destabilization after each cycle. Accelerated stability study predicts the long-term stability study.

10.5.4 Long-term stability study

It is also known as "real-time stability study." Long-term stability study of the bigels is performed as per the ICH guidelines. The storage temperature of the formulations is

decided by evaluating the drug-containing product for upto 2 years or more (0, 3, 6, 12, 18, and 24 months or upto 36 months) by incubating the samples at different probable storage temperatures. The bigels are evaluated for pH, color, homogeneity, consistency, phase separation, and overall appearance or any other sign of destabilization at regular intervals during the stability study duration.

10.5.5 Optical microscopy

The mutual disposition of organic and aqueous phases within the bigels is studied using different types of microscopy techniques, which include confocal microscopy, scanning electron microscope (cryo-SEM), transmission electron microscopy, and phase contrast microscopy, etc. Confocal microscopy is used to identify the oil and/or aqueous phases by incorporating fluorescent dyes in the two phases (Lupi et al., 2015). Patel et al. (2015) converted the bigels into freeze-fractured samples to study the distribution pattern of aqueous and organic phases using cryo-SEM. Kodela et al. (2017) reported that the bigels containing higher proportions of the oleogels possessed bigger-sized globular structures and further increment of the oleogel concentration resulted in irregularly shaped globular structures. Moreover, the oleogel proportion in some samples beyond a critical oleogel concentration resulted in the formation of bicontinuous bigels (Kodela et al., 2017). A schematic diagram representing the microscopic arrangements of different types of bigels has been shown in Fig. 10.2.

10.5.6 Droplet size distribution

The droplet size distribution is a critical factor in bigel formulations for drug absorption and colloidal stability. The smaller droplet size provides large interfacial surface area, which increases the drug absorption and provides excellent colloidal stability and vice versa (Yan et al., 2011). The droplet size distribution of the dispersed phase in bigels is analyzed using size analysis tools such as ImageJ software. Droplet size distribution is determined by calculating the width of the droplet size distribution; a dimensionless number also known as SPAN number (Eq. 10.1) (Liu et al., 2012).

$$\text{SPAN} = (D_{90} - D_{10})/D_{50} \tag{10.1}$$

where D_{10}, D_{50}, and D_{90} represents the cumulative particle size distribution of 10%, 50%, and 90% particles, respectively (Piacentini et al., 2014).

10.5.7 Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FTIR) spectroscopy evaluates the functional groups present in the bigel formulations (Singh et al., 2014a). Most of the functional groups of molecules absorb infrared rays in a range between 4000 and 1500 cm^{-1}. Therefore, the spectrum is recorded in this range to determine the lipophilic and the hydrophilic nature of the colloidal mixtures (Rehman and Zulfakar, 2017). The absorption bands

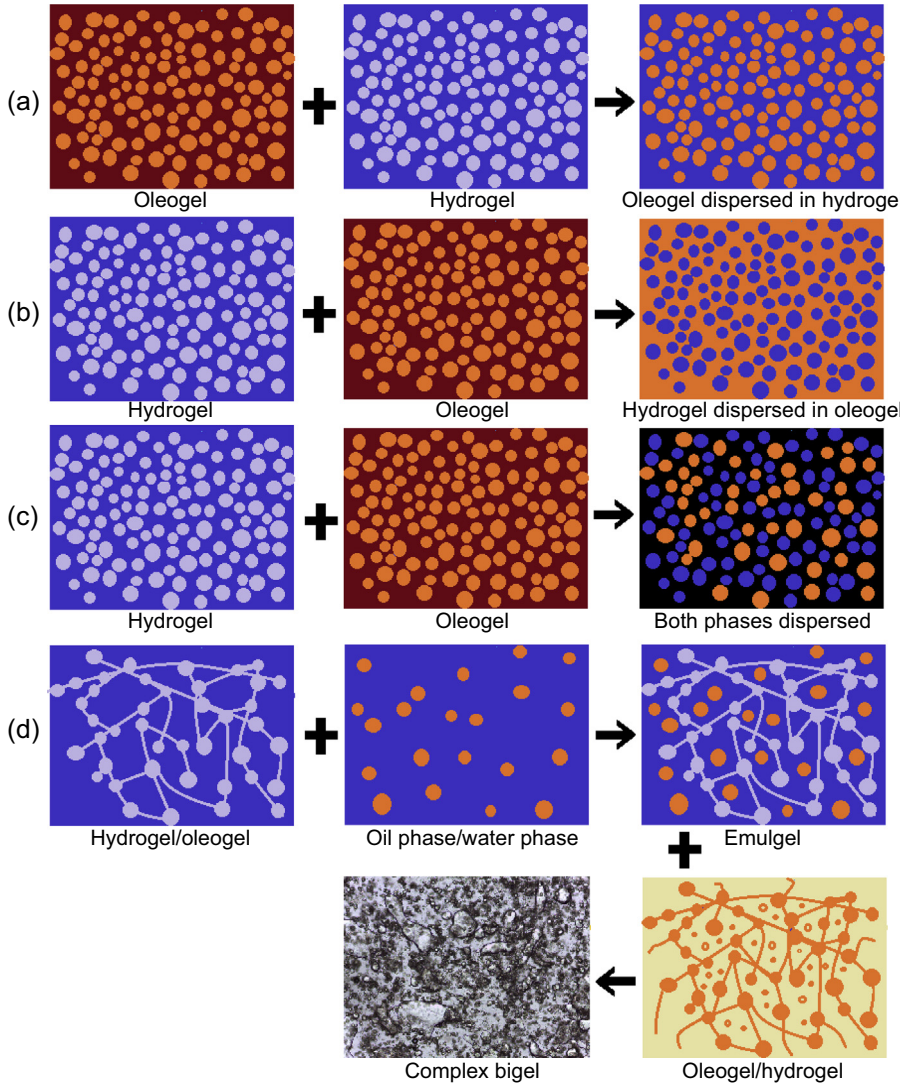

Figure 10.2 Schematic diagram representing microscopic arrangements of different types of bigels. (a) Oleogel dispersed in hydrogel, (b) hydrogel dispersed in oleogel, (c) both phases are dispersed, and (d) Complex bigel.
Modified from Di Michele, L., et al., 2014. Aggregation dynamics, structure, and mechanical properties of bigels. Soft Matter; Lupi, F., et al., 2015. Olive oil and hyperthermal water bigels for cosmetic uses. Journal of Colloid and Interface Science 459, 70–78.

below 1500 cm^{-1} are generally referred to as the "fingerprint region" of the spectrum, which is an indicative of deformation, bending- and ring-vibration (Schmitt and Flemming, 1998). Attenuated total reflection is a helpful analytical tool for identifying the chemical composition directly in the undisturbed state without any requirement of

sample preparation (Harrick, 1967). Generally, a broad hump is observed in the range of 3300−3200 cm^{-1} which is mainly attributed to the intra- or intermolecular hydrogen bonds within the hydrogel. Kodela et al. (2017) reported that the incorporation of the oleogel within the agar hydrogel resulted in the formation of the bigels bringing about a considerable decrease in the intra- and intermolecular hydrogen bonding of the agar gels. Rehman and Zulfakar, (2017) also reported reduced transmittance in the region of 3330 cm^{-1} as the oleogel ratio inside the colloidal mixture is increased from 10% to 50% (Rehman and Zulfakar, 2017).

10.5.8 Mechanical properties

Various mechanical properties of the bigels such as viscosity and stress relaxation are studied by various instruments, viz. viscometer, rheometer, and mechanical tester. A cone-and-plate viscometer is used to record the viscosity of the bigel. Generally, the measurement is done at room temperature (25°C) with shear rate ranging between 20 and 100 s^{-1} (Neves et al., 2009). The measured data are fitted using the Ostwald-de Waele Power Law. Ostwald-de Waele Power Law is used to represent the viscosity profiles of the non-Newtonian fluids. The model is represented by Eq. (10.2).

$$\tau = K \cdot \gamma^{n} \tag{10.2}$$

where, τ is the shear stress, γ is the shear rate, K is the flow consistency coefficient, and "n" is the power law (or rate) index. The n-value is a measure of the shear-thinning property.

Patel et al. (2015) reported that the rheology of the bigels increased synergistically when organic and aqueous phases were mixed together. This synergistic effect on the rheology of bigels might have resulted in due to the interpenetrating network of the organic and the aqueous phases. The bigel did not form at an aqueous gel concentration higher than 50% of its proportion with organogel. At higher ratios of an aqueous gel, viscous sols were formed (Patel et al., 2015). Similarly, Di Michele et al. (2014) reported the arrested demixing of the binary colloidal mixtures. The two-component gel so formed showed enhanced rheological properties due to the formation of multiple smaller clusters resulting from the rearrangement of the particles under deformation. One-component gels form only single compact cluster (Di Michele et al., 2014).

Various mechanical properties (such as stress relaxation and spreadability) of the bigels are analyzed using the static mechanical tester. The stress relaxation study is performed by moving the probe into the bigels upto a certain distance after detecting a defined force. This results in the increase of the force to a maximum value, named as F_0. The probe is allowed to remain undisturbed at the said distance for a defined time. Initially, the force values decrease at a faster rate and later on reach a plateau phase

called as "residual force," F_r. Percent stress relaxation of the formulations is calculated using Eq. (10.3).

$$\% \text{ relaxation} = \left(\frac{F_0 - F_r}{F_0}\right) \times 100 \tag{10.3}$$

10.5.9 Thermal properties

Thermal properties of the bigels are studied using differential scanning calorimetry (DSC). The experiment is performed under an inert N_2 gas atmosphere using aluminum crucibles with pierced aluminum lid. The formulations (~15 mg) are hermetically sealed in aluminum pans, with pierced lids. The melting and crystallization events are recorded in a defined temperature range (e.g., 25−150°C) under an inert (nitrogen) atmosphere (Souza et al., 2014). The change in the entropies during the melting event is calculated by using Eq. (10.5).

$$\Delta G = \Delta H_m - T_m \Delta S \tag{10.4}$$

where, ΔG is Gibbs' free energy.

ΔG is zero at the melting point of the material, though the enthalpy and the entropy of the sample increase. The melting occurs at the point when ΔG of the liquid becomes lower than the solid (Lam and Rogers, 2011).

10.5.10 Electric conductivity

The electrical property of the bigels is measured to understand their conductivity profiles. The electrical profiles of the bigels can be measured using a computer-controlled impedance analyzer. The data are measured in a desired frequency range (e.g., 0.1 Hz−1 MHz) at room temperature (Lupi et al., 2015). The conductivity of the formulations helps in understanding their transport behavior under the influence of current (Singh et al., 2014b). Also, it helps in understanding the microstructural arrangement of the bigel system. The bigels containing a higher proportion of hydrogel shows higher conductivity due to the ions present in the water phase. O/W bigels show conductive behavior, whereas, W/O bigels show insulative behavior, i.e., approximately zero electrical conductivity (Lupi et al., 2015).

10.5.11 In vitro release study

Franz's diffusion cell is used to perform in vitro release study. The volume required for dissolution, pH of dissolution media, temperature, and stirring speed is selected accordingly. The study is performed for a predefined time period. The drug aliquot is collected at different time points, filtered through a 0.45 μm millipore filter, and is assayed using UV-Vis spectrophotometer or HPLC. Various mathematical models

(zero-order, first-order, Higuchi and Korsmeyer-Peppas model) are used to fit the release kinetics of the drug. The amount of drug release is affected by many factors such as (including but not limited to) initial water content, drug loading, electrostatic interaction between drugs, lipid bilayers, and swelling capacity.

Zero-order kinetics represents the delivery systems that release the drug from the delivery vehicle at constant rate, e.g., reservoir type delivery systems. This can be expressed by Eq. (10.5) (Pal et al., 2013):

$$M_R = M_0 + kt \tag{10.5}$$

where, M_R is the amount of drug released/present in the dissolution media, M_0 is the initial amount of the drug present in the dissolution media, "k" is proportionality constant, and "t" is the time at which M_R has been released.

First-order release kinetics suggests a constant rate of release of the drug over the period of time. This is mathematically represented by Eq. (10.6) (Pal et al., 2013; Barzegar-Jalali et al., 2008):

$$\ln M_R = \ln M_0 + kt \tag{10.6}$$

where, M_R is the amount of drug released/present in the dissolution media, M_0 is the initial amount of the drug present in the dissolution media, "k" is the release constant, and "t" is the time at which M_R has been released.

Higuchi, in 1961 proposed a model to explain the release of the drug from the matrix-type delivery vehicle. The matrix-type delivery vehicle is based on the uniform dispersion of drug particles within the insoluble homogenous polymer matrix. The model suggests that the solutes diffuse through Fickian diffusion from the polymer matrix. The model may be represented by Eq. (10.7) (Dash et al., 2010).

$$M_R = k_H \times t^{0.5} \tag{10.7}$$

where, M_R is the amount of drug released/present in the dissolution media, k_H is the Higuchain release constant, and "t" is the time at which M_R has been released.

Korsmeyer et al. (1983) gave an equation to understand the drug-release pattern from a polymeric system. First 60% of the drug-release data are fitted with Korsmeyer-Peppas model (Eq. 10.8) to understand the drug-release mechanism (Korsmeyer et al., 1983).

$$M_t/M_\infty = Kt^n \tag{10.8}$$

where, M_t/M_∞ is the fraction of the drug released at a time "t," "k" is the release rate constant, and "n" is the release exponent. The n-value characterizes different types of release for cylindrical-shaped matrices. The n value of 0.45 corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to case II (relaxational) transport, and $n > 0.89$ to super case II transport.

10.5.12 Photostability study

Photostability testing is done to identify the stability of the formulation in the presence of light. This study helps in determining the primary and the secondary packaging of the final product. Photostability testing of the drug-loaded bigel is done as per ICH guideline Q1B (Guideline, 1996). ICH guidelines (Q6) suggest the sample exposure to light ensuring an overall illumination of not less than 1.2 million lux hours and not less than 200 Wh/m^2 (Guideline, 1996). The product is kept in four conditions, i.e., by wrapping in aluminum foil (control), by packing in a carton, by keeping in primary packing container with label, and by keeping in primary packing container without label (worst condition). After completion of exposure to the defined light, the product is evaluated in terms of appearance, pH, assay, and impurities generated, if any.

10.5.13 In vitro skin permeation studies of drug-loaded bigels

The study is performed by acute skin toxicity testing using rat's skin (Takeuchi et al., 2011). The rat's back is shaven and two fields of 1 cm^2 dimension are marked. One field is left untreated and the other site is rubbed gently with the drug-loaded bigel formulations (~1 mL). Another rat treated with a blank bigel is considered as control. The rats are observed at predefined time intervals (e.g., 1, 24, and 48 h) for any developed sign of local and systemic toxicity. Draize scoring system is used to calculate the irritation score (Boddu et al., 2014).

10.6 Applications

In recent years, numerous bigel systems are being investigated for various types of applications. Mostly, these systems are used as vehicles for controlled release of the active ingredient in topical and transdermal applications. Andonova et al. (2017) prepared bigels using carbopol hydrogel and sorbitan monostearate (SMS)—almond oil based organogel for medicated topical applications with a moisturizing effect. Various hydrogel:organogel ratios (80/20, 70/30, and 60/40 w/w) were evaluated to form stable bigel formulations. The formulation prepared with the hydrogel:organogel ratio of 60/40 w/w was found to be unstable during the early stage of storage due to syneresis in the almond oil organogel. According to authors, this syneresis in the bigel formulation was due to the imbalance of the interfacial tension between almond oil and SMS and the gravitational force. The rest two formulations, prepared at hydrogel:organogel ratios of 80/20 and 70/30 w/w, were stable and showed very similar mechanical properties (e.g., viscosity, spreadability), bimodal droplet size distribution, and droplet mean diameter. The in vivo results did not show any signs of skin toxicity (Andonova et al., 2017).

Singh et al. (2014c) prepared guar gum and sesame oil—based bigels for controlled release of ciprofloxacin. The bigel containing higher organogel proportion

possessed higher thermal stability. The developed bigels were biocompatible and demonstrated good antimicrobial activity against *Bacillus subtilis*. Bigels demonstrated controlled drug release governed by the zero-order diffusion kinetics (Singh et al., 2014c). Further, Singh et al. (2014e) developed metronidazole-loaded bigels for vaginal drug delivery using SMS−sesame oil organogel and carbopol 934 hydrogel. Metronidazole-loaded bigels showed diffusion-mediated drug release and good antimicrobial activity against *Escherichia coli* (Singh et al., 2014e).

Sagiri et al. (2015) synthesized bigels by mixing gelatin hydrogel and stearic acid−based organogel using hot emulsification method. Stearate organogels, prepared by mixing stearic acid in sesame oil and soya bean oil, were used to prepare bigels. Leaching of the internal phase from the bigels was found to be minimum as compared to the emulgels. Confirmation regarding the presence of organogel matrix within the bigels was accomplished using different techniques such as XRD, FTIR, and DSC. Mucoadhesive nature and mechanical stability of the developed bigels were better in comparison to emulgels. The in vitro release of ciprofloxacin showed non-Fickian diffusion from the bigel matrices (Sagiri et al., 2015).

Due to the inherent electroconductive nature, bigels can be used as a carrier for iontophoretic drug delivery. Singh et al. (2014c) reported iontophoretic delivery of metronidazole from guar gum and sesame oil−based bigels for topical application. The drug release was found to be lower in the bigels containing higher concentration of oleogel and followed zero-order release kinetics (Singh et al., 2014c). Further, carbopol 934 and sesame oil−based bigel showed 13%−38% increase in the release of metronidazole under the influence of constant current source (Singh et al., 2014a). They also evaluated metronidazole-loaded carbopol-based bigels for iontophoretic drug delivery applications (Singh et al., 2014d). Moreover, they revealed that the developed bigels were electroconductive in nature and showed higher bulk resistance and lower drug release (Singh et al., 2014d).

Behera et al. (2014) evaluated starch and nonstarch polysaccharide-based bigels prepared by mixing Span 40 and sunflower oil oleogel with aqueous polysaccharide sol as a matrix for controlled delivery of metronidazole and probiotics. The encapsulated probiotics were found to be tolerant of the gastric and the intestinal environment compared to the free cells (Behera et al., 2014). Behera et al. (2015b) explored metronidazole-loaded bigels prepared by mixing Span 40−sunflower oil oleogel with aqueous gels of water-soluble synthetic polymers (e.g., polyvinyl alcohol and polyvinyl pyrrolidone) as a controlled delivery vehicle. The drug release was found to be slower in the bigels containing a higher concentration of polymer (Behera et al., 2015a). Behera et al. (2015b) further prepared bigels by mixing Span 40−sunflower oil oleogels and protein-based hydrogels. The stability of the bigels was found to be improved due to the addition of proteins. The developed bigels showed good antimicrobial activity against *E. coli* and proposed their use as

matrices for controlled drug release (Behera et al., 2015c). In 2015, the same group also prepared bigels by mixing sunflower oil−span 40 oleogel with natural gum−based hydrogels as matrices for delivery of antimicrobial agent (Behera et al., 2015b). Wakhet et al. (2015) presented a comparative study of gelatin−agar based phase-separated hydrogel, emulgel, and bigel. They reported that emulgels and bigels were comparatively more crystalline and mechanically stable than the phase-separated hydrogels and can be suitable candidates for drug-delivery applications (Wakhet et al., 2015).

Almeida et al. (2008) prepared physically stable bigels by varying the composition of polyacrylic acid hydrogel and oleogels. The resulted bigels exhibited cooling effect and good spreadability properties of hydrogels with enhancing emollient and moisturizing effect (Almeida et al., 2008). Rehman et al. (2014) reported fish oil−containing bigel prepared using natural (sodium alginate) and synthetic (hydroxypropyl methylcellulose) polymers as topical and transdermal drug-delivery systems. The developed bigels showed pseudoplastic behavior with excellent spreadability and adhesiveness. The permeation and the drug flux were also higher as compared to hydrogels. Fish oil also helped in increasing the drug permeation (Rehman et al., 2014). Rehman et al. (2015) developed a colloidal bigel containing fish oil as an anti-inflammatory agent for controlled release of imiquimod, a drug used in the treatment of many skin-related diseases. By performing NMR and computerized molecular modeling, they tried to decipher the mechanism of imiquimod transport (Rehman et al., 2015). Rehman et al. (2017) further reported an imiquimod-loaded novel bigel system by mixing carbopol hydrogel and fish oil oleogel in different proportions for controlled drug delivery. The developed bigel showed higher drug availability inside the skin as compared to the individual hydrogel and oleogel formulations. The drug followed quasi-Fickian diffusion mechanism for its controlled release. The antitumor effects were enhanced and the tumor progression was also reduced, which may be associated to the combined effect of imiquimod and fish oil. The combination also increased the expression of interleukin-10, an anti-inflammatory cytokine, which acts against skin cancer (Rehman and Zulfakar, 2017). Patel et al. (2015) reported the use of fumed silica, which possesses oil-structuring properties, to generate triglyceride solvent-based soft matter systems such as organogels and bigels. Bigel was formed on addition of water gel to the structured oil. The developed bigels possessed better gel strength compared to the organogels, whereas the thixotropic recovery was weaker (Patel et al., 2015). Kodela et al. (2017) prepared ciprofloxacin hydrochloride−loaded bigels based on agar hydrogel and stearyl alcohol oleogel mixed in different proportions. Microscopic analysis confirmed the formation of the biphasic bigels and bicontinuous bigel at lower and higher proportions of oleogel, respectively (Kodela et al., 2017). A summary of applications of the bigel-based systems has been tabulated in Table 10.1.

Table 10.1 Applications of the different bigel systems

Organic phase	Aqueous phase	Application	References
Span 60, cholesterol, silicic acid–based oleogel	Carbopol 934–based hydrogel	Moisturizing effect of oleogel	Almeida et al. (2008)
Sorbitan monostearate–almond oil based organogel	Carbopol 940 dissolved in propylene glycol, ethanol, and purified water	Topical application exhibiting moisturizing effect	Andonova et al. (2017)
Sorbitan monostearate–sesame oil organogel	Carbopol 934 hydrogel	Treatment of bacterial vaginosis (model drug metronidazole)	Singh et al. (2014e)
Sorbitan monostearate–sesame oil organogel	Carbopol 934 hydrogel	Iontophoretic delivery systems (model drug metronidazole)	Singh et al. (2014d)
Span 40–sunflower oil oleogel	Aqueous gels of water-soluble synthetic polymers (e.g., polyvinyl alcohol and polyvinyl pyrrolidone)	Controlled delivery vehicle (model drug metronidazole)	Behera et al. (2015a)
Sorbitan monostearate as the gelator in sesame oil	Guar gum–based hydrogel	Topical drug delivery systems (model drug Ciprofloxacin)	Singh et al. (2014c)
Fish oil oleogel	Carbopol hydrogel	Enhanced antitumor effects, reduced tumor progression, and increased antitumor activity against skin cancer	Rehman and Zulfakar (2017)
Sorbitan monostearate as the gelator in sesame oil	Guar gum–based hydrogel	Iontophoretic delivery systems (model drug ciprofloxacin)	Singh et al. (2014a)
Stearic acid in sesame oil and soy bean oil	Gelatin hydrogel	In vitro release of ciprofloxacin	Sagiri et al. (2015)
Span 80: Tween 80 surfactant mixture as the liquid gelator and sunflower oil as the organic phase	Gum acacia as the ionic gum and guar gum as nonionic gum	Iontophoretic delivery systems (model drug metronidazole)	Sahoo et al. (2015)

Organogel with policosanol as organogelator and glyceryl stearate as emulsifier in extra virgin olive oil	LM pectin	Cosmetics and pharmaceuticals	Lupi et al. (2016)
Organogel containing Span 60, cetyl alcohol, or lecithin-pluronic as organogelators in soya bean oil	Hydroxypropyl−methylcellulose (HPMC) based hydrogel	Transdermal drug delivery	Ibrahim et al. (2013)
Sunflower oil−span 40 oleogel	Natural gum−based hydrogels	Controlled drug release	Behera et al. (2015b)
Fish oil oleogel	Natural (sodium alginate) and synthetic (hydroxypropyl methylcellulose) polymer	Topical and transdermal drug delivery	Rehman et al. (2014)
Fish oil oleogel	Carbopol hydrogel	Controlled drug release	Rehman and Zulfakar (2017)
Stearyl alcohol oleogel	Agar hydrogel	Controlled drug release	Kodela et al. (2017)
Span 40−sunflower oil oleogels	Protein-based hydrogels	Controlled drug release	Behera et al. (2015c)
Span 40 and sunflower oil oleogel	Aqueous starch and nonstarch polysaccharides sol	Controlled delivery of metronidazole and probiotics	Behera et al. (2014)
Bovine serum albumin	Gelatin	Drug delivery and tissue engineering	Blumlein and McManus (2015)

10.7 Conclusion

In recent years, bigels are gaining importance due to its widespread applications in food, pharmaceuticals, and cosmetic industry. The presence of beneficial properties of both, hydrogels and oleogels, improves patient compliance along with the loading capability of both hydrophilic and lipophilic drugs. So far, researchers have explored the bigel systems mainly for controlled drug delivery for topical applications. These systems have been explored in academic research but commercial products are still not established. Therefore, further research needs to be conducted to address the potential issues such as stability, toxicity, and bioequivalence of bigels to establish them as ideal controlled delivery vehicles for topical applications.

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Synthesis and biomedical applications of filled hydrogels

11

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11.1 Introduction

Gel-based systems have received much attention in the pharmaceutical, food, and biomedical industries during the last couple of decades (Kickelbick, 2007). This can be attributed to the fact that gels may be easily modulated to have varied consistencies, as for example, ointments, sauces, and dessert dressing. Such formulations appear as semisolid formulations, which can be easily spreadable (Sivaraman et al., 2017). On the contrary, wound dressings, scaffolds, and jell-O appear as solid matrices at room temperature (Steed, 2011). Every formulation has different types of applications. The properties of the gels can be modulated by incorporating a second phase within it (Aichinger et al., 2017). Such systems have been regarded as filled gels (de Lavergne et al., 2016). If the external phase is aqueous in nature, then the gel system is regarded as filled hydrogels. In this review, we will discuss about the different categories of filled hydrogels, their methods of preparation, and their applications.

Broadly, the filled hydrogels have been categorized into two categories, namely, oil/fat-filled hydrogels and water-in-water type of filled hydrogels (McClements, 2017). The oil/fat-filled hydrogels are formed when the oil/fat droplets are trapped within a continuous matrix of hydrogels. It might be necessary to add emulsifiers (e.g., sorbitan monopalmitate, sorbitan monooleate, polyglycerol polyricinoleate, Tween 20, and Tween 80) so as to result in the formation of oil/fat droplets of uniform size within the continuous hydrogels matrix. This is necessary because the distribution of the droplet sizes within the hydrogels matrix plays an important role in altering the physical and the mechanical properties of the filled hydrogels (Li et al., 2015). It is important to note that if the internal phase of the oil/fat-containing filled hydrogels is liquid in nature, then the samples are regarded as emulsion gels (also known as emulgels). On the contrary, if the internal phase is semisolid in nature, the samples are regarded as bigels because both the phases are semisolid in nature (Ullah et al., 2015). Recently, oleogel-based samples have been reported for varied applications (Patel, 2015). Many researchers have incorporated the oleogels as the internal phase of the hydrogels. As oleogels are semisolid in nature, such oleogel-in-hydrogel—based filled hydrogels are also categorized as bigels (Patel et al., 2014). In many cases, oleogels are formed by fluid-filled matrix mechanism. In such type of samples, a surfactant

Polymeric Gels. https://doi.org/10.1016/B978-0-08-102179-8.00011-9

is solubilized within an apolar solvent (Xenakis et al., 2016). Addition of water into the solution of the surfactant in apolar solvent results in the generation of self-assembled microarchitectures, which result in the formation of a three dimensional (3D) network. This network helps immobilizing the apolar solvent. A classical example of this kind of sample is lecithin-based organogels (Elnaggar et al., 2014). Such filled systems are regarded as filled organogels, as the aqueous phase is the internal phase. Because, in this study, we are discussing about filled hydrogels, such fluid-filled organogels will not be discussed further.

Apart from the emulsion-based filled hydrogels, another kind of filled hydrogels has been explored extensively by many researchers. In such a system, both the internal and external phases are aqueous in nature (Ahmed, 2015). Such kind of systems is formed when protein and polysaccharide solutions are mixed together. It is reported that the formation of such kind of filled hydrogels may occur either via segregative phase separation or associative phase separation. If the polysaccharide used has either a neutral or similar charge as that of protein molecules, then segregative phase separation is prevalent. Associative phase separation happens when the protein and the polysaccharide molecules bear opposite electrical charge. Because the formation of such filled hydrogels are dependent on the interactions among the protein and the polysaccharide phases, based on their net electrical charges, the formation of such kind of systems is dependent on the local environmental conditions (pH and ionic strength) (Le et al., 2017). Usually, the associative interactions result in the formation of coacervates. Many researchers have explained this kind of hydrogels as water-in-water type of emulsions (Esquena, 2016).

Another type of filled hydrogels is prepared by entrapping particles (micro or nano) within the hydrogel matrices. This type of hydrogels usually consists of particles (micro or nano) made up of polymers or inorganic microparticles, which are subsequently suspended into a continuous matrix of hydrogel (Ullah et al., 2015). The particles may be of matrix type or it may contain oil within its core (core−shell particles). Fig. 11.1 summarizes the different types of filled hydrogels.

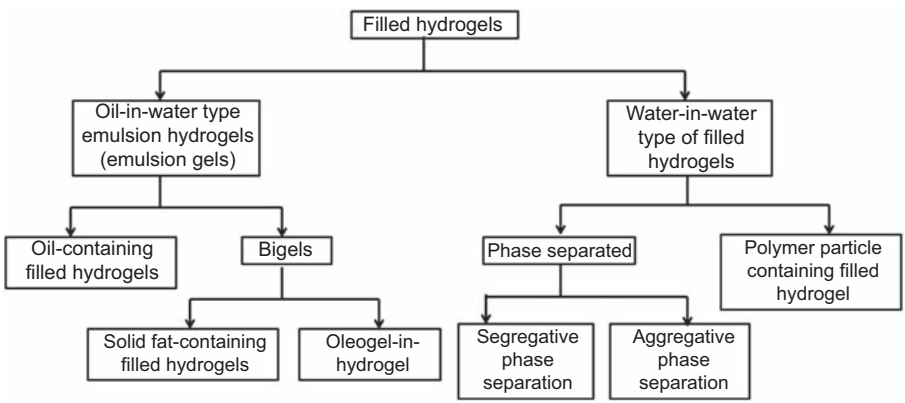

Figure 11.1 Classification of filled hydrogels.

11.2 Methods of preparation of the filled hydrogels

As discussed in the previous section, we have seen that the filled hydrogels can be categorized into a large number of categories. The preparation of the samples of these categories of filled hydrogels requires specific techniques. Hence, it becomes necessary to discuss about the different types of methods of preparation of the filled hydrogels.

11.2.1 Preparation of emulsion gels

For the preparation of the emulsion gel, a primary oil-in-water emulsion is first developed. The oil phase consists of the bioactive agents, which need to be delivered within the human body. The bioactive agent may either be of therapeutic or nutraceutical importance. The aqueous phase consists of either proteins or surfactants (usually nonionic surfactants such as Tween 20). The protein and the surfactants are used as emulsifiers, which help in stabilizing the interface between the oil and the aqueous phases. The oil phase is dispersed into the emulsifier-containing aqueous phase and homogenized thoroughly. This results in the formation of the coarse emulsions. Some researchers have reported to process the course emulsions in a microfluidizer (Jafari et al., 2007). This step allows the conversion of the coarse emulsions into fine emulsions. It is important to note that the size of the internal droplets of the primary emulsion developed not only alters the physical properties of the emulsion gels but also play a significant role in tailoring the release profiles of the bioactive agents from the emulsion gels. Hence, proper care should be taken to decide the size distribution of the internal droplets, bearing in mind the end application of the developed product. After the primary emulsion is formed, the external aqueous phase is jellified using proper gelling agents (e.g., starch, sodium caseinate, gelatin, etc.) (Pintado et al., 2015). The gelling process may be induced either by physical gelation method (Fig. 11.2) or by chemical cross-linking technology (Fig. 11.3) (Pandey et al., 2016; Soltani and Madadlou, 2016). The physical gels are formed due to the physical interactions among the polymeric materials (usually entanglement of the polymer chains and hydrogen bonding) (Singh et al., 2016). Under shear, once these physical forces are overcome, such type of formulations or samples undergoes a semisolid to liquid type of phase transition. Such phenomenon is regarded as shear-thinning phenomenon (Behera et al., 2015). On the contrary, in the emulsion gels, where chemical cross-linking is carried out to achieve gelation, they form permanent hydrogels (Negru et al., 2010). The permanent hydrogels do not show shear-thinning phenomenon (Appel et al., 2012).

Solid fat−containing filled hydrogels are prepared in a similar manner as that of emulsion gels. Because the solid fats are solid at room temperature, they are converted into its liquid form at higher temperature so as to allow the processing of the samples. Hence, the solid fat−containing filled hydrogels are prepared by homogenizing the solid fat and the aqueous phase at temperatures higher than the melting temperature of the solid fat (McClements, 2010). Oleogel-in-hydrogel−based filled hydrogels are prepared in a similar process as that of solid fat−containing filled hydrogels. This is because oleogels also appear as solid sample at room temperature and undergoes melting at higher temperatures.

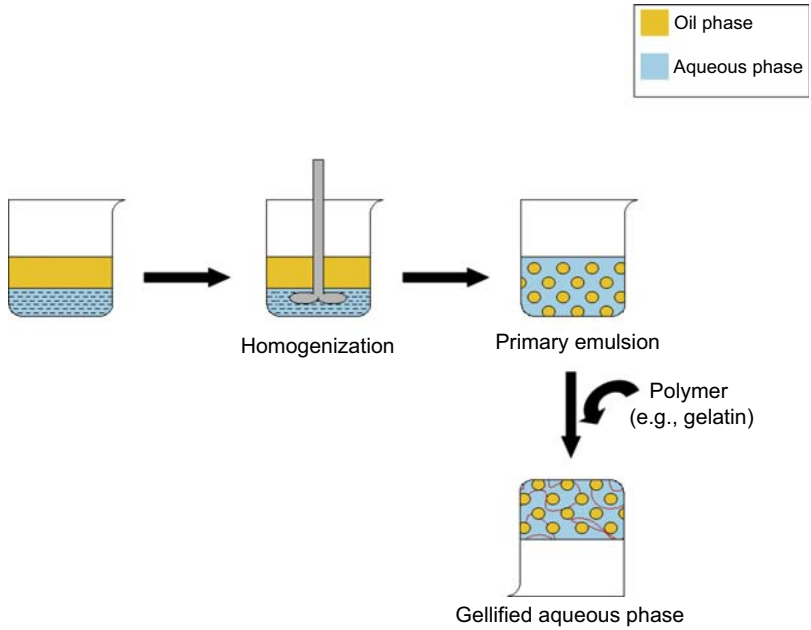

Figure 11.2 Preparation of the filled hydrogels by physical gelation method.

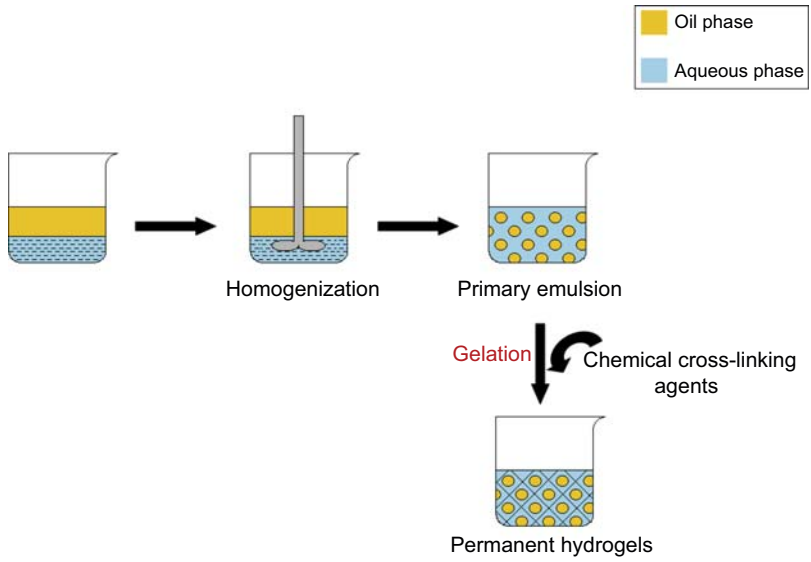

Figure 11.3 Preparation of the filled hydrogels by chemical cross-linking technology.

11.2.2 Preparation of water-in-water type of filled hydrogels

Water-in-water type of filled hydrogels is usually categorized into phase-separated and polymer particle−loaded filled hydrogels. The former is predominant when there are interactions among the two types of biopolymers used. The occurrence of the phase separation has been reported due to the fact that the contribution of the entropy of such systems is greater than the contribution due to the enthalpy (Doublier et al., 2000). The affinity between the polymers and the solvent plays an important role during the preparation of the phase-separated systems. After the two polymer mixtures are mixed, if the polymers have repulsive forces acting against each other, then, segregative phase separation is reported to occur (Le et al., 2017). This can be explained by the thermodynamic incompatibility among the two phases of the polymers (Thongkaew et al., 2015). This phenomenon results in the formation of two distinct phases, namely, polymer 1−rich phase and polymer 2−rich phase. In such hydrogels, the interactions between the solvent and each of the polymers are stronger in comparison to the polymer−polymer and solvent−solvent interactions (Vis et al., 2014). On the contrary, if the polymer−polymer interactions are stronger, associative phase separation (coacervation) occurs. Such kind of interactions is predominant when both the polymers have opposite charges. Protein−polysaccharide based mixed hydrogels usually form such kind of phase-separated systems (Fig. 11.4). Phase-separated hydrogels have been reported to be formed by mixing protein (e.g., gelatin) and polysaccharide (e.g., starch, carboxymethane cellulose) solutions. They are usually formed by mixing a solution of protein and polysaccharide solutions at temperatures greater than 50°C. When the temperature is lowered, the strong associative forces among the protein−protein molecules and the polysaccharide−polysaccharide molecules are promoted than the protein−polysaccharide interactions. Interestingly, in such hydrogels, the proportion of the protein phase has been reported to be higher as compared to the polysaccharide phase. Hence, such kind of system results in the

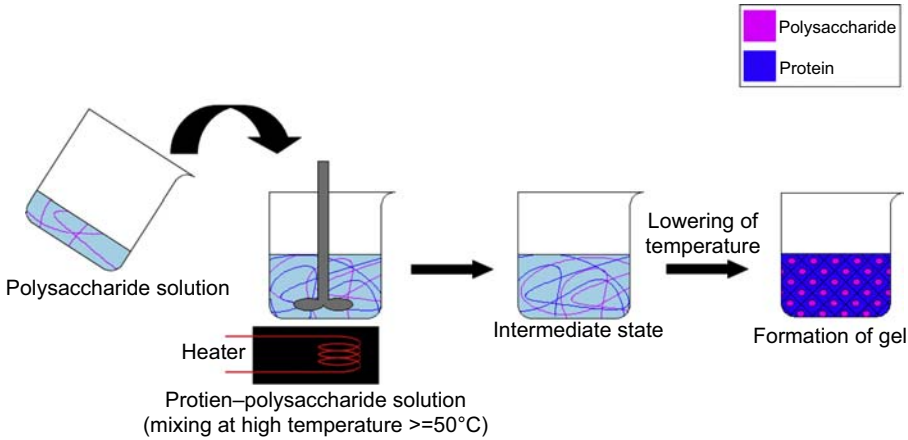

Figure 11.4 Preparation of water-in-water type of filled hydrogels.

formation of dispersed polysaccharide phase with a continuous protein phase. The coacervate system is formed when the pH of the protein−anionic polysaccharide mixture is slightly lower than the isoelectric point of the proteins. In such cases, the protein assumes an overall cationic charge, whereas, the anionic polysaccharides retain their net negative charge. This results in the formation of a polymer-rich phase (coacervates) and a solvent-rich phase (Doublier et al., 2000).

The polymer particle−containing filled hydrogels are prepared by entrapping polymer micro- or nanoparticles (matrix type) within the hydrogel matrices (Fig. 11.5). Ribeiro et al. (2013) reported the synthesis of matrix type chitosan microparticles loaded within dextran hydrogels. The authors reported the development of chitosan microparticles by ionotropic gelation. In this study, sodium tripolyphosphate was used as the ionic cross-linking agent for the chitosan matrix. The chitosan solution (in acetic acid) was prepared and loaded into a plastic syringe. The microparticles were prepared by electrospinning method, collected, and transferred into an aqueous solution of sodium triphosphate. The harvested microparticles were washed with distilled water. The prepared microparticles were then suspended into an oxidized dextran solution in phosphate buffer saline. Thereafter, the oxidized dextran phase was chemically cross-linked using adipic acid dihydrazide.

In a separate study, Jeon et al. (2015) proposed the synthesis of coacervate microparticle (matrix type) loaded hydrogels. For the preparation of the particle-loaded hydrogels, the authors employed the principle of phase separation. In the study, the authors synthesized oxidized-cum-methacrylated alginate (OMA), which was used as the polysaccharide phase. Methacrylated gelatin (MelMA) was used as the protein phase. The aqueous solutions of both the polymers (oxidized-cum-OMA and MelMA) were mixed together. Due to the thermodynamic incompatibility among the polysaccharide and the protein phase, micrometer-scale coacervates were formed. Concurrently, the aldehydic groups of the derivatized alginate reacted with the amine groups present within the derivatized gelatin molecules by photo−cross-linking mechanism. This resulted in the formation of polysaccharide-based coacervates (microparticles) immobilized within a continuous protein phase.

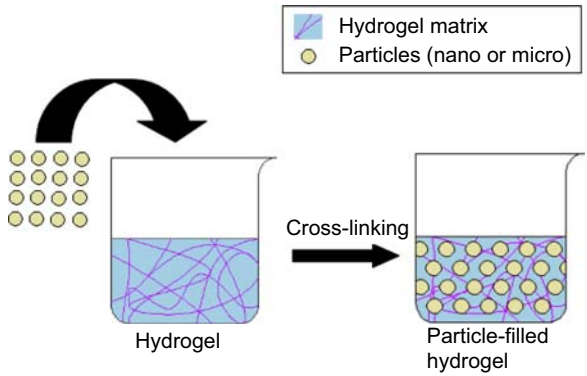

Figure 11.5 Preparation of particle-filled hydrogels.

Park et al. (2009) synthesized magnetic nanoparticle-incorporated filled hydrogel microparticles for enzyme immobilization. The hydrogel microparticles were prepared from polyethylene glycol (PEG) of varied molecular weight by free radical polymerization.

The enzyme named peroxidase was covalently linked on the surface of the magnetic nanoparticles using 3-aminopropyltriethoxysilane. Finally, photopatterning was implemented to incorporate the enzyme-linked nanoparticles within the hydrogel microparticles.

The particle-filled hydrogels have also been prepared using inorganic microparticles (e.g., $CaCO_3$ and hydroxyapatite) instead of polymer particles. Han et al. (2017) reported the development of a novel polyacrylamide (PAM)/hydroxyapatite composite hydrogel for bone tissue engineering applications. The hydroxyapatite was prepared using a peroxide route as reported in the literature with slight modifications. Then the formation of PAM/hydroxyapatite suspension was achieved using ammonium persulfate as the initiator. The suspension was subjected to gelation, followed by washing with water and freeze-drying to obtain the composite.

11.3 Applications

Hydrogels have found extensive applications in pharmaceutical, food, and biomedical industries due to their semisolid nature. Further, these polymeric architectures are highly biocompatible in nature. Filled hydrogels are a class of hydrogels, which consist of a dispersed phase within a continuous hydrogel matrix. As discussed previously, the dispersed phase may either be oil, fat, microparticle, etc. In this section, an attempt will be made to discuss about the applications of the different filled hydrogel–based systems in tissue engineering and pharmaceutical and nutraceutical industries (Table 11.1).

11.3.1 Oil-containing filled hydrogels

The delivery of lipophilic active agents via oral route has found applications in food and pharmaceutical industries. The lipophilic compounds have been encapsulated within hydrogel matrices. This is due to the fact that such systems have been reported to improve the shelf-life of the product by preventing the decomposition of the bioactive agents during the storage. Further, the hydrogels may be designed to release the bioactive agents either in the mouth or in the specific region of the gastrointestinal tract. In food industry, such formulations have been developed as the carrier for flavors. Oil-filled hydrogels have been used for reducing the fat content in food (Chung et al., 2013). Such systems have also been explored for the delivery of the antimicrobial agents, which can be used for maintaining a good oral health.

Filled hydrogel particles have been reported to improve the oxidative stability of the lipophilic compounds entrapped within the hydrogel matrix (Salcedo-Sandoval et al., 2015). Zhang et al. (2015a) reported the development of the filled hydrogel particles for the encapsulation of lipid droplets. The hydrogel matrix was prepared by employing electrostatic interaction among the caseinate and alginate biopolymers. The authors

Table 11.1 Applications of the different filled hydrogel-based systems in tissue engineering, pharmaceutical, and nutraceutical industries

Type of filled hydrogel	Internal phase	External phase	Application	References
Oil-containing filled hydrogels	Emu oil nanoemulsion	Carbopol gel	Drug delivery	Jeengar et al. (2016)
	Olive oil/sunflower oil/light liquid paraffin	Low methoxy pectin and Kondagogu gum	Drug delivery	Bera et al. (2017)
	Khardal oil, zanjabeen oil, and podina oil	Carbopol 940	Drug delivery	Saleem and Idris (2016)
Bigels	Stearate-based organogel	Gelatin-based hydrogel	Drug delivery	Sagiri et al. (2015)
	Organogel with an extra virgin olive oil as solvent, policosanol as organogelator, and glyceryl stearate as emulsifier.	LM pectin	Cosmetics and pharmaceuticals	Lupi et al. (2016)
	Organogel with soyabean oil as solvent and span 60, acetyl alcohol or lecithin-pluronic as organogelators	Hydroxypropyl-methylcellulose (HPMC)—based hydrogel	Transdermal drug delivery	Ibrahim et al. (2013)
	Bovine serum albumin	Gelatin	Drug delivery and tissue engineering	Blumlein and McManus (2015)

Phase-separated hydrogels	Starch	Gelatin	For organoleptic properties in food products	Firoozmand and Rousseau (2013)
	Amylopectin	Gelatin		Yadav et al. (2016)
	Carboxymethyl Chitosan	Gelatin	Antimicrobial drug delivery	Pandey et al. (2016)
	Maltodextrin	Gelatin	Controlled delivery of fluorescent markers	Khan et al. (2011)
Particle-filled hydrogel	Lipid nanoparticles (trimyristin,tyloxapol, and thiomersal)	Alginate microbeads	Carrier of lipophilic compounds in pharmaceutical and food products	Strasdat and Bunjes (2013)
	Zein core and whey protein shell	Casein and alginate microgel	Stability improvement of food ingredients	Zou et al. (2016)
	Gold and iron nanoparticles	Alginate	Tissue engineering	Blaeser et al. (2016)
	Hydroxyapatite nanoparticles	N-isopropyl acrylamide, acrylamide, and monoacryloxyethyl phosphate	Bone tissue engineering	Watson et al. (2015)

were able to develop filled hydrogel particles of required dimensions and improved stability when the pH of the biopolymer solutions used were adjusted in between 4 and 5 (isoelectric point of caseinate). Under this condition, both the aforesaid polymers acquired a net negative charge. It was proposed by the authors that the formation of the stable hydrogel particles could be explained due to the improved electrostatic interaction between both the biopolymers used. The authors found that when the proportion of alginate-to-caseinate ratio was high, filled hydrogel particles with desired property could be obtained. The proposed hydrogel particles were developed with an intention to release the lipid droplets, when the particles are placed in the mouth. Unfortunately, the prepared particles were stable only in the pH range of 4 and 5. Outside this pH range, the particles became unstable. The authors have reported that this disadvantage can be overcome by cross-linking the hydrogel matrix. In another study, the authors prepared temperature-triggered oil-filled microgels using gelatin−casein polymer composite (Zhang et al., 2015b). The hydrogels were formed due to the electrostatic interaction among the casein and gelatin molecules. An increase in the temperature above 35°C resulted in the disintegration of the microparticles. This was due to the fact that gelatin gels undergo melting at 35°C. The disintegration of the polymer matrix resulted in the triggered release of the oil droplets. The authors reported that the prepared filled hydrogels can be explored as the delivery systems for delivering bioactive agents in the oral cavity.

Zeeb et al. (2015b) reported the development of calcium alginate-based filled hydrogel beads. The prepared beads were used as the delivery system for lipid droplets. The authors reported that the polymer concentration and the pH of the polymeric solution, used for the development of the filled hydrogel beads, played an important role in tailoring the release behavior of the lipid droplets. The alteration in the polymer concentration resulted in the tailoring of the pore size of the hydrogel matrices. On the contrary, the alteration in the pH could result in the change in the electrostatic interaction among the lipid and the alginate molecules. Though, the beads were stable in a wide range of pH conditions (as one would expect in the gastrointestinal tract), these particles could be susceptible to degradation in the distal portion of the gastrointestinal tract. Hence, the authors reported that such systems would be used successfully as colonic delivery systems. The authors reported that the prepared hydrogel beads can also be used for developing trigger release system, where the electrostatic interactions among the lipid and the calcium alginate molecules can be tailored. In another study, the authors reported that the emulsion technique used for the preparation of the filled hydrogel beads can play an important role in the retention and the release properties of the oil droplets from the filled hydrogel beads (Zeeb et al., 2015a). The authors demonstrated this activity using curcumin as the lipophilic bioactive agent and calcium alginate as the hydrogel matrix.

Mun et al. (2015a) reported the development of starch-based filled hydrogels. The authors used β-carotene as the lipophilic bioactive agent. β-carotene is an inactive precursor of vitamin A and has been used in pharmaceutical industry for its pro-vitamin A activity. The authors studied the effect of starch molecules, which were extracted from mung bean and rice. They used whey protein isolates and Tween 20 as the surfactants. It was found that the bioaccessibility of the β-carotene molecules

was significantly improved when it was encapsulated within the starch-based gels. Interestingly, the increase in the bioaccessibility of the β-carotene molecules was observed in both types of the starch. The use of the emulsifier of different chemical compositions did not negatively affect the bioaccessibility of the β-carotene. Among the emulsifiers, when whey protein isolates were used for the emulsification process, a significant increase in the bioaccessibility of the β-carotene molecules was observed. The authors concluded that the prepared filled hydrogels can play an important role in deciding the fate of the lipid molecules in the gastrointestinal tract. In a separate study (Mun et al., 2015b), the authors reported that the bioaccessibility of the β-carotene molecules was significantly higher when it was incorporated within the filled hydrogels as against when it was incorporated within the emulsions and hydrogels. Komaiko and McClements (2015) reported that nanoemulsion-filled hydrogels can be explored as functional food gels for the delivery of the lipophilic bioactive agents (Komaiko and McClements, 2015). Nanoemulsions are inherently thermodynamically stable and generally transparent formulations. The designing of such systems did not significantly alter the rheological and the optical properties of the gelatin dessert.

Singh et al. (2015) developed nimorazole-containing topical emulgel formulations. Nimorazole is a radiosensitizer and is used for the treatment of hypoxic tumors. The prepared formulations are expected to deliver the drug nimorazole across the skin to the hypoxic tumor site. After the radiosensitization is achieved, the radiotherapy process can be initiated. It is expected that the side effects associated with the conventional roots of radiosensitizers can be avoided by this method.

Shen et al. (2015) developed an emulgel-based ocular delivery system for the ocular delivery of cyclosporine A (Shen et al., 2015). In this study, the authors have used carbophil as the aqueous gelling agent and castor oil was used as the dispersed phase. The stability of the emulgels was found to be consistent for a period of 3 months. It was observed that the ocular retention time and the bioavailability of the drug was considerably improved when the drug was delivered using the emulgel formulation. Further, the emulgel formulations did not induce ocular irritation. Based on the results obtained in the study, the authors suggested that the prepared emulgels can be explored as the topical ocular delivery systems.

Varma et al. (2014) developed carbopol-based emulgels. A combination of the liquid paraffin kollicream 3C and kollipher CS20 was used as the dispersed oily phase. The prepared emulgels were tested as a probable delivery system for calciprotriol. Calciprotriol is one of the commonly used medications for the treatment of psoriasis. The authors found that the prepared emulgels showed sufficient physical and drug release properties to be used as topical drug delivery systems. The use of the penetration enhancers (PEG and isopropyl alcohol) was able to improve the drug permeation properties. The authors reported that the development of calciprotriol gel is difficult to make due to its hydrophobic nature. But, the employment of the emulgel formulation as a delivery system could allow them to make stable formulations. Hence, it is expected that the emulgel-based formulations can be explored successfully for the delivery of calciprotriol and other hydrophobic drugs. In the year 2015, Sawant and Mohite (2015) reported the development of cabopol 934 and carbopol 940-based emulgel formulations for the delivery of itraconazole (Sawant and Mohite, 2015).

Itraconazole is an antifungal drug. The authors found that the antifungal activity of the prepared itraconazole formulations was superior to that of the commercially available itraconazole formulations. The emulgel formulations did not induce skin irritation, marked by no formation of edema and erythema on the rabbit skin.

Pinheiro et al. (2016) developed amphotericin B loaded emulgels for treating skin leishmaniasis. Amphotericin B is one of the drugs of choice for the treatment of leishmaniasis. The formulations were challenged against the strains of *Leishmania major* MHOM/L/80/Friendlin of *Leishmania major*. Based on the study, the author reported that the prepared formulations can be explored for the treatment of cutaneous Leishmaniasis. Recently, various authors have reported the synthesis of emulgels for various drugs (e.g., diclofenac diethylamine, oxiconalzole, piroxicam) (Preeti and Suresh, 2015; Gadad et al., 2017; Gu, 2017).

11.3.2 Bigels

Singh et al. (2014b) reported the development of guar gum and sesame oil—based bigels as matrices for drug delivery systems. The prepared bigels were semisolid in nature and showed advantages over the conventional gels. In this study, the authors used sorbitan monostearate as the gelator for sesame oil. The authors loaded ciprofloxacin within the bigels. They reported that as the amount of organogel was lowered, there was a subsequent increase in the release of ciprofloxacin from the bigels. The release of the drug was found to be via zero-order diffusion kinetics. Because ciprofloxacin is an antimicrobial drug, the authors found good antimicrobial efficacy for the prepared bigel formulations. Based on the results they concluded that the prepared bigels could be explored as topical drug delivery systems.

Sahoo et al. (2015) reported the development of bigels using ionic and nonionic gums. In the study, the authors have used gum acacia as the ionic gum, whereas, guar gum was used as the nonionic gum. The internal phase of the bigels was fluid-filled organogel, prepared using span 80: tween 80 surfactant mixture as the liquid gelator and sunflower oil as the organic phase. The authors loaded the formulations with metronidazole, an antimicrobial drug. The prepared gels were explored as matrices for iontophoretic delivery systems. It was observed that the ionic bigels (where gum acacia was used as the gelling agent for the aqueous phase) showed better drug release properties as compared to the guar gum bigel. This was explained by the polyelectrolytic nature of the gum acacia.

Recently, Rehman and Zulfakar (2017) reported the development of fish oil—based bigel systems. The bigels were used for the delivery of imiquimod. The prepared bigels showed good antitumour activities. It was found that the bigels were able to markedly reduce the serum cytokine levels and also reduce tumor progression. It is expected that the proposed imiquimod-loaded bigel delivery systems can be used for the treatment of skin cancer.

11.3.3 Phase-separated hydrogels

The phase-separated hydrogels are prepared by the combination of two polymer aqueous solutions. The interaction between these polymers whether physical (such

as Van der Waals forces, hydrophobic interaction, and hydrogen bonding) or chemical (such as covalent bonds) (Liu and Urban, 2010) results in either attraction or repulsion. The attraction signifies that the polymers have more affinity toward each other than the affinity between solvent−solvent and solvent−polymer interactions. This results in the formation of two phases (aggregative phase separation), in which, one phase is rich in biopolymers and other is rich in the solvent. On the other hand, the repulsion implies that the solvent−polymer interaction overcomes the affinity between solvent−solvent and polymer−polymer (segregative phase separation) interaction (Doublier et al., 2000). On account of their properties, these polymer-in-polymer filled hydrogels are registering their significant presence in different fields such as pharmaceuticals, tissue engineering, etc.

The development of cell delivery systems, which are biocompatible and possess the ability to mimic the 3D tissue environment, has gained much importance in recent years for tissue engineering and regenerative medicine applications. Chen et al. (2011) reported the fabrication of phase-separated microbeads from the blends of naturally derived polymers, namely chitosan and fibrinogen. The microbeads were explored as a 3D matrix for the delivery of human fibroblast cells (Chen et al., 2011). After thrombin-mediated polymerization of fibrin and physical gelation of chitosan, the microbeads were formed using phase separation, employing polydimethylsiloxane as an emulsifier. In the microbeads, chitosan helped to maintain the structural integrity and to avoid mechanical damage of the microbeads. On the other hand, the cell proliferation and attachment was enhanced by the natural protein, fibrin. Considering these properties, the authors proposed that the developed formulations can be used in tissue regeneration applications.

Singh et al. (2014a) reported the formation of gelatin and polysaccharide (such as sodium carboxymethyl cellulose, maltodextrin, and dextran)-based stable phase-separated hydrogels. The author characterized these prepared hydrogels by performing microscopic analysis, mechanical testing, and impedance spectroscopy studies. To propose its application as a drug delivery agent, metronidazole was loaded in the gels and its release profile was examined. Further, its antimicrobial activity was tested against *E. coli* and *B. subtilis*. The results of the drug release and antimicrobial studies suggested the potential of the developed hydrogels to deliver therapeutic agents in the vaginal lumen of the sexually active females for the treatment of sexually transmitted disease. The authors further suggested that the release profile of the drug can be controlled by tailoring the composition of its constituents.

Shaw et al. (2015) synthesized biocompatible gelatin−tamarind gum (TG)/carboxymethyl tamarind gum (CMT) based phase-separated hydrogels. On analyzing and comparing the mucoadhesive property of both the hydrogels, the author suggested that TG-based hydrogel was more interactive with mucin in comparison to the CMT-based hydrogel. Both the gels were found to be hemocompatible and cytocompatible. The antimicrobial activity of the hydrogels was tested against *E. coli* using ciprofloxacin as the model drug. The swelling profile of the hydrogels was found to be pH dependent, and hence, can be useful in pH triggered drug delivery.

Shaw et al. (2017) proposed the development of stable phase-separated hydrogel films, combining gelatin−TG/CMT with PEG (used as plasticizer). The characterization

of the films was performed using mechanical and impedance analysis studies. The stress relaxation properties were found to be dependent on the composition of the formulations. The cell proliferation capability of the developed films was examined using human keratinocytes (HaCaT cells) as the representative cells. The results suggested that the polysaccharide-containing films showed better cell viability in comparison to the control. The drug-loaded films exhibited good drug release and antimicrobial properties. Based on the results, the authors concluded that the developed formulations have huge potential for applications in skin tissue engineering.

Mechanical strength, water absorbency, and cell adhesive capability are some of the most important criteria for selecting a particular scaffold in tissue engineering applications. In the last few decades, much attention has been paid by the researchers for exploring the suitability of the phase-separated hydrogels as scaffolds by tailoring their properties according to the requirements in in vivo and in vitro tissue-engineering conditions (Przeradzka et al., 2017). Przeradzka et al. (2017) synthesized phase-separated hydrogel networks consisting of PEG, PCL, poly(D,L-lactide), and poly (trimethylene carbonate) macromers using stereolithography technique. The prepared formulations had excellent mechanical properties such as controlled pore size, toughness, and stiffness (Przeradzka et al., 2017). The phase separation of the developed formulations was evident from the atomic force microscopy and X-ray diffraction studies. These networks did not fail under compression and were able to return to their original dimensions after undergoing reequilibration in water. Considering the abovementioned properties, the authors suggested that the prepared phase-separated hydrogels can act as a suitable candidate for developing soft tissue engineering related implants such as intervertebral discs and manisci.

11.3.4 Polymer particle–filled hydrogels

Although the hydrogels possess attractive physical properties, their application in drug delivery is often restricted due to the rapid drug release profile, caused by their high water content (Ribeiro et al., 2013). Also, a limited amount of drug is loaded in the hydrogels to avoid the risk of potential harmful side effects on the patients because of the exposure to high drug concentrations (Ribeiro et al., 2013). Researchers have suggested the incorporation of different micro- and nanoparticles within the hydrogel matrix to overcome the abovementioned limitations of the hydrogels (Ribeiro et al., 2013; Acharya et al., 2010).

Hydrogels are usually unable to deliver hydrophobic drugs due to their hydrophilic nature. However, Joung et al. (2007) reported the fabrication of polymer particle–filled thermosensitive hydrogels for controlled delivery of hydrophobic drugs. Initially, poly(lactic-*co*-glycolic acid) (PLGA) particles were produced using an oil-in-water emulsion method. Indomethacin (a hydrophobic drug) was mixed with the PLGA particles. A 16% chitosan–pluronic (CP) solution was converted into a temperature-sensitive hydrogel by raising the temperature above a lower critical solution temperature, and the indomethacin-laden PLGA particles were homogeneously distributed in the CP hydrogel. It was found that the resulting formulation released 30% of the loaded drug in 25 days, which was nearly half in comparison to the release

from the PLGA particles only. This, in turn, suggested the controlled release of the drug due to the influence of the CP matrix on the degradation and the release of the drug from the PLGA particles.

The immobilization of enzymes onto or into various organic and inorganic materials has always been an economical route for their repeated usage. Hydrogels have been explored for enzyme immobilization due to their matrix-like structure (Park et al., 2009). However, the leaching of its enzymes from the hydrogels often limits its applications. Park et al. (2009) proposed an innovative approach to employ hydrogel microparticles instead of bulk hydrogels for indirect enzyme immobilization. In this study, peroxidase (model enzyme) was immobilized on the surface of the magnetic nanoparticles. The peroxidase-linked nanoparticles were then encapsulated within the different shapes of PEG-based hydrogel microparticles using photopatterning. The enzyme was able to continue its activity for 1 week without any leaching from the hydrogel microparticles. Based on this result, the authors concluded that the proposed method of enzyme immobilization may be used for biosensor and bioreactor applications by embedding suitable biomolecules inside the hydrogel microparticles.

The use of implantable biosensors is mainly hindered by the inflammatory response of the immune system toward these devices. The inflammatory response can be restrained using continuous local delivery of an antiinflammatory drug by providing a drug coating on it (Wang et al., 2010). Wang et al. (2010) synthesized a pH-sensitive molecularly imprinted polymer nanosphere/hydrogel composite for potential application as a coating in implantable biosensors to enhance their biocompatibility. The performance of the developed coating was tested using dexamethasone-21 phosphate disodium (DXP) as a model anti-inflammatory drug. The proposed composite coating was found to exhibit superior controlled release of DXP as compared to the pure hydrogel thereby, increasing the life span of the implantable biosensors.

Delivery of the therapeutic agents to specific sites in the brain is hindered due to the restrictions such as exposure time, spatial and temporal release, and compatibility of the delivery system with the brain environment (Lampe et al., 2011). Lampe et al. (2011) reported the preparation of PLGA microparticles−filled PEG hydrogel. The developed hydrogel was exploited for the delivery of two neurotrophic factors (NF), namely, brain-derived neurotrophic factor and glial-derived neurotrophic factor at two different locations of the brain (i.e., striatum and substantia nigra). The results suggested that the PLGA/PEG biocompatible device successfully delivered the above-mentioned NFs with time-coordinated release profile and lower inflammatory response. Thus, the authors concluded that the developed hydrogel can act as suitable candidate for drug delivery and tissue-engineering applications. Ribeiro et al. (2013) reported the development of dextran-based hydrogels embedded with chitosan microparticles for wound-healing applications. The microparticles were loaded with epidermal and vascular endothelial growth factors to aid the wound-healing process. The authors investigated the carriers' morphology using scanning electron microscopy, the cytotoxicity profile and the degradation by-products using in vitro assays, and the performance in the treatment of skin burns using in vivo experiments. The monitoring of the wound-healing process was performed using macroscopic and

histological analyses. The macroscopic analysis exhibited that the duration of wound healing was shorter in the animals subjected to microparticles-embedded hydrogels than that of the control groups. The absence of reactive inflammatory reactions in the skin lesions was evident from histological analysis. The in vitro and in vivo experiments revealed that the degradation by-products were biocompatible in nature. Based on the abovementioned results, the authors proposed that the developed filled hydrogel can be used for the controlled delivery applications. Jeon et al. (2015) reported the formation and characterization of coacervate-loaded photo−cross-linked hydrogels for bone tissue engineering applications. The hydrogels were prepared by mixing oxidized OMA and MelMA in aqueous solution at a range of pH values and room temperature. The developed hydrogels facilitated the concurrent formation of drug-laden microdroplets and the incorporation of stem cells in the coacervate hydrogels. Based on these properties, the authors proposed that the developed formulation can act as a novel platform for therapeutic applications.

Apart from the polymer particles, inorganic microparticles such as $CaCO_3$ and hydroxyapatite have also been conjugated with hydrogels to develop novel biomedical materials for controlled delivery applications (Wang et al., 2008). Wang et al. (2008) proposed the development of hydrophilic alginate/$CaCO_3$ microparticles−based hybrid hydrogel system for the delivery of drugs and proteins. The author fabricated porous $CaCO_3$ microparticles loaded with the hydrophobic drug ibuprofen (IBU). The hybrid hydrogel was prepared by mixing the IBU-loaded porous $CaCO_3$ microparticles with the alginate polymer in the presence of D-glucono-D-lactone. The study suggested the improvement in the mechanical strength and sustainable drug release profile of alginate polymer by integrating $CaCO_3$ microparticles as the structural component, which can unlock applications of this hybrid hydrogel in the delivery of less water-soluble substances.

11.4 Conclusion

In the last few decades, hydrogels have registered wide spread applications in various fields, where the material has to be in contact with the aqueous environment due to its the gellike properties and hydrophilic nature of its constituents. Among the different types of hydrogels, a new class of hydrogels called “filled hydrogels” has received special attention of the researchers. Filled hydrogels consists of a hydrophilic polymer as an external phase accommodating an internal phase of materials such as oils, natural polymers such as protein and polysaccharide, other gels, microparticles and nanoparticles, depending on the type of the application. These internal and external phases may be stabilized by either physical or chemical cross-linking. Over the last few decades, significant improvement has been observed in the development of innovative hydrogel formulations such as filled hydrogels for the controlled delivery and tissue regeneration applications. However, further research needs to be conducted to address potential issues such as acidic degradation products and rapid release profiles of hydrogels to establish them as an ideal controlled delivery and tissue regeneration platform (Huynh et al., 2011).

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Polymeric gels for tissue engineering applications

12

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12.1 Introduction

Extracellular matrix (ECM) is composed of various components such as collagen, proteoglycans, low and high molecular weight proteins, and soluble factors; altogether, they create a cellular microenvironment. It is well understood that the natural environment of the cell that supports, synthesizes, and constantly remodels the matrix, which is connected to the cell via cell surface receptors, whereby the cellular responses and activities such as proliferation, migration, regulating growth and cell–cell communication, and differentiation become turn on (Frantz et al., 2010). Tailoring of an in vitro cell culture environment that mimics the tricky nanoscale meshwork of native ECM is essential to develop a tissue or organ. Though tissues and organs can be reconstructed with different strategies, combining patient's cells with polymeric scaffold is considered the most appealing approach toward success. In this approach, cells isolated from patient's biopsy are incorporated into three-dimensional (3D) polymer scaffolds that act as an equivalent to the ECM in vivo. These scaffolds are delivered to the desired site of the patient's body along with the patient's cells that provide a structure and space for new tissue formation and potentially control the structure and function of the engineered tissue (Putnam and Mooney, 1996; Marler et al., 1998). Many reports are available on the prospective of polymeric hydrogel to mimic natural ECM and to get engineered native-like ECM to provide cells with rational cues for diagnostic and therapeutic studies (Geckil et al., 2010).

Many tissues such as tendon, ligament, cartilage, bone, skin, and artery are being developed and near clinical use (Niklason et al., 1999; Oberpenning et al., 1999; Pomahač et al., 1998; Ma and Langer, 1999; Lin et al., 1999). Fundamentally, polymer scaffold is the critical component of all tissue-engineering approaches. The polymer can possibly replace many functions of tissue ECMs. Synthetic polymers including poly(glycolic acid), poly(lactic acid) (PLA), and their copolymers (poly(lactic-co-glycolic acid) (PLGA)) and aliphatic polyesters are the most widely used polymers in tissue engineering (Kim and Mooney, 1998), which has been approved by FDA and used in medical applications for a long time. However, the disadvantage of these polymeric scaffolds is the required incisions to enable implantation of the constructs with or without cells. A promising alternative to scaffold–cell delivery for tissue engineering is the polymeric hydrogels that can be injected into the site by a minimally invasive procedure. Apart from this, the

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structural resemblance to the macromolecular-based extracellular components and the biocompatibility made hydrogels an attractive form of polymers for tissue engineering applications (Jhon and Andrade, 1973). In this chapter, the critical design properties and types of hydrogels to be used in tissue engineering are discussed. We also present latest examples to demonstrate the different application of polymeric hydrogels in the field of tissue engineering.

12.2 Design properties

The complex ECM network supports cells by creating an environment in which cells' receptors assimilate signals from ECMs. These signals control the cell functions and behavior while concurrently sending out signals from the cells to build and remodel their ECM microenvironment. The ECMs consist of a large variety of macromolecular proteins whose precise arrangement, specific structures, and composition vary from tissue to tissue (Gullberg et al., 2012), which is considered as the crucial factors that affect overall structure and biomechanical properties of the formed matrix. Also, these macromolecular components' precise arrangement, specific structures, and composition can influence and modulate the signals communicated to cells from the ECM (Kirkpatrick and Selleck, 2007; Rozario and DeSimone, 2010). Thus, it is greatly desirable to design scaffolds to mimic the natural ECM, structurally and biologically (Ma, 2008; Chen and Hunt, 2007; Tibbitt and Anseth, 2009). There are many factors that have to be considered during the synthesis of the required hydrogel scaffold materials, majorly physical, biological, and mass transport properties required for each specific application. These design variables are identified and decided by the in vivo environment into which the scaffold is typically implanted. Scaffolds intended for cell encapsulation must get gelled without being destructive to the cells and toxic to the cells and the neighboring tissue on post-gelling, facilitate nutrients and oxygen diffusion, and possess sufficient mechanical integrity and strength (Lim, 1984). Here, the physical, mass transport, and biological properties are categorized based on mechanical properties, gel formation dynamics, and diffusion requirements of the gel (Lim, 1984).

12.2.1 Physical properties

The successful synthesis of a hydrogel scaffold is mainly decided by the physical properties of the material and it includes (1) mechanisms of gel formation, (2) mechanical properties, and (3) degradation dynamics and behavior. These properties of the polymeric hydrogel are primarily decided by the intrinsic properties of the main chain and size, amount and type of cross-linking molecules, and by environmental conditions.

12.2.1.1 Mechanism of gel formation

The mode of scaffold delivery and cell/biomolecule encapsulation is dictated by mechanisms of gel formation and dynamics. Mixing with bioactive factors or cells

prior to injection and gel formation in situ is widely accepted to avoid cell death during the hydrogel fabrication by cross-linking reagents, increased temperature, and unfavorable pH. It has been demonstrated that injectable forms of alginate (Paige et al., 1995; Marler et al., 2000; Alsberg et al., 2001; Lee et al., 2001b), chitosan (Chenite et al., 2000), P(PF-co-EG) (Suggs and Mikos, 1999; Suggs et al., 1999; He et al., 2000), and PEO (Jeong et al., 1997; Elisseeff et al., 1999) can be delivered with bioactive molecules and cells that gelled in vivo. This approach would be successful only if there is a control on liquid flow properties and gel formation rates during pre- and post-gelling (Drury and Mooney, 2003).

12.2.1.2 Mechanical properties

Hydrogels are insoluble hydrophilic networks of cross-linked polymers (Fig. 12.1) (Ahmed, 2015) akin to the physical structure of native ECM (Peppas et al., 2006). One of the reasons for its wide acceptability in tissue engineering applications is its similar biomechanical property to the many native tissues (Burdick, 2009). Mechanical properties of polymeric hydrogels can be modulated by fine-tuning the parameters such as polymers used, their concentrations, and the cross-linking density (Wenger et al., 2007; Anseth et al., 1996). Biocompatible hydrogel scaffolds can be obtained by selecting biocompatible synthetic or natural polymers and cross-linkers (Rivest et al., 2007). To provide volume maintenance function, scaffolds must be able to bear the load. Meanwhile on the microscopic level, cell growth and differentiation, and further tissue formation are completely dependent on mechanical cues (Kim et al., 1999; Butler et al., 2000; Cowin, 2000; Sikavitsas et al., 2001). Mechanical properties of a scaffold depends on compressibility, tensile strength, failure strain, elasticity, and viscoelastic behavior, which can be controlled by polymer and

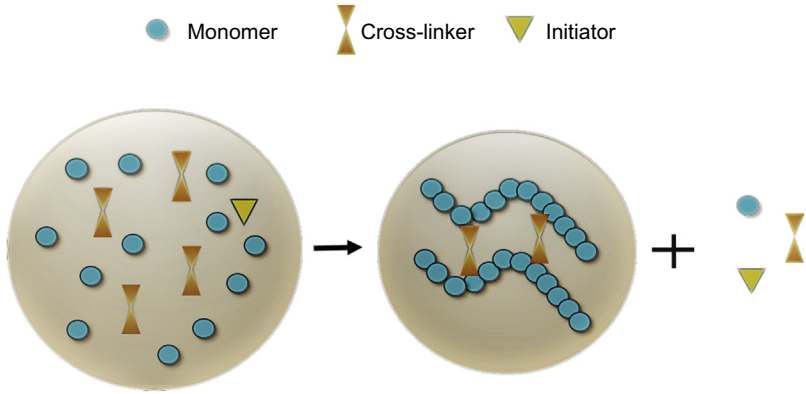

Figure 12.1 Schematic diagram of three integral part of hydrogel preparation. After cross linking hydrogel mass needs to be washed to remove impurities left from the preparation process. These include non-reacted monomer, initiators and cross-linkers.
Adapted from Ahmed, E.M., 2015. Hydrogel: preparation, characterization, and applications: a review. Journal of Advanced Research 6, 105−121 with permission.

cross-linker characteristics, gelling conditions such as temperature, pH, swelling, and degradation (Anseth et al., 1996). For example, increasing lengths of G blocks and an increase in ratios of G to M subunits contribute to the mechanical strength and compressive modulus of alginate (Smidsrød and Skja, 1990). Additionally, both equilibrium shear modulus and compressive modulus increased when there was an increase in volume fraction from 1% to 3% of alginate (LeRoux et al., 1999). Also comparable increment observed in compression modulus of poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) hydrogels with an increase in weight fraction from 10% to 40% (LeRoux et al., 1999; Stammen et al., 2001). Hydrogel mechanical properties are also affected by the cross-linker type and density. Furthermore, the mechanical strength is dependent on ionic concentration and type of cross-linker as evidenced by an increase in mechanical strength of alginate hydrogels, when it is cross-linked with higher concentration of ions as well as with divalent ions that have a higher affinity for alginate (Smidsrød and Skja, 1990). Similarly, cross-linker density is also a determinant of mechanical shear modulus of covalently cross-linked alginate (Lee et al., 2000b). At the same time, mechanical strength of the gel decreases up on swelling of the hydrogels (He et al., 2000; Anseth et al., 1996; Bryant and Anseth, 2002). However, the gel strength and swelling can be controlled independently by varying cross-linker type and density in covalently cross-linked alginate hydrogels (Lee et al., 2000b). Gel becomes weak if the hydrogel degrades or dissolved (LeRoux et al., 1999; Bryant and Anseth, 2002), unless tissue ingrowth acts to strengthen them (Bryant and Anseth, 2002) or these properties are decoupled (Lee et al., 2000c). The preferred degradation kinetics of scaffold depends and varies on the specific tissue engineering application.

12.2.1.3 Degradation dynamics and behavior

In vivo, ECM is subjected to constant remodeling by cells with maintaining balance between matrix synthesis and degradation by a variety of enzymes called matrix metalloproteinase and other group of enzymes (Tibbitt and Anseth, 2009; Hong et al., 2007). Applications where both tissue regeneration and release of biomolecules or growth factors are concerned, the rate of scaffold degradation and new tissue formation should occur concomitantly and to be adequate for the controlled or desired release of growth factors or bioactive molecules (Drury and Mooney, 2003). Thus, it is essential to understand the rate and the mechanism by which each hydrogel is being degraded. The hydrogel network structure design orchestrates the proper degradation of hydrogel scaffolds (Lee and Mooney, 2001). There are mainly three basic mechanisms of degradation of hydrogels; they are hydrolysis, enzymatic cleavage, and dissolution. Firstly, hydrolysis occurs at a persistent rate in both in vivo and in vitro, and it is worth to note that for hydrolytically labile gels such as PEG−PLA copolymer, the degradation rate can be modulated only by altering the material composition but not the environment (Saito et al., 2001). Most of the synthetic hydrogels come under this category and are degraded through ester linkage hydrolysis (Suggs and Mikos, 1999; Saito et al., 2001; Metters et al., 2000). Secondly, enzymatic degradation, which is purely determined by the number

of cleavage sites present in the polymer and the enzymes type and quantity of which is available in the scaffold environment where is being implanted (West and Hubbell, 1999; Mann et al., 2001). Natural polymers such as chitosan and collagen are all degraded by enzymatic degradation (Alberts, 1994; Lee et al., 1995; Vårum et al., 1996; Tomihata and Ikada, 1997). The rate of dissolution depends on the presence of ions in the site of implantation as exemplified by ionically cross-linked alginate (LeRoux et al., 1999), where it normally undergoes dissolution (LeRoux et al., 1999) though it can also go through controlled hydrolysis after partial oxidization (Bouhadir et al., 2001).

12.2.2 Mass transport properties

Hydrogel scaffolds architecture for tissue engineering must have sufficient porosity to possess a large surface area to volume ratio. This highly interconnected pores facilitate uniform distribution cells, ingrowth, and assist the neoangiogenesis in the matrix (y Leon, 1998) and also ensures that all cells are within 200 μm from the blood supply for better mass transfer of oxygen and soluble nutrients (Yang et al., 2001; Salgado et al., 2004). Sufficient exchange of gases, nutrients, cells, and waste products with in, out, or into the tissue-engineered scaffold are the determinant factors for a successful construct. In a hydrogel scaffold, major mass transport occur through diffusion and nanoporous structure of a hydrogel, which is a decisive factor in the rate of diffusion and that is decided by polymer fraction, size, and cross-linker concentration (Hersel et al., 2003; Currie et al., 2001). Subsequently, the rate of diffusion is further affected by the size and molecular weight of diffusant (defined by Stokes radii) with respect to the pores.

However, molecules with higher molecular mass, including fibrinogen, albumin, and myoglobin cannot diffuse freely into the hydrogel scaffold systems. Rate of diffusion in alginate hydrogel is further decreased with increases in alginate or Ca^{2+} concentration, and or the extent of gelation. Similarly, with increased concentration of glutaraldehyde as a cross-linker, chitosan gels showed decreased rate of diffusion of molecules (Bryant et al., 2004). Remarkably, for the charged molecules of alginate, the rates of diffusion are size-dependent and are influenced by charge interactions with the alginate chains that are negatively charged. Similarly, it happens with other charged polymers too (Hersel et al., 2003; Chang et al., 2001). Eventually, the kind of application of hydrogel scaffold for tissue engineering decides the diffusion requirements and followed by choice of polymer (El-Sherbiny and Yacoub, 2013).

12.2.3 Biological properties

Hydrogels have been widely accepted and applied for tissue engineering due to their compositional, natural, structural closeness to that of ECM. Furthermore, advantage of tunable biocompatibility, structural integrity, cellular adhesions, molecular responses, and biodegradability also played a major role in making them attractive for these applications (Slaughter et al., 2009). The hydrogel scaffold must support

cellular functions such as proliferation, differentiation, and further formation of tissue in the body, without eliciting a severe or chronic inflammatory response. Polymeric hydrogels in use are generally nontoxic to the cells and neighboring tissue. Both hyaluronic acid (HA) and collagen integrate favorably with the body with no cross-species immunological issues (Alberts et al., 1994; Lee et al., 2001c). PEO is an FDA approved hydrogel, whereas P(PF-co-EG) is reported slightly toxic to cells in vitro, though it does not elicit considerable inflammatory response in vivo (Suggs et al., 1999). Both alginate and chitosan are also biocompatible in its pure forms (Lee et al., 1995; Ueno et al., 2001; Zhang et al., 2001), but it is important to note the debate on the immunogenicity of alginate (Klöck et al., 1997). Even though hydrogels have many strategic features, some exhibit poor biocompatibility and researchers are trying to improve their biocompatibility by different conjugation approaches (Beenken-Rothkopf et al., 2013; Darnell et al., 2013; Popa et al., 2014; Aziz et al., 2015) and by modulating ionic charges (Silva-Correia et al., 2013; Zhu and Marchant, 2011). For instance, viscoelastic and swelling behavior of alginate hydrogel are also controlled by cations, but biocompatibility can be severely affected by the presence of cations (Hyland et al., 2013; Lee et al., 2013b). Most of the polymeric hydrogels do not support cell adhesion immediately with an exception of collagen, the major ECM protein. Cells failed to adhere due to the hydrophilicity of hydrogel and because they are devoid of receptors to hydrogel-forming polymers. Additionally, these hydrogel surfaces do not support the absorption of major ECM components such as fibronectin, vitronectin, and laminin readily (Hahn et al., 2006). To tackle this problem, researchers designed a highly specific cell-binding surface that is covalently coupled to the entire ECM protein (Hahn et al., 2006; Rosiak and Ulański, 1999) or cellular receptor−binding peptide sequences (Hahn et al., 2006; Tang et al., 2007), arginine−glycine−aspartic acid (RGD) to the polymer. The cellular adhesion of PEG (Magnani et al., 2000; Duflo et al., 2006) as well as alginate (Tang et al., 2007; Peattie et al., 2006) has been enhanced after the addition of RGD peptide. Similarly, coupling of growth factors to regulate the cellular functions is also common in tissue engineering with hydrogels. For example, PEG has been tethered with TGF-β for the enhancement of ECM synthesis in smooth muscle cells (Duflo et al., 2006). Instead, alginate was covalently attached by oligopeptide from BMP-2 for the enhanced migration of osteoblast into the gel and subsequent calcification of the hydrogel construct (Drury and Mooney, 2003), alternatively several factors can also be assimilated into hydrogels to upregulate tissue formation (Kirker et al., 2002).

12.3 Applications of polymeric gels in tissue engineering

12.3.1 Three-dimensional scaffolds

The advantages of 3D culture over 2D have been proven by many studies for tissue development in the field of regenerative medicine. Such 3D culture systems are developed for many human tissues, including brain, kidney, intestine, and stomach (Lancaster et al., 2013; McCracken et al., 2014). For the first time, about four decades

ago, collagen matrices are used for growing mammary spheroids (Emerman et al., 1977; Emerman and Pitelka, 1977). Subsequently, a first attempt was made to develop an in vitro mammary morphogenesis in a 3D matrigel culture with mouse epithelial cells by Barcellos-Hoff et al. (1989).

Owing to their water retention property, hydrogels resemble the native soft tissue. High biocompatibility and ability to facilitate mass transport of soluble metabolites, oxygen, and nutrients make them attractive substrate for cell encapsulation (Zhu and Marchant, 2011; Lee et al., 2008). A multicellular 3D cell culture model in a collagen hydrogel was constructed in the 1990s that was intended of repairing vascular tissues. Over the past several decades, in vitro tissue model reconstruction in tissue engineering relied on hydrogels to mimic native tissue (Lutolf et al., 2003). Cell encapsulation in 3D hydrogel is a more realistic approach toward tissue development. This 3D scaffolding better simulates that cells experience in vivo, compared to conventional 2D plate tissue culture, due to the tissue-like properties of hydrogel. Synthetic polymeric hydrogels can be modulated in terms of stability or degradation as they have a network that can be swelled in water and can be degraded through proteolysis or hydrolysis according to the formation of new tissue. A list of polymers such as HA (Khetan and Burdick, 2009), PVA, PEG, and PLGA have been widely used. The mechanical strength of hydrogels is often adjusted by controlling the cross-linking density. Importantly, a key requirement for the replication of functional organs and tissues is the comprehensive knowledge of the organization and composition of their components, based on the in vivo model (Yanagawa et al., 2016).

12.3.2 Injectable scaffolds

Conventional methods such as scaffold-based or preformed hydrogels in tissue engineering face the problem of invasive implantation procedures that increases the threat of infections and other issues that can lead to rejection of the scaffold. To overcome these problems, injectable hydrogels were introduced as they can easily or with less invasive procedure reach the defect site even in very deep tissues (Patenaude et al., 2014). Injectable hydrogels have been prepared from both synthetic and natural biodegradable polymers. Naturally derived polymers, but not all, have better interaction with cells along with enhanced cellular function in terms of proliferation and differentiation (Stevens and George, 2005). Natural polysaccharides such as alginate, cellulose, chitin, chondroitin sulfate, chitosan, dextran sulfate, HA, pectin, etc., proteins such as collagen, fibrin, gelatin, heparin, etc., and synthetic polymers such as polypeptides, polyphosphazenes, polyesters, etc. are widely used as injectable hydrogels (Flory, 1953). On the other hand, their tunable mechanical strength and degradation profile make them good substrate for tissue engineering applications (Drury and Mooney, 2003). To improve the mechanical properties of injectable hydrogels, attempts have been made for development of combinatorial system of both natural and synthetic polymers (Sionkowska, 2011). Various chemical and physical cross-linking methods, such as self-assembly, photo-induced, electrostatic Michael addition, click chemistry, disulfide cross-linking, Schiff-base

cross-linking, and pH/thermo-responsive cross-linking are few methods used to prepare injectable hydrogels. The major factors such as charge, hydrophilicity, biocompatibility, biodegradability, strength, and porosity are also to be considered to a greater extent while designing an injectable hydrogel system (Sivashanmugam et al., 2015).

Tissue engineering is a promising approach for repairing various tissues including bone and cartilage. Among the available scaffolds, injectable hydrogels have exhibited highest application potential for bone and cartilage tissue engineering. Their resemblance to the native ECM, high water content, porosity, and ability to take shape of irregular defects are the key properties that make injectable hydrogels attractive for tissue engineering applications (Liu et al., 2017). Because hydrogels resemble the retained rich water-containing environment of cartilage for lubrication effect, it is considered as a better form of a biomaterial for cartilage tissue engineering or repair (Eyrich et al., 2007; Park and Lee, 2014).

The chitosan is structurally related to glycosaminoglycans (GAGs) of tissues. So, it has been widely accepted in the form of hydrogel that can be injected for cartilage repair (Jin et al., 2009; Di Martino et al., 2005; Naderi-Meshkin et al., 2014). A tough chitosan−gelatin hydrogel has been fabricated by Shen et al. (2015) through an in situ precipitation method. Apart from the enhanced mechanical properties, it also exhibits excellent biodegradability and biocompatibility. Chitosan−HA based hydrogel, formed by Schiff-base reaction, was encapsulated with articular chondrocytes that showed excellent cell adhesion and proliferation as well as exhibited good mechanical stability (Liang and Kiick, 2014). Fibrin and alginate, polymers of natural origin, were successfully used for chondrocyte encapsulation in an attempt to regenerate cartilage tissue (Jaikumar et al., 2015; Casu, 1985). Additionally, collagen gel constructs encapsulated with chondrocytes showed superior expression of type II collagen and aggrecan along with GAG, the major ECM component of cartilage (Tae et al., 2007).

Alginate is one of the injectable hydrogels, which is most studied among the biomaterials for bone tissue engineering (Venkatesan et al., 2015). Matsuno et al. (2008) have reported a novel injectable hydrogel with β-tricalcium phosphate beads and alginate that can be applied for bone defects. This hydrogel has been implanted subcutaneously along with Mesenchymal Stem Cells (MSCs) and demonstrated that the scaffold favorably supports bone differentiation. It has been shown that injectable calcium silicate/sodium alginate hybrid hydrogel efficiently promotes osteogenic and angiogenic cell adhesion, proliferation, and differentiation (Han et al., 2013). Injectable chitosan hydrogel was also used in bone tissue engineering (Ma et al., 2010). Dessì et al. (2013) have developed a β-glycerophosphate cross-linked thermosensitive chitosan hydrogel reinforced with β-tricalcium phosphate by physical interactions. The hydrogel supports cellular proliferation and functional activities and undergoes a sol−gel transition in vivo at physiological temperature with typical rheological properties (Liu et al., 2017). Recently, synthetic injectable hydrogels such as methoxy polyethylene glycol-b polycaprolactone block copolymer (Jang et al., 2016), *N*-isopropylacrylamide, or gelatin microparticle-composite hydrogel (Vo et al., 2016) are used for tissue engineering of bone. To meet the

challenge of poor mechanical properties of hydrogel compared to natural bone, a three-component injectable thermosensitive hydrogel composite, composed of PEG−PCL−PEG copolymer triblock, nanohydroxyapatite, and collagen, has been developed (Fu et al., 2012). This composite hydrogel has excellent thermosensitivity and good interconnected porous structure.

Nucleus pulposus (NP) region of intravertebal disk contains proteoglycans, thin network of collagen, and 70%−90% water (Gloria et al., 2012). Injectable hydrogels are also being used for IVD regeneration. A mixture of dodecylamide, a photo−cross-linkable ester, and two HA derivatives have been used successfully for nucleus regeneration as injectable hydrogel. The mixture supported for cell growth and ECM production and exhibited similar viscoelastic properties to that of native NP (Gloria et al., 2012). In a different study, an indigenously developed injectable amidic alginate hydrogel showed good viscoelastic properties compared to nondegenerated human lumbar NP under dynamic conditions (Leone et al., 2008). Additionally, the gel also exhibited synthesis of all major ECM components. It is reported that the native phenotype of NP cells has been maintained in injectable hydrogels prepared from gellan gum (Silva-Correia et al., 2013; Oliveira et al., 2010).

12.3.3 Bioactive molecule delivery

Bioactive factor delivery is considered as a promising strategy to solve many existing problems in tissue engineering and can be used as a treatment strategy for a variety of human diseases. This field has been fostered significantly with the development of novel biomaterials and new technologies (Storrie and Mooney, 2006; Lee et al., 2011, 2013a; Slaughter et al., 2009). Generally, the growth factors with low molecular weight (Tessmar and Göpferich, 2007), anticancer drugs (DuVall et al., 2009; Elstad and Fowers, 2009), and genetic agents (Dang and Leong, 2006) are categorized under the term "bioactive factor," which are key factors that play a major role in treating various diseases, enhancing tissue regeneration and guiding cellular functions. Hydrogels hold abundant potential in tissue engineering and pharmaceutical applications due to its cross-linked hydrophilic polymeric 3D networks (Huynh et al., 2011; Van Tomme et al., 2008; Van Vlierberghe et al., 2011). Hydrogels are of great interest in the field of drug or bioactive delivery due to their ability to locally deliver therapeutics in vivo in a sustained spatiotemporally controlled manner (Nguyen and Alsberg, 2014).

These molecules generally undergo enzymatic degradation in vivo and also nonspecific uptake by tissues when delivered in a larger dose to the patients via oral or intravenous route (Drury and Mooney, 2003). Therefore, to circumvent this problem and to promote specific delivery, there is a need of a matrix-encapsulated system for sustained, localized, and controlled delivery of the molecule. Polymer hydrogel matrices are considered appropriate for this purpose.

Natural and synthetic polymers are being used by engineering them for specific therapeutic delivery applications. Ionic and chemical cross-linked, environmentally responsive, and biomolecule recognition polymeric hydrogels have been prepared and used for various bioactive molecules delivery (Nguyen and Alsberg, 2014). For

instance, self-assembling polymers in response to temperature is the most common technique of fabricating hydrogels for bioactive factor delivery on changing the temperature (Cohn et al., 2003). PNIPAm is a popular thermo-gelling substance that remains soluble in aqueous solution at room temperature and precipitates above 32°C (Wu and Wang, 1998).

Various proteins including VEGF, an angiogenesis promoting growth factor, have been delivered by encapsulating them in ionically cross-linked alginate gels (Fig. 12.2), and it was observed that the bioactivity of this matrix-delivered VEGF was greater than VEGF delivered alone due to its stabilization via alginate interaction (Peters et al., 1998; Elçin et al., 2001; Lee et al., 2000a). Osteogenesis-promoting growth factor, BMPs, has also been loaded in many polymeric scaffolds. BMP-2 has been encapsulated by photo−cross-linking into PLA−PEG and PLA−DX−PEG

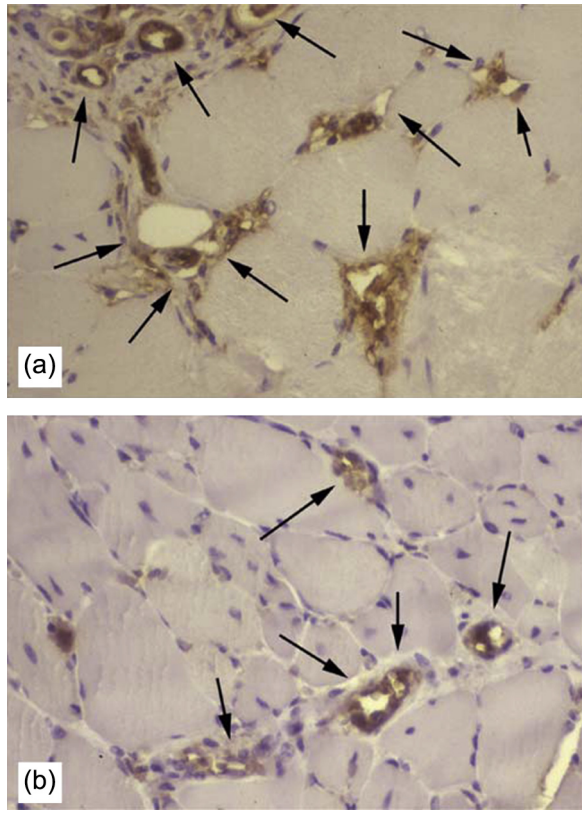

Figure 12.2 VEGF release from alginate scaffolds resulting in angiogenesis: (a) with out mechanical stimulation of alginate gels and (b) with mechanical stimulation of alginate gels. *Arrows* indicate blood vessel formation in the muscle tissue surrounding the implanted gels. Reprinted from Lee, K.Y., Peters, M.C., Anderson, K.W., Mooney, D.J., 2000a. Controlled growth factor release from synthetic extracellular matrices. Nature 408, 998 with permission, copyright Macmillan Magazines Ltd.

systems and was released according to the polymer degradation profile (Saito et al., 2001). Certain hydrophobic drugs such as doxorubicin are encapsulated in hydrophobic polymers and stabilized via hydrophobic interactions in aqueous solutions (Jeong et al., 2000). Another drug molecule, dexamethasone corticosteroid has been loaded in HA−tyramine macromers and the cross-linking density of the hydrogel determined the release rate of the drug (Kim et al., 2011). Heparin, a GAG found in the ECM, has a high affinity for various growth factors, and thus several hydrogel systems have been formulated using heparin as it has a characteristic property to protect the growth factors from degradation and has a slow release profile along with retaining its bioactivity (Fig. 12.3) (Wissink et al., 2000; Gospodarowicz and Cheng, 1986). Certain research groups have exploited the use of widespread mechanical signals in the body such as compression in the cartilage and bone, tension in the muscle and tendons, and shear stress in the blood vessels to facilitate the release of certain bioactive factors. VEGF, trypan blue, and methylene blue were released from Ca^{2+} cross-linked alginate by compression (Lee et al., 2001a).

12.3.4 Cell delivery

Polymers as scaffolds play a vital role as temporary ECM but rarely mimic the native in vivo environment to promote tissue growth. The emerging polymer scaffolds are

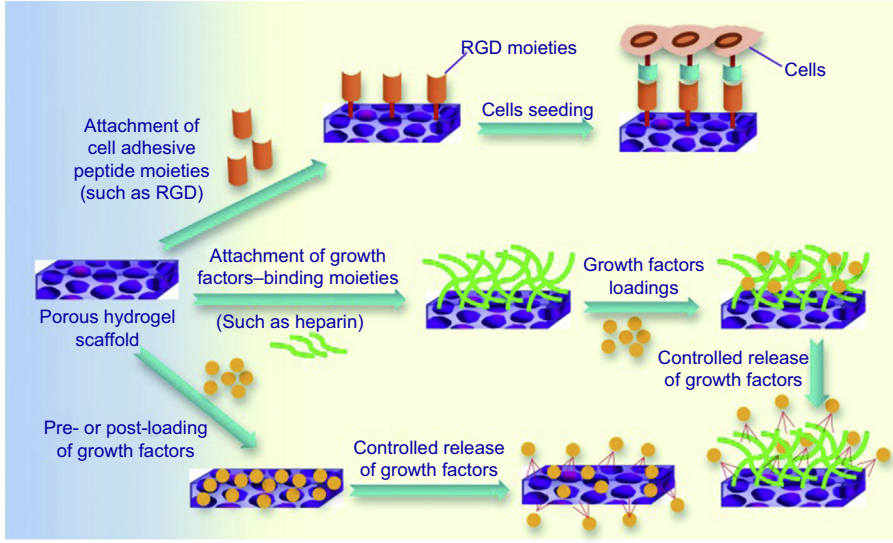

Figure 12.3 Some approaches for selective enhancement of surface characteristics of hydrogel scaffolds toward increasing surface cells attachment and controlled release of regulatory growth factors. *RGD*, arginine−glycine−aspartic acid.
Reprinted from El-Sherbiny, I.M., Yacoub, M.H., 2013. Hydrogel scaffolds for tissue engineering: progress and challenges. Global Cardiology Science and Practice, 38 with permission.

now capable of holding the growth factors that help in cell proliferation and tissue development. Polymeric scaffolds as cell delivery vehicles are believed to be the most promising therapeutic approach to treat various disease conditions. For polymeric scaffolds to function as cell delivery vehicles, they should be able to encapsulate either cells or required growth factors that help to repopulate a defect site (Fig. 12.3). Collagen being the major protein component in ECM is considered the ideal scaffold for tissue engineering. Collagen-based scaffolds are used in various tissue engineering applications such as cartilage regeneration, long bone fractures, enhancing angiogenesis, and also in reconstruction of renal glomerular tissue (Malafaya et al., 2007). Apart from collagen and its derivatives, recently, chitosan-based materials are gaining attention in the field of orthopedic tissue engineering. Scaffolds fabricated using chitosan are widely used in cartilage regeneration due to its structural similarity with GAGs present in native cartilage (Suh and Matthew, 2000). Due to the cationic nature and predictive degradation rate of chitosan-based materials, they bind growth factors and can release them in a controllable manner (Di Martino et al., 2005). It was also reported that chitosan has been used to modify surface properties of titanium implants, which enhances the osteointegration (Lee et al., 2002).

Alginate is potentially used as cell delivery systems to enhance growth in different tissues and is widely used in cartilage engineering. It has been mixed with chondrocytes and was either injected or implanted in the defect site and the chondrocytes were viable and produced ECM components (Paige et al., 1995). Alternatively, alginate can also be used as an injectable material for filling the irregular defects. Mechanically, these scaffolds are weak and hence should be combined with other polymers that can strengthen the mechanical properties of these hydrogels. Recently, alginate is combined with silk to develop innovative microcarriers for MSCs adhesion and proliferation. Results indicated that MSCs rapidly adhered onto the surface of microcarriers and also preserved their multilineage differentiation potential (Perteghella et al., 2017). Alginate along with HA can be used to fill the critical size defects that help in regeneration, which would not heal otherwise (Drury and Mooney, 2003). The potentiality of alginate hydrogels has also been explored in the area of nerve grafting and as scaffolds to promote hepatocyte function (Mosahebi et al., 2001; Glicklis et al., 2000).

12.3.5 Space filling

Polymers are used extensively in the field of regenerative medicine not only as cell carriers and 3D scaffolds to direct the cellular organization but also as acellular systems. They have been used in many applications without incorporating cells as space filling agents. The term space filling is used for the group of scaffolds that are used to provide bulk support, prevent adhesion, or functions as bioglue when applied in the desired site. Polymers that can maintain their structural integrity and volume over a desired period are primary candidates for space filling agents. To provide bulk support as a bulking material, the biocompatible polymer can be directly injected into the site before or after gelation or as a scaffold with minimal invasion. As an illustration, such materials are used in healing the corneal damage,

vesicoureteral reflux, and to treat urinary incontinence. Scaffolds that are developed using alginate and collagen are prominently used as bulking agents. Alginate modified using RGD has been successfully implanted to mice model with minimal immune response (Loebsack et al., 2001). Due to faster degradation of the material, there is a need of multiple injections to maintain the functionality. Cross-linking by glutaraldehyde partially reduce the degradation time but cannot completely inhibit it (Drury and Mooney, 2003).

For the polymers to behave as antiadhesives there should not be any receptors present on the material for the cells to attach. Synthetic polymers are the suitable materials as cells lack receptors to them and proteins are not readily adsorbed onto their surfaces. Hence, they are excellent materials that have an ample scope in this area. PVA/PEG can be used to prevent postoperative peritoneal adhesions, which are frequent complications post abdomino-pelvic surgery. The efficacy of PVA as an antiadhesive agent was reported by implanting a PVA/Gel membrane between cecum and peritoneal wall in rat and the adhesion significantly reduced when compared with the control (Bae et al., 2014). Adhesions that develop after gynecological surgery are the leading cause of secondary female infertility worldwide. Techniques such as laparoscopy reduce the extent of adhesions, but it does not decrease the incidence of adhesions. Therefore, there is a dire need of antiadhesive agents. PEG is used to protect arteries from intimal thickening after damage (Hill-West et al., 1994; West and Hubbell, 1996). The major negative aspect with the available membrane adhesions barriers is the difficulty of handling them during the surgery. To overcome this, sprayable PEG antiadhesion barrier was developed and was proven to be effective in rodent and porcine models (ten Broek et al., 2012).

Other than bulking material and antiadhesives, polymers are also used as biological glue to seal small wounds. Most commonly used fibrin sealant is a biologic tissue adhesive that can be used independently to seal wound where sutures cannot stop bleeding or would enhance bleeding (Spotnitz et al., 1997). Photo−cross-linked chitosan gels are now being explored for their use as bioglue; they also appear to be nontoxic in vitro (Ono et al., 2000).

12.3.6 Three-dimensional bioprinting

3D bioprinting, a process that uses cell-laden hydrogels as bioink and places them accurately at desired positions to ensure proper cell−cell interaction, cell−cell communication, and cell−ECM interaction, is still in a rudimentary stage and researchers are investigating various bioinks for the printing process.

Polymers, especially hydrogels have been contemplated as suitable bioinks owing to their inherent properties that include appropriate viscosity, cytocomptible cross-linking mechanisms, mechanical, hydration, and biological interaction properties. Both natural and synthetic polymers possess a variety of advantages and limitations (Carrow et al., 2015); the natural polymers for example collagen, gelatin, alginate, HA, fibrin, etc. mimic the attributes of ECM effectively and possess certain cell-binding moieties and do not require harsh treatment such as high temperature and pH during the fabrication process, thus allowing enhanced cell responses. But their

innate properties are difficult to alter for effective printing (Das et al., 2013). On the other hand, the synthetic polymers such as PCL, PLLA, and PEG have properties that can be adjusted for efficacious printing but they lack certain cell-binding moieties, which help in effective cell adhesion to the material. To overcome these drawbacks, researchers are blending the synthetic polymers with natural polymers (Nakamura et al., 2010; Schuurman et al., 2011; Skardal et al., 2010). For instance, copolymerization of enzymatic degradation sites from natural polymers with synthetic polymers has seen to be beneficial for cell adhesion and migration (West and Hubbell, 1999).

In some cases, two natural polymers have also been blended together to get a desired effect. For example, fibrin and collagen have been used in conjunction for cell-laden bioprinting in case of skin wounds healing. Amniotic fluid−derived stem cells and bone marrow−derived mesenchymal stem cells were incorporated in the blend and gelation was achieved by printing thrombin alternatively (Skardal et al., 2012). HA, a major component of the ECM enriched in the connective tissue, is seen as a potential polymer for 3D printing due to its excellent biodegradability and viscoelastic properties, but its high water retention properties and high solubility limit its usage so researchers are adopting strategies such as cross-linking polysaccharide chains with hydrophobic moieties. This has been achieved by blending HA along with photo−cross-linkable derivative of dextran, HEMA (hydroxyethyl methacrylate−derivatized dextran), such constructs have been used for chondrocytes (Pescosolido et al., 2011).

Certain naturally occurring polymers such as chitosan, starch, soy protein are being used for 3D printing, but incorporation of cells in such constructs is not feasible due to the harsh printing or post-printing conditions and research is being done to circumvent these issues as such polymers can play an instrumental role to regenerate the functional tissue (Carrow et al., 2015).

Newer strategies to develop hybrid systems instead of blending polymers is also an excellent alternative; here the synthetic polymer is used as a structurally supporting scaffold and cells embedded in natural polymer is used as filler (Carrow et al., 2015). For example, PCL, a synthetic polymer has excellent biocompatibility, strength, and degradation capabilities and is widely used in such systems (Schuurman et al., 2011; Xu et al., 2012). The 3D-printed polymers can even be functionalized with nanotubes to better replicate the microenvironment, and this provides better mimicking potential for the construct. For instance, a research group from the United States chemically functionalized 3D-printed PLLA with multiwalled carbon nanotubes, and this construct has shown to mimic collagen and furthermore induced stem cell differentiation into osteogenic and chondrogenic lineages.

Most recently, the use of decellularized extracellular matrix (dECM) is gaining attention in 3D bioprinting, motivated from the ECM−cell interaction in vivo. The dECM hydrogels have been using as bioink in the field of 3D bioprinting, and in a such kind of attempt Pati et al. (2014) had recreated the complexity of native ECM in the form of dECM hydrogel (Figs. 12.4 and 12.5). The advantage of dECM hydrogel as bioink is that it does not require cross-linkers and allow the cells within the gel to remodel the ECM. The study revealed the ability of dECM hydrogel to

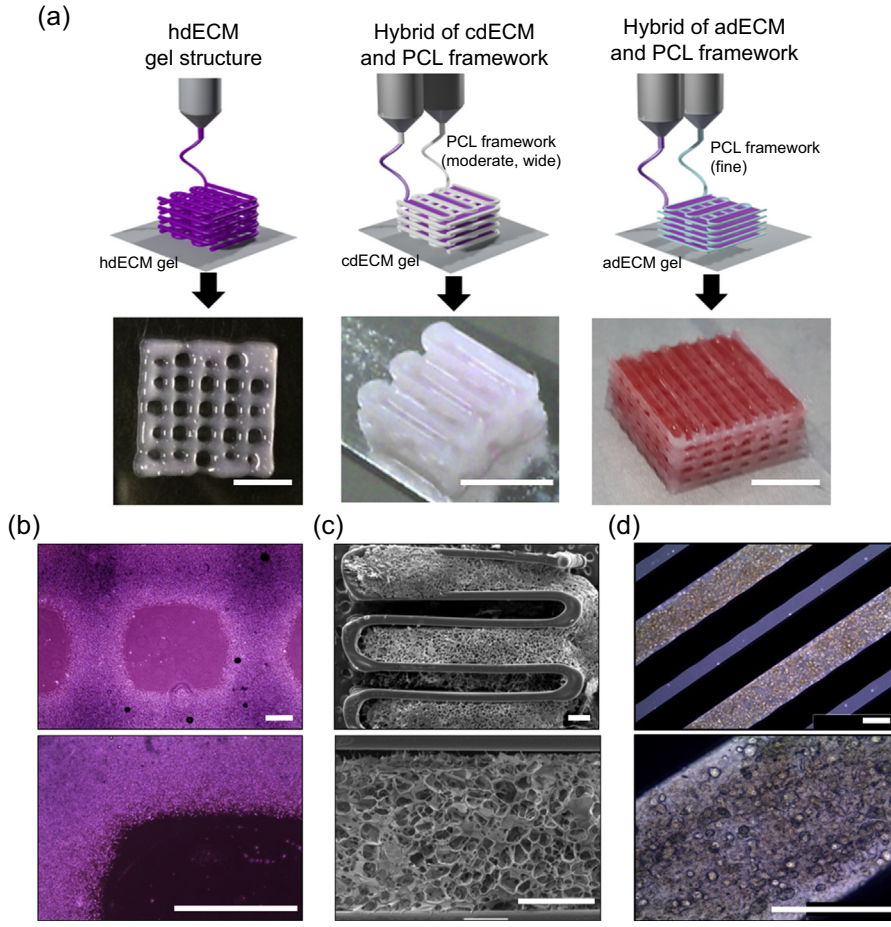

Figure 12.4 Tissue-printing process with decellularized extracellular matrix bioink. (a) Heart tissue construct was printed with only heart dECM (hdECM). Cartilage and adipose tissues were printed with cartilage dECM (cdECM) and adipose dECM (adECM), respectively, and in combination with PCL framework (scale bar, 5 mm). (b) Representative microscopic images of hdECM construct (scale bar, 400 μm), (c) SEM images of hybrid structure of cdECM with PCL framework (scale bar, 400 μm) and (d) microscopic images of cell printed structure of adECM with PCL framework (scale bar, 400 μm).
Reprinted from Pati, F., Jang, J., Ha, D.-H., Kim, S.W., Rhie, J.-W., Shim, J.-H., et al., 2014. Printing three dimensional tissue analogues with decellularized extracellular matrix bioink. Nature Communications 5, 3935 with permission.

support the lineage specific commitment of the stem cells toward either chondrogenic or adipogenic lineages. Along with the same set of studies, they also observed that heart tissue dECM hydrogel as a bioink facilitates the functional maturation of rat myoblasts (Pati et al., 2014, 2015). dECM retained cell adhesion proteins, fibrous proteins, GAGs, and remnant growth factors and thereby mimic the native

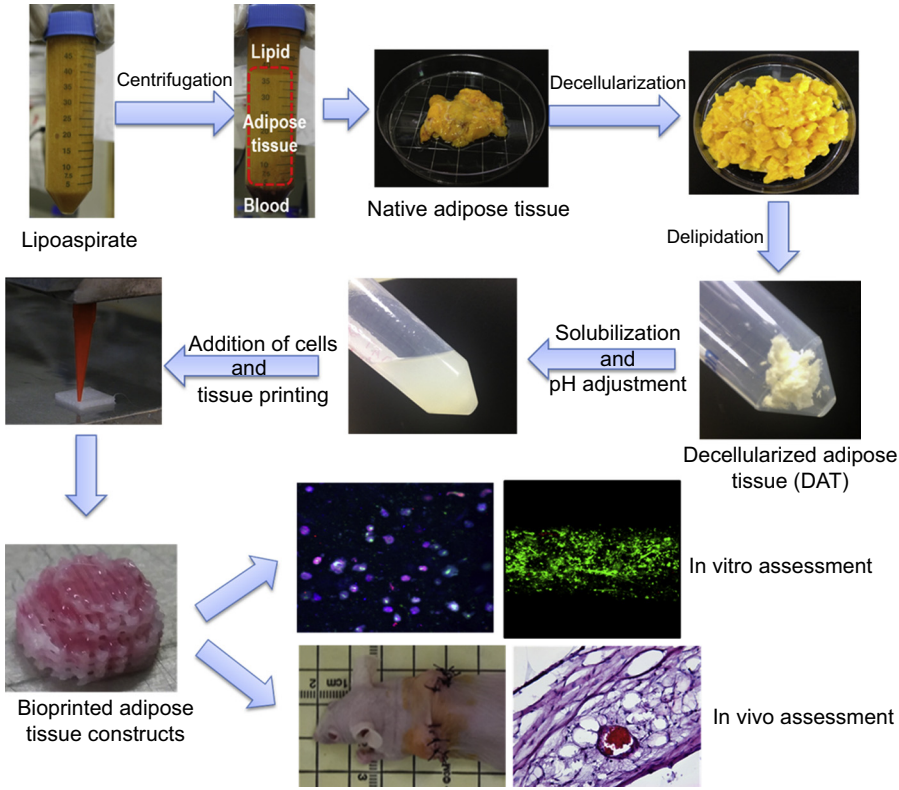

Figure 12.5 Schematic elucidating preparation of decellularized adipose tissue (DAT) bioink and printing of dome-shaped adipose tissue constructs and their in vitro and in vivo evaluations.
Reprinted from Pati, F., Ha, D.-H., Jang, J., Han, H.H., Rhie, J.-W., Cho, D.-W., 2015. Biomimetic 3D tissue printing for soft tissue regeneration. Biomaterials 62, 164−175 with permission.

microenvironment and enable cells to preserve their original functionalities (Crapo et al., 2011; Ott et al., 2008, 2010; Song et al., 2013; Uygun et al., 2010), when implanted in cardiac tissue (Quarti et al., 2011), skeletal muscle (Valentin et al., 2010; Turner et al., 2010; Wolf et al., 2012), and the peripheral nervous system (Nagao et al., 2011). It is already proven that a hydrogel formed from enzymatically degraded and solubilized ECM would retain some of the biologic activity found in the intact ECM. Though it is not possible to mimic all the facets of ECM, it would be a step forward toward the design and development of a hydrogel biomaterial closer to native ECM. In short, the dECM bioinks hold great promise for in vitro and in vivo tissue development and would successfully be used as an alternative to chemically cross-link hydrogel inks even though extensive research is demanded to increase the mechanical properties.

12.4 Future directions and conclusion

Most of the reported or existing studies focused on the characterization, compositional and physical properties, and effect of polymeric hydrogel on cells and toxicological reactions, which can reduce the immune rejections. There are many biomaterials available, which are proven biocompatible and can be used for tissue engineering applications. It is worth to note that most of the biocompatibility studies have been performed in vitro, where research cannot consider the complex immune reactions. Though an effective biocompatible scaffold has been a major goal of tissue engineering researches for many years, the goal is still far away and we need to focus on the growing need of an effective polymeric hydrogel material that can be applied in the field of tissue engineering and regenerative medicine (Naahidi et al., 2017). Furthermore, engineering of smart hydrogels that can take shape according to the special requirements, cells proliferation, and subsequent ECM deposition is also equally important as it can accelerate the translational research in this field. Advancement in the technology to develop nanofibrillar structures might also help to approach this field more realistically. Additionally, perhaps most importantly, the unmet mechanical property of polymeric hydrogel is still a major challenge in the application of polymeric hydrogel for specific applications such as cartilage and bone tissue engineering (Liu et al., 2017), which need to be addressed with at most priority.

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Polymeric gels for the controlled drug delivery applications

13

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13.1 Introduction

Hydrogels are composite of a solid (a polymer) and a liquid (water), which are a class of cross-linked polymers that absorb large quantities of water due to their hydrophilic nature. Hydrogels are generally prepared from hydrophilic monomers; hydrophobic monomers are also sometimes used to manipulate the properties of the hydrogel for the specific applications. In general, the hydrogel synthesis requires monomer, initiator, and cross-linker. The hydrophilic polymers without cross-linking are called hydrosol (soluble in water). Hydrosols cannot retain their shape, whereas the hydrogels does due to the intermolecular cross-links, which makes the restricted movement of polymer chains within the hydrogel. The method of cross-linking polymer chains depends on the type of monomer and the final application (Peppas, 2010).

Synthetic hydrogels are generally produced via bulk, solution, and inverse dispersion methods. While the first two reactions are homogeneous, the inverse dispersion method is conducted in the dispersed and continuous phases. Among the homogeneous polymerizations, the solution reaction is highly preferred because of the ease of controlling the reaction conditions. Most of the high-swelling hydrogels are produced in this way. The hydrogel properties can be modulated by varying the synthetic factors, such as reaction vessel, reaction time, reaction temperature, monomer type, type of cross-linker, and concentration of monomer, initiator, and cross-linker.

Efficiency of the hydrogels is usually defined by their degree of swelling where solubility of the hydrogels is prevented by elastic forces, which originate from the network cross-linking, and the swelling capacity of hydrogels can be determined by the amount of space available inside the hydrogel network to accommodate water. Hydrogel swelling starts with the polymer−water interactive forces, so the more hydrophilic the polymer makes the strong the polymer−water interaction.

Hydrogels can be used as a swelling agent or as a delivery platform for active compounds in various fields as shown in Fig. 13.1. During the past few decades, hydrogels have been tremendously used for biomedical applications such as drug delivery systems (DDSs) (Wang et al., 2012). However, the translation of both the enhanced permeability and retention effect and ligand recognition into the clinic still remains challenging. In fact, Fickian diffusion, which governs the release of drug from the carrier, is not specific to cells, tissues, or organs; therefore, efficient strategies are required

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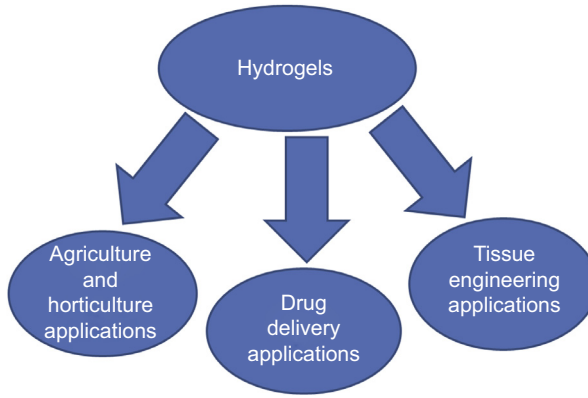

Figure 13.1 Applications of hydrogels.

to control the release of drug. The alternative includes switch "on/off" mechanism, which in principle allow tailored release of molecules with excellent spatial, temporal, and dosage control. This approach would be feasible through the design of stimuli-responsive systems that recognize their microenvironment and react in a dynamic way, mimicking the responsiveness of living organisms (Mura et al., 2013). Therefore, herein we highlight the synthesis of stimuli-responsive polymers and their application as a controlled drug delivery agent.

13.2 Synthesis of stimuli-responsive hydrogels

As an intriguing material, hydrogels have gained great interest during the last few decades due to their excellent biocompatibility and ability to absorb and release molecules during the swelling−deswelling processes. Moreover, these biocompatible polymeric networks can be made responsive to various stimuli. This is a promising approach because of their reversibility of sol−gel transition and/or volume-phase transition in response to external physical or chemical stimuli, such as pH, temperature, and light (Verdejo et al., 2011; Li et al., 2012; Sánchez-Ferrer et al., 2013; Vashist et al., 2014). These unique properties make them very attractive as a drug delivery agent.

13.2.1 Thermoresponsive hydrogels

Thermoresponsive hydrogels are the most widely employed smart polymers because it is the easiest parameter to manipulate and their characteristic properties of a reversibly alterable phase (or volume) transition, which occurs in response to the change in temperature. Poly(ethylene glycol) (PEG) is a biodegradable, nontoxic, and nonantigenic and immunogenic polymer. Therefore, PEG-based thermosensitive hydrogels have been widely studied for the biomedical applications. PEG could be copolymerized

with poly(lactide) (PLLA/PDLLA), poly(lactic acid-co-glycolic acid) (PLGA), poly(e-caprolactone) (PCL), poly(*N*-isopropylacrylamide) (PNIPAAm) with structures of the AB diblock type, ABA or BAB triblock type.

13.2.1.1 Poly(N-isopropylacrylamide)/Poly(ethylene glycol) copolymer (PNIPAAm-b-PEG)

In the year 1997, Topp et al. first synthesized PNIPAAm-b-PEG and studied their micellation behavior. Though the synthesis was reported in 1997, the first, PNIPAAm/PEG-based hydrogel was reported in 1999 by Lin et al. (Lin and Cheng, 2001). Synthesis of PNIPAAm/PEG involved a ceric ion redox system to produce radicals at the terminal carbons of PEG. These radicals are used for the polymerization of NIPAAm as shown in Fig. 13.2.

13.2.1.2 Poly(ethylene glycol)–grafted polyesters

Though the PNIPAAm/PEG hydrogels were very stable to form the gels by induced temperature, problem araised while coming for biomedical applications. For the biomedical applications, the material must be biocompatible and biodegradable, PNIPAAm-based hydrogels were biocompatible but not biodegradable. Therefore, researchers started to explore on polyesters that have very good mechanical property to sustain in the physiological environment.

13.2.1.2.1 PLGA-b-PEG

Prof. Kim's research group initially explored about the sol–gel transition behavior of PEG–PLGA–PEG triblock copolymer aqueous solutions (Jeong et al., 1999a, 2000a). General synthesis is presented in Fig. 13.3. Glutaric anhydride was ring opened by PEG to get PEG–COOH. It was further reacted with epoxy-terminated PEG. The intermediate product was further reacted with D,L-lactide and glycolide to get the final PLA/PEG copolymer. It was observed that 21%–25% of polymer solution

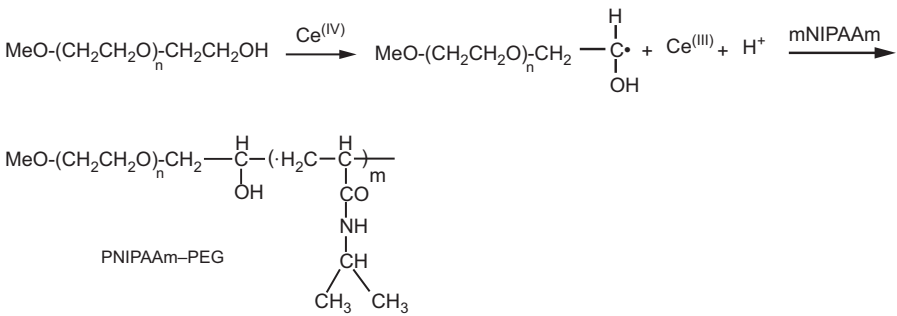

Figure 13.2 Synthesis of Poly(N-isopropylacrylamide)/Poly(ethylene glycol) copolymer Reproduced with permission Topp, M., Dijkstra, P.J., Talsma, H., Feijen, J., 1997. Thermosensitive micelle-forming block copolymers of poly (ethylene glycol) and poly (N-isopropylacrylamide). Macromolecules 30, 8518–8520.

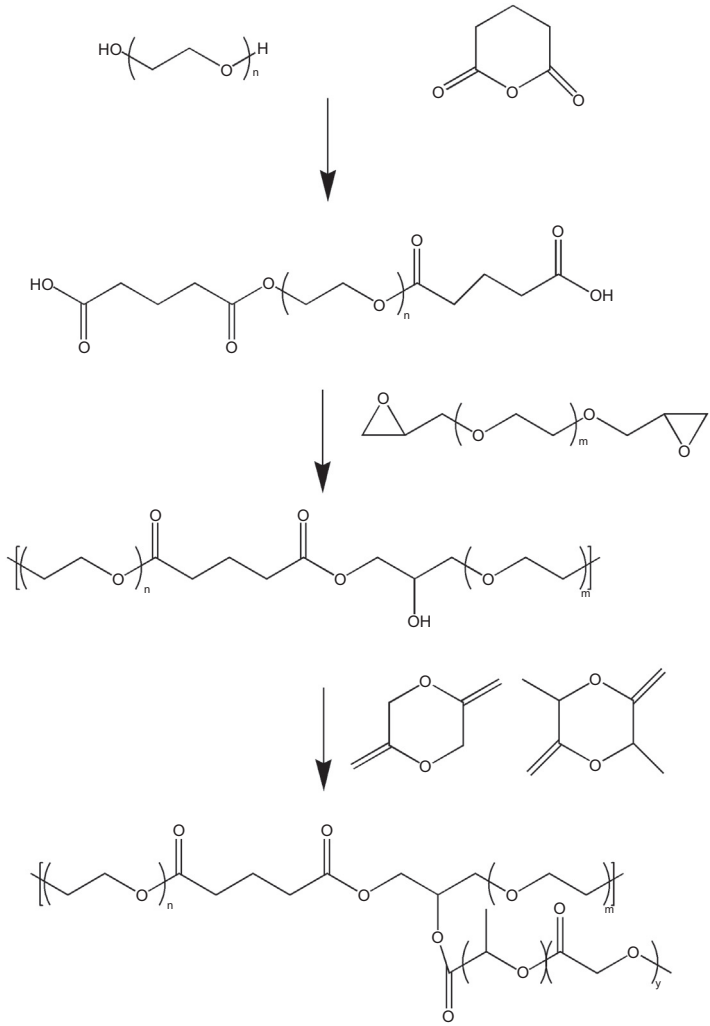

Figure 13.3 Synthesis of PEG-g-PLGA.
Reproduced with permission Jeong, B., Kibbey, M.R., Birnbaum, J.C., Won, Y.-Y., Gutowska, A., 2000b. Thermogelling biodegradable polymers with hydrophilic backbones: PEG-g-PLGA. Macromolecules 33, 8317−8322.

is required to form the hydrogel (Jeong et al., 2000b). Followed by this Lee and workers synthesized triblock copolymers of PLGA−PEG−PLGA and produced the hydrogels out from the triblock copolymer. They hypothesized that these triblock copolymer forms micelles, and the grouped micelles bridged between micelles because the hydrophobic PLGA end blocks can be located in different micelles. Further these micelles interconnect each other and forms hydrogels (Shim et al., 2002). Prof. Jing group extensively studied the gelation behavior of PLGA/PEG copolymers in recent

times. Yu et al. (2011) reported the influence of LA and GA sequence in the PLGA block on the properties of thermogelling of PLGA/PEG. They found that the gelling behaviors of the aqueous solutions of PLGA−PEG−PLGA triblock copolymers can be tailored by adjusting the sequence structure in the PLGA block (Yu et al., 2011). Recently, they also studied the effect of molecular weight in the thermogelation. The block copolymer with a relatively low $Đ_M$ exhibited a gel-to-sol transition (normal gelling) or sol-to-gel transition on heating (reverse gelling), depending on molecular composition including the length of PEG block and a large $Đ_M$ induced a reentrant physical gelation with gel-to-sol-to-gel transitions. Their observation revealed that the hydrophilic blocks can influence the macroscopic self-assembly of amphiphilic block copolymers to a large extent, which will affect the gelation (Chen et al., 2015).

13.2.1.2.2 PLA-b-PEG

Jeong et al. (1997) initially proposed the synthesis of hydrogels from PEG-b-PLLA. Synthesis of the copolymer involved two steps. First, block of PEG-b-PLLA was synthesized by ring opening of L-lactide using monomethoxy poly(ethylene oxide). Then the diblock copolymer was coupled by hexamethylene diisocyanate (Fig. 13.4). These block copolymers formed gel at lower temperature and became sol at higher temperature. By changing the biodegradable block length, the sol−gel transition temperature can easily be manipulated. Further, Tew and coworkers have reported hydrogel formation from $PLLA_{72}$−PEG_{202}−$PLLA_{72}$ copolymers from 20% (wt/wt) solutions. It was also reported that very long chain of PLLA did not allow the gelation (Tew et al., 2005). In another study it was reported that the physical and mechanical properties of PLA−PEG−PLA micelles and hydrogels were shown to change with respect to change in the PEG block length and micellar structures (Abebe and Fujiwara, 2012). Then, stereocomplex-induced gelation of PLLA/PEG and PDLA/PEG block copolymers was also reported (Li, 2003). At the same time, sterocomplexed multiarm PLLA/PEG and PDLA/PEG hydrogels were also prepared. It was also found that the critical gelation concentration required for star-shaped PLLA/PEG is lower than the linear PLLA/PEG copolymer (Hiemstra et al., 2005, 2006).

13.2.1.2.3 PCL-b-PEG

Though the copolymers of PCL/PEG were explored in 1980, their association properties in aqueous solution especially gelation were explored by Martini et al. in the year 1994.

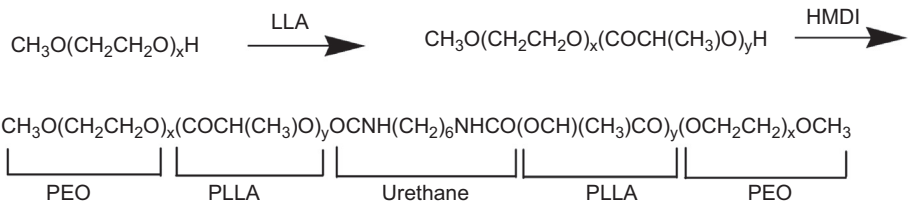

Figure 13.4 Reaction scheme for the synthesis of triblock copolymer of PEG-b-PLLA. Reproduced with permission Jeong, B., Bae, Y.H., Lee, D.S., Kim, S.W., 1997. Biodegradable block copolymers as injectable drug-delivery systems. Nature 388, 860.

Fig. 13.5 shows an example of synthesis of PCL−PEG−PCL triblock copolymer, where caprolactone monomer was initially ring opened by hydroxyl group of PEG (Liu et al., 2008). In the recent years, Qian group extensively studying the properties of PCL/PEG hydrogels and their biomedical applications (Ni et al., 2014; Gong et al., 2009).

13.2.1.2.4 Poly(ethylene glycol)/chitosan

Tran et al. produced the in situ hydrogels of tyramine-conjugated chitosan derivatives (figure) using horseradish peroxidase and hydrogen peroxide (H_2O_2). Here, initially they synthesized p-nitrophenyl carbonate ester (NPC) containing PEG in presence of triethylamine. Further they coupled tyramine into the PEG-NPC. Then rutin-conjugated chitosan−poly(ethylene glycol)−tyramine synthesized in two steps where first chitosan was coupled with NPC-PEG-TA, further carbodiimide chemistry was used to couple the chitosan grafter polymer into the rutin (Fig. 13.6) (Tran et al., 2011).

13.2.2 Photosensitive hydrogels

13.2.2.1 PLA-b-PEG

Triblock PLA-*b*-PEG-*b*-PLA copolymer with acrylate end groups was originally described by Sawhney et al., but the extensive study started once the Hubbell et al. and other researchers explored their possibility of biomedical applications (Sawhney et al., 1993). Synthesis of this PLA-*b*-PEG-*b*-PLA copolymer with acrylate end groups involves ring opening of lactide by the PEG and followed by the reaction with acryloyl chloride triethyl amine (Fig. 13.7). Acryl groups are photosensitive. Therefore when the aqueous polymer solution was exposed to UV, photopolymerization occurred and the hydrogels were formed (Sawhney et al., 1993).

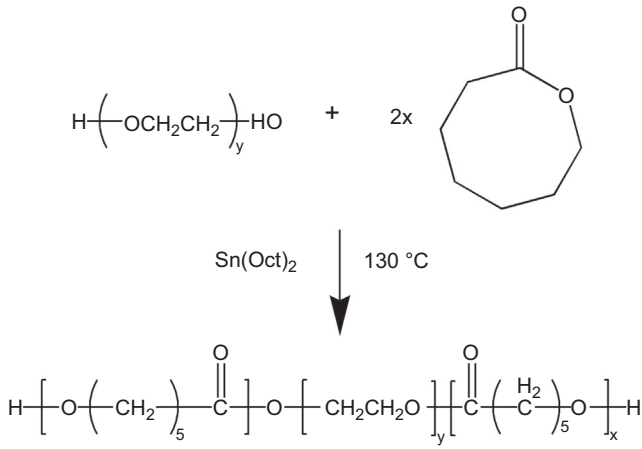

Figure 13.5 Synthesis scheme of PCL-b-PEG copolymer.
Reproduced with permission Liu, C.B., Gong, C.Y., Huang, M.J., Wang, J.W., Pan, Y.F., Zhang, Y.D., et al., 2008. Thermoreversible gel−sol behavior of biodegradable PCL-PEG-PCL triblock copolymer in aqueous solutions. Journal of Biomedical Materials Research Part B: Applied Biomaterials 84, 165−175.

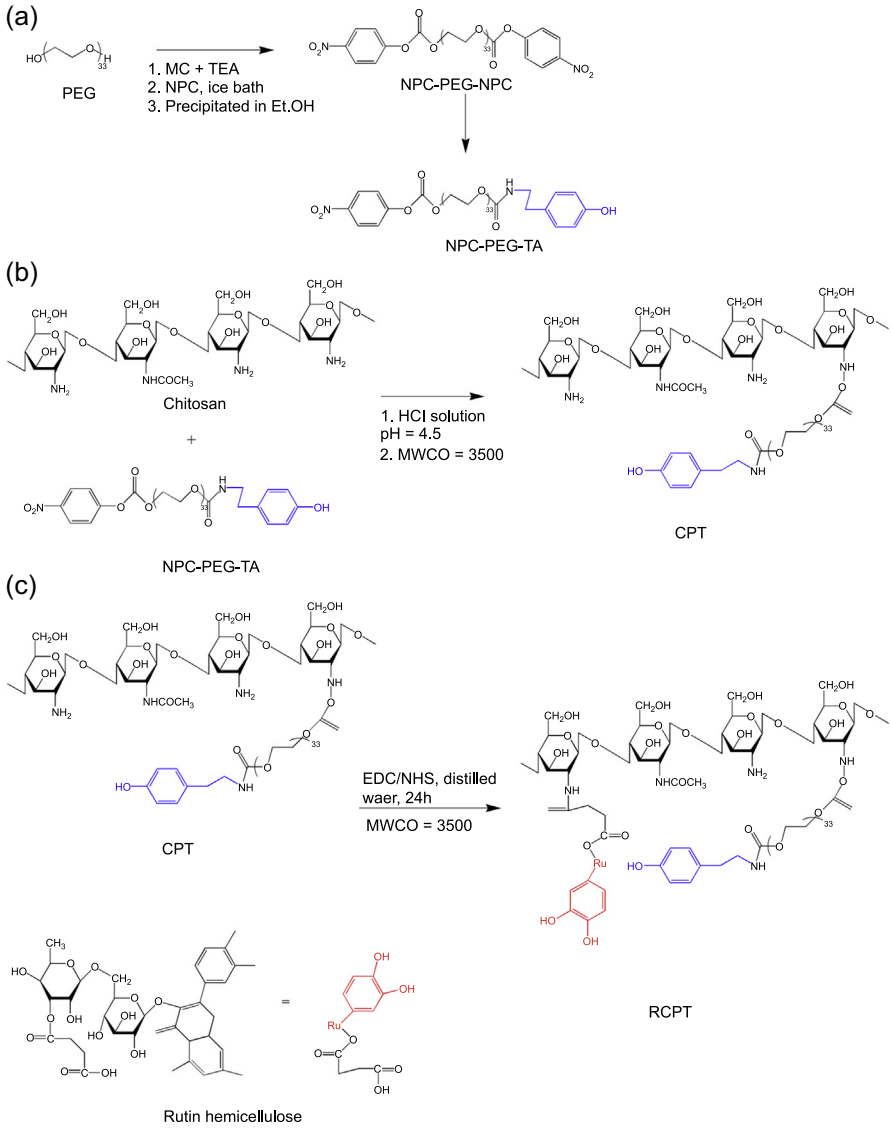

Figure 13.6 Synthetic schemes of polymers: (a) NPC-PEG-TA, (b) CPT, and (c) RCPT. Reproduced with permission Tran, N.Q., Joung, Y.K., Lih, E., Park, K.D., 2011. In situ forming and rutin-releasing chitosan hydrogels as injectable dressings for dermal wound healing. Biomacromolecules 12, 2872−2880.

13.2.2.2 Chitosan-based hydrogel

Ona et al. in the year 2000 reported the photosensitive chitosan-based hydrogels. They introduced both azide and lactose moieties into the chitosan molecule through a two-step condensation reaction. In the first condensation reaction, lactobionoic acid was

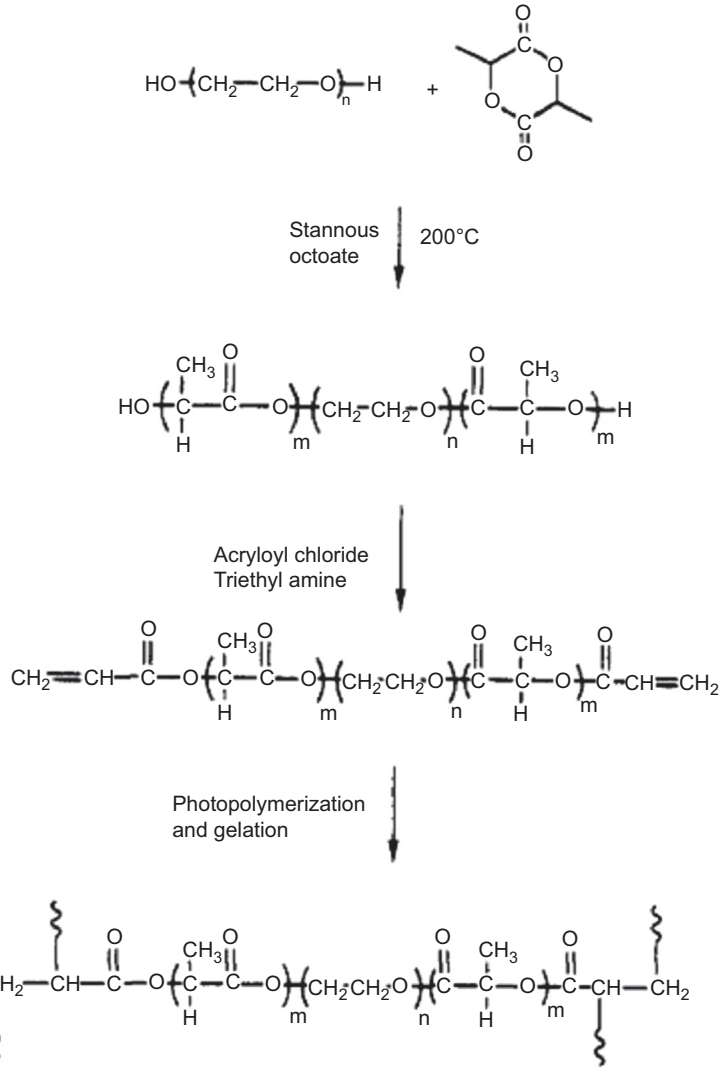

Figure 13.7 Reaction scheme for the synthesis of polymerizable PEG-co-poly(α-hydroxyacid) di- and tetraacrylates and hydrogels.
Reproduced with permission Sawhney, A.S., Pathak, C.P., Hubbell, J.A., 1993. Bioerodible hydrogels based on photopolymerized poly (ethylene glycol)-co-poly (.alpha.-hydroxy acid) diacrylate macromers. Macromolecules 26, 581−587.

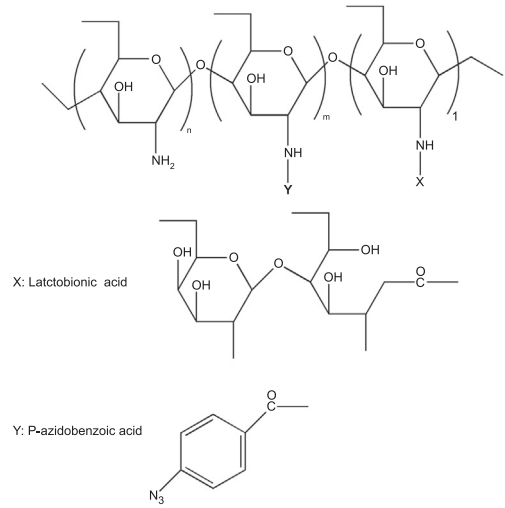

Figure 13.8 Chemical structure of photocrosslinkable Az−CH−LA.
Reproduced with permission Ono, K., Saito, Y., Yura, H., Ishikawa, K., Kurita, A., Akaike, T., et al., 2000. Photocrosslinkable chitosan as a biological adhesive. Journal of Biomedical Materials Research Part A 49, 289−295.

coupled onto chitosan (CH-LA) then azidobenzoic acid was coupled onto the CH-LA. Fig. 13.8 shows the chemical structure of Az−CH−LA. The Az−CH−LA was photopolymerized using UV radiation (Ono et al., 2000).

13.2.3 pH-responsive hydrogels

13.2.3.1 Chitosan/palmitic acid

Chiu et al. (2009) synthesized chitosan/palmitic acid polymer, which will make hydrogel by pH triggering. Earlier, Jiang et al. (2006) produced a novel micelle system from the palmitic acid−grafted chitosan, where palmitic anhydride and chitosan reacted together to get the final product as shown in Fig. 13.9.

13.2.4 Dual-responsive hydrogels

Dual responsive hydrogels will respond to two stimuli at the same time for example, pH- and temperature-sensitive hydrogels that respond to change in pH and temperature and make them injectable to hydrogels. These types of hydrogels have unique advantages over single stimuli responsive hydrogels because they prevent the gelation within the injection needle and form a better ionic complex with therapeutic agents. Injectable pH- and temperature-sensitive hydrogels are biocompatible and biodegradable, and their drug-release behavior can be triggered by changing the chemical structure of the pH- and temperature-sensitive polymers, leading to enhanced specificity of drug delivery and less side effects. Shim et al., in 2005 first synthesized the dual pH- and temperature-responsive copolymer to produce hydrogels from sulfamethazine oligomer-b-poly(ε-caprolactone-co-lactide)-b-pol(ethylene glycol)-b-sulfamethazine

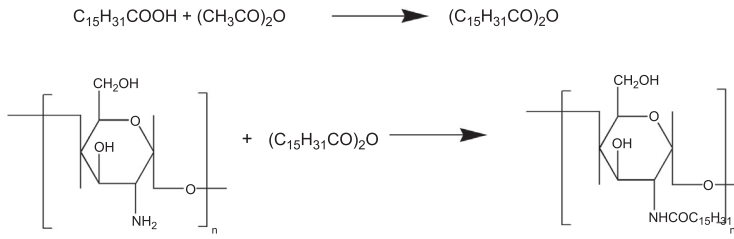

Figure 13.9 The synthesis of *N*-palmitoyl chitosan.
Reproduced with permission Jiang, G.-B., Quan, D., Liao, K., Wang, H., 2006. Novel polymer micelles prepared from chitosan grafted hydrophobic palmitoyl groups for drug delivery. Molecular Pharmaceutics 3, 152−160.

oligomer (OSMs−PCLA−PEG−PCLA−OSMs). Synthesis involved, first synthesis of sulfamethazine, a member of sulfonamide family, was derivatized to a polymerizable acryloyl monomer, which served as a pH-sensitive component. Then PCLA-PEG-PCLA was synthesized using PEG as a ring-opening agent. Further, the carboxylic group in OSM was activated and coupled with OH groups at both ends of PCLA-PEG-PCLA, yielding the final block copolymer, OSM−PCLA−PEG−PCLA−OSM as shown in Fig. 13.10.

13.3 Drug delivery systems

DDS is an interface between the patient and the drug, and it is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of the release of drugs in the body (Jain, 2008).

13.3.1 Drug delivery routes

Drugs may be introduced into the human body by various anatomical routes. They may be intended for systemic effects or targeted to various organs and diseases. The choice of the route of administration depends on the disease, the effect desired, and the product available. Drugs may be administered directly to the organ affected or given systemically and targeted to the diseased organ (Jain, 2008).

13.3.1.1 Parenteral drug delivery

Parenteral drug delivery involves introduction of substances into the body by routes other than the gastrointestinal tract (GIT), which is by injection via subcutaneous, intramuscular, intravenous, or intra-arterial routes. Parenteral administration of the

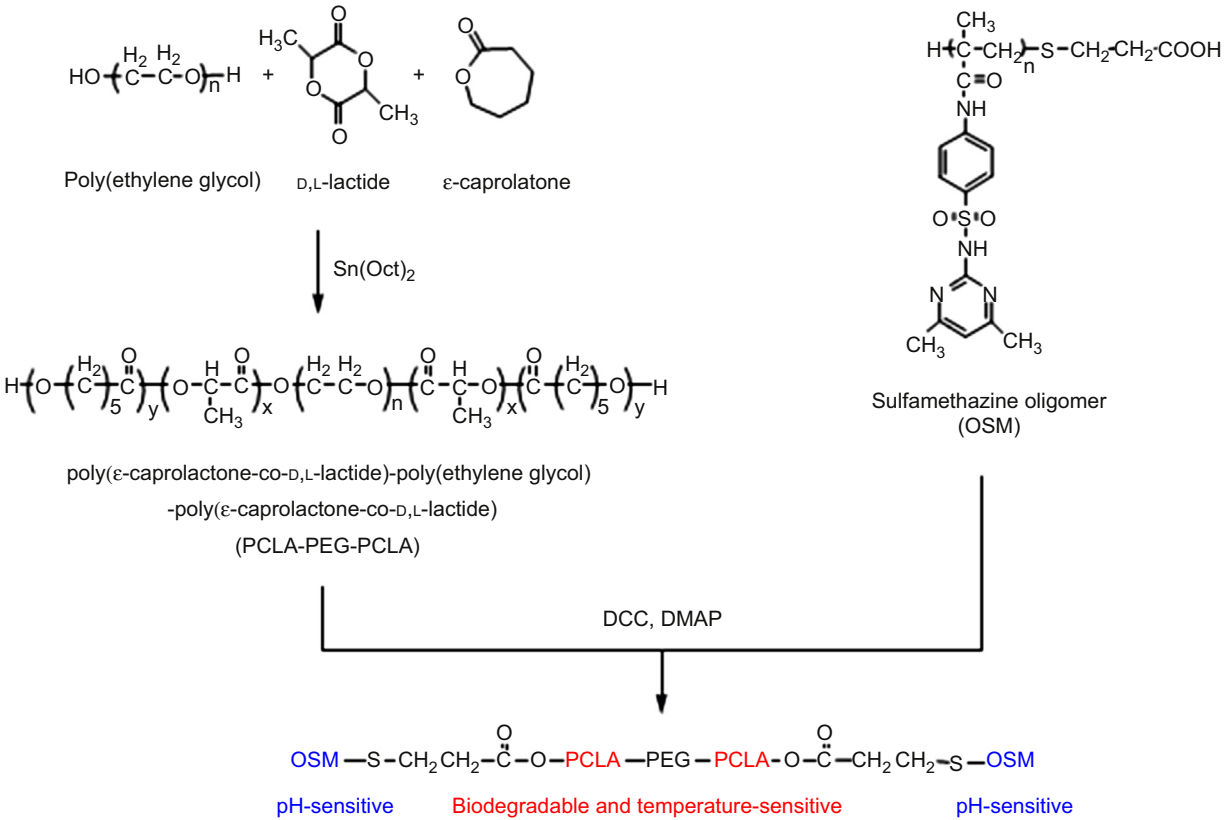

Figure 13.10 pH- and temperature-sensitive anionic pentablock copolymer (OSM−PCLA−PEG−PCLA−OSMs). Reproduced with permission Shim, W.S., Yoo, J.S., Bae, Y.H., Lee, D.S., 2005. Novel injectable pH and temperature sensitive block copolymer hydrogel. Biomacromolecules 6, 2930−2934.

drugs is the most commonly used invasive method of drug delivery in the medical practice because of the following advantages:

1. Rapid onset of action.
2. Predictable and almost complete bioavailability.
3. Provision of a reliable route for drug administration even when patients are very ill, who are not able to ingest anything orally.

This mode of drug delivery also has flaws such as patient compliance when injection is applied and limited sustained release of drug molecules such as protein products.

13.3.1.2 Nonparenteral drug delivery

13.3.1.2.1 Oral drug delivery

Oral route of drug administration is the widely used conventional technique of drug delivery. The ease of administration and widespread acceptance makes it widely used by the patients. Major limitations of oral route of drug administration are as follows:

1. Drugs taken orally for systemic effects have variable absorption rates in the body, which may be unpredictable. This has led to the development of sustained-release and controlled-release systems.
2. Drugs taken orally will directly enter into digestive tract and ubiquitous digestive enzymes present in the digestive tract can degrade some drugs well before they reach the site of absorption into the bloodstream.
3. Many macromolecules and polar compounds cannot effectively traverse the cells of the epithelial membrane in the small intestines to reach the bloodstream. Therefore, their use is limited to local effect in the GIT.
4. Oral route may not be suitable for drugs targeted to specific organs. Despite disadvantages, the oral route remains the preferred route of drug delivery because of the acceptance of the patients.

13.3.1.2.2 Transdermal drug delivery

Transdermal drug delivery involves the delivery of drugs through skin. It includes the following categories of drug administration:

1. Local application formulations, e.g., transdermal gels.
2. Transdermal patches.
3. Minimally invasive methods of transdermal drug delivery, e.g., needle-free injections

13.3.1.2.3 Transmucosal drug delivery

Mucous membrane covers all the internal passages and orifices of the body, therefore drugs can be introduced at various anatomical sites. Major anatomical sites are buccal, nasal, and rectal. Movement of molecules across the mucous membranes occurs by diffusion. This technique offers few advantages such as rapid absorption when compared with oral administration and bypassing liver metabolism.

Mucoadhesive controlled-release devices can improve the effectiveness of transmucosal delivery of a drug by maintaining the drug concentration between the effective and toxic levels, inhibiting the dilution of the drug in the body fluids, and allowing targeting and localization of a drug at a specific site.

13.3.1.2.4 Buccal and sublingual routes

Buccal or sublingual route can be used for the patients who have swallowing difficulties. Buccal absorption is dependent on lipid solubility of the drug, the salivary pH, and the partition coefficient (which is an index of the relative affinity of the drug for the vehicle than for the epithelial barrier). A large partition coefficient value indicates a poor affinity of vehicle for the drug. A small partition coefficient value means a strong interaction between the drug and the vehicle, which reduces the release of the drug from the vehicle. The ideal vehicle is the one in which the drug is minimally soluble. Buccal drug administration has the following attractive features:

1. Quick absorption into the systemic circulation with rapid onset of effect due to absorption from the rich mucosal network of systemic veins and lymphatics.
2. The tablet can be removed at any point of time, in case of an undesirable effect, and the tablet can remain for a prolonged period in the buccal cavity, which enables development of formulations with sustained-release effect.
3. Oral mucosal absorption avoids the first pass hepatic metabolism.

Buccal route have few limitations as follows:

1. The tablet must be kept in place and not chewed or swallowed, which is not convenient for all the patients.
2. Excessive salivary flow dissolves the drug very fast so that it may be washed away from its position.
3. A bad-tasting tablet will have a low patient acceptability.

Some of these limitations can be overcome by the use of a patch containing the drug that is applied to the buccal mucosa or by using the drug as a spray.

13.3.1.2.5 Nasal drug delivery

Drugs have been administered nasally for several years both for topical and systemic effect. Topical administration includes agents for the treatment of nasal congestion, rhinitis, sinusitis, and related allergic and other chronic conditions. Intranasal route is chronically used for drugs that are ineffective in oral administration, which require small doses and rapidly enters into the circulation. The rate of diffusion of the compounds is influenced by the physicochemical properties, which occurs through the nasal mucous membranes.

Advantages of nasal drug delivery includes:

1. Rapid absorption, usually within half an hour.
2. Avoidance of the effects of gastric stasis and vomiting.
3. Avoidance of first pass effect that occurs after absorption of drugs from the GIT.
4. Ease of administration by the patients, who are usually familiar with nasal drops and sprays.
5. Higher bioavailability of the drugs compared to oral delivery.

Disadvantage of nasal drug delivery includes:

1. The nasal route of delivery is not applicable to all drugs. Hydrophilic macromolecules are not absorbed well because of poor membrane permeability, rapid clearance, and enzymatic degradation in the nasal cavity.

13.3.1.2.6 Colorectal drug delivery

Although drug administration to the rectum in human beings is there since 1500 BC, majority of patients are reluctant to administer drugs directly via this route. The idea of colorectal drug delivery was developed from basic physiological reaction that food passes through the small intestine within few hours but it remains in the colon for 2−3 days. However, the colon is the safe for slow absorption of drugs, thus the ingested materials will remain in the colon for longer time. Importantly, the drugs must be active against the numerous bacterial species present in the colon, which are mainly anaerobes and possess a wide range of enzymatic activities. Local treatment of colonic pathologies, such as ulcerative colitis, colorectal cancer, and Crohn's disease, is more effective with the delivery of drugs to the affected area.

Advantages of the rectal route of administering drug are as follows:

1. Relatively large amount of the drug can be administered.
2. This route is safe and convenient particularly for the infants and the elderly. Especially, treatment of emergencies such as seizures in infants when the intravenous route is not available.
3. Drugs absorbed from the lower part of the rectum bypass the liver mechanism.
4. Degradation of the drugs is much less in the rectal lumen than in the upper GIT.

Disadvantages are as follows:

1. Some hydrophilic drugs (such as antibiotics, peptide drugs) are not easily absorbed from the rectum, so enhancers are required.
2. Drugs may cause rectal irritation and sometimes proctitis with ulceration and bleeding.

13.4 Applications of hydrogels in drug delivery

Hydrogels can be used to deliver the drug of interest to various parts of human body based on its composition as explained in Fig. 13.11. The composition of the polymeric material will decide the route of delivery.

13.4.1 Drug delivery from hydrogel depots

Temperature-sensitive hydrogels will be in sol state at room temperature, which facilitate the incorporation of bioactive molecules such as drugs and when the temperature was raised upto the physiological temperature Lower critical solution temperature (LSLT) they form immediate gel. The LCST allows the polymers to be injected into the body in a liquid state that is followed by gelation at a physiological temperature to form a cross-linked hydrogel, thus allowing them to serve as a drug delivery carrier (Elias et al., 2015).

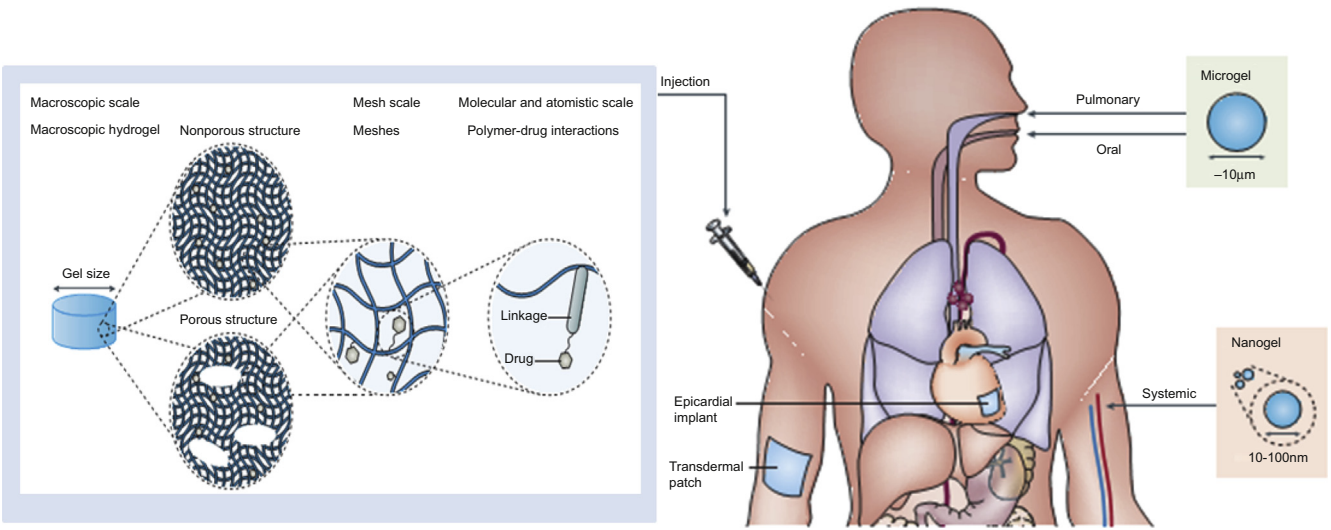

Figure 13.11 Delivery route of hydrogels: hydrogels are used for the delivery of drugs into the body. Injectable hydrogels that can be delivered via syringe–needle injection include in situ-gelling hydrogels. Apart from in situ hydrogels physical and chemically cross-linked hydrogels can be used for the oral, nasal, and transmucosal delivery.
Reproduced with permission Li, J., Mooney, D.J., 2016. Designing hydrogels for controlled drug delivery. Nature Reviews Materials 1, 16071.

Kang et al. in the year 2006 reported doxorubicin (DOX) release from a thermosensitive and biodegradable poly(organophosphazene) hydrogel, which was prepared using a physical mixing method that increased the solubility of DOX in the aqueous polymer solution 40-fold compared with that in phosphate buffer solution. They also found that DOX loaded in the hydrogel in high concentrations could be released at a faster rate than that observed using low concentrations of DOX (Kang et al., 2006).

One of the ideal thermosensitive polymers is poly(*N*-isopropyl acryl amide). Unfortunately, poly-NIPAAm is not suitable for biomedical applications due to its nonbiodegradable nature. Triblock poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) copolymers, PEO−PPO−PEO, known as poloxamers or pluronics, have shown gelation at body temperature at higher concentration. The highest concentrations of a surfactant, however, lead to significant cytotoxicity and, furthermore, they increase the plasma cholesterol and triglycerol levels in rats after intraperitoneal injection (Wasan et al., 2003).

Therefore, biodegradable and negligible cytotoxicity showing PLA/PLGA-b-PEG-b-PLA/PLGA copolymer could be an efficient polymer to form the hydrogels at stimulated temperature and sustained release of drug molecule from the gel matrix. Hydrophobic drugs can be loaded into PLGA-b-PEG-b-PLGA or PLA-b-PEG in a sol state and injected into a diseased site, which gradually releases drug over a prolonged time period and as well as degrades into nontoxic by-products. PLGA-b-PEG-b-PLGA sol−gels have been studied for systemic drug delivery, e.g., peptides after subcutaneous injection, by far the most significant clinical progress has been made in localized control of cancer along with surgery and radiation (Elstad and Fowers, 2009). MacroMed developed thermosensitive biodegradable polymers based on ABA and BAB triblock copolymers, wherein A denotes the hydrophobic polyester block and B denotes the hydrophilic PEG block. They also found that low molecular weight polymers of this polymer class are water-soluble and yield a temperature-dependent reversible gel−sol transition. The aqueous polymer solution of PEG−PLA−PEG (M_n 5000−2040−5000) was loaded with fluorescein isothiocyanate dextran (FITC−dextran) at 45°C and then injected into animals to form a gel at body temperature and the release was observed over 10−20 days in the blood (Jeong et al., 1997, 1999b). In another study, Gao et al. demonstrated the improvement of docetaxel (DTX) solubility 6.85 ± 3.26 μg/mL in water using PLGA−PEG−PLGA copolymer solution (20%). The drug-loaded polymer became gel at 32°C and sustained release of DTX from PLGA−PEG−PLGA was observed for more than 3 weeks. The controlled release rate was due to the clear micelle formation and the drug was encapsulated by the polymeric micelles. Further, intratumoral injection (i.t.) of DTX from the PLGA−PEG−PLGA gel system in A-549 lung tumor−bearing BALB/cA mice exhibited one i.t. injection of the thermosensitive hydrogel containing DTX was comparable to three i.v. injections of DTX in inhibiting tumor growth in mice (Gao et al., 2011). In a recent study, Shang et al. reported the human calcitonin (hCT), a peptide drug release from PLGA-b-PEG-b-PLGA hydrogels. hCT was sustainedly released over 3 weeks in the 20 wt% hydrogel (Shang et al., 2017).

MacroMed's formulation ReGel has entered into market, which contains 23% (w/w) ABA-triblock copolymer (PLGA−PEG−PLGA) in phosphate-buffered saline (pH 7.4) that is loaded with paclitaxel. This oncogel that contains a concentration of

6 mg/g ReGel releases the paclitaxel for about 6 weeks. The clear advantage is the ability to solubilize the hydrophobic drug molecules paclitaxel and its prolonged release for more than 50 days (Packhaeuser et al., 2004; Zentner et al., 2001).

Local delivery of drug release from hydrogels has been mostly studied against cancer. Surgical procedure remains the most important treatment option for cancer though considerable research efforts have been made in anticancer drug development and formulation using nanomaterial. Despite the curative potential of surgical resection for localized early stage cancer, undetected micrometastases and undefined tumor margins remain as major challenges faced by surgeons. Therefore, localized drug delivery by polymeric delivery systems as an adjunct to surgery will be an efficient strategy to cure cancer (Wolinsky et al., 2012). It was best exemplified by Gliadel wafer for brain tumors (Matthes et al., 2007). The endoscopic ultrasound-guided injection of PLGA-b-PEG-b-PLGA sol−gel is another strategy for the local cancer drug delivery, particularly for the pancreatic cancer, where patients cannot undergo resection of pancreas (Matthes et al., 2007; Cho et al., 2016). Cho et al. (2016) have written a detailed review on the commercial sol−gels for the cancer treatment. More recently, Marta Cerruti group reported the mucoadhesive chitosan hydrogels for the local delivery to rectal for treating ulcerative colitis. They used the model drug Sulfasalazine which was loaded into catechol−chitosan polymer that further formed hydrogel once it was injected to the rectum of mouse (Xu et al., 2017).

Variations in pH are known to occur at various body sites, such as the GIT, vagina, and blood vessels. This information provided a strong base to develop the hypothesis pH-responsive drug release (Gupta et al., 2002). pH-sensitive hydrogels have been most frequently used to develop controlled release formulations for oral administration. The pH in the stomach (<3) is quite different from the neutral pH in the intestine, and such a difference is large enough to elicit pH-dependent behavior of hydrogels. Most commonly studied ionic polymers for pH-responsive behavior are poly(acrylamide), poly(acrylic acid), poly(methacrylic acid) (PMAA), poly(diethylaminoethyl methacrylate), and poly(dimethylaminoethyl methacrylate) (Pitt et al., 1985). The polycationic hydrogels have minimal swelling at neutral pH, thus minimizing drug release from the hydrogels. This property has been used to prevent release of foul-tasting drugs into the neutral pH environment of the mouth. In a study, seigal et al. demonstrated that caffeine was not released from the hydrogels of methyl methacrylate and *N*,*N*′-dimethylaminoethylmethacrylate at neutral pH, where the release was observed at zero order in pH 3−5 (Bazban-Shotorbani et al., 2017; Siegel et al., 1988). In another study, patel et al. showed the release of antibiotics in the acidic condition from semi-interpenetrating network of cross-linked chitosan and PEO hydrogels for the site-specific delivery of antibiotics against *Helicobacter pylori* (Patel and Amiji, 1996).

pH-sensitive hydrogels have been mostly studied for the insulin delivery. In general, parenteral route is preferred for the insulin delivery because oral administration has several drawbacks such as insulin inactivation by proteolytic enzymes in the GIT, low permeability through intestinal membrane, hyperinsulinemia, pain, allergic reactions, and low patient compliance (Aoki et al., 2005). Moreover, parenteral route may not replicate the normal dynamics of endogenous insulin release, resulting in failure to achieve long-lasting glycemic control in diabetic patients (Alexander et al., 2013; Agarwal and Khan, 2001; Takale et al., 2017). Hydrogels-based insulin delivery

is an another alternative strategy to overcome the problems observed in the parenteral mode of delivery. Mi et al. showed the efficient insulin release at acidic pH and reduced release at neutral pH from self-assembled NPs producing pH-responsive trimethylated chitosan (TMCS)-*g*-PGA. Further, they also showed that the usage of TMCS alone as a carrier for insulin is limited due to its reduced intrinsic mucoadhesivity (Prabaharan, 2008). PEG-grafted PMAA (P(MAA-g-EG)) are another class of polymeric hydrogels mostly studied for the insulin delivery. Herein, the hydrogels exhibit pH-responsive swelling and changes in the network pore structure due to reversible formation/dissociation of interpolymer complexes. In acidic environment, due to H-bond formation between carboxylic acid protons of PMAA and oxygens of PEG, temporary physical cross-links might form to entrap and protect insulin. At higher ionic strength, pH $>$ 5.2, as in upper small intestine, pendant acidic groups dissociate due to ionization and the network swells. In addition, these hydrogels enhanced insulin transport across the intestinal mucosa as well as decreased the mucosal membrane resistance in Caco-2 monolayers without any appreciable cytotoxicity (Morishita et al., 2002). Various review articles have been published on hydrogels for the insulin delivery. Interested readers may read them for the detailed survey (Chaturvedi et al., 2013; Mukhopadhyay et al., 2012).

pH-responsive hydrogels also have been explored for the oral vaccine delivery to avoid the disadvantage of the currently available vaccines such as poor usability and incomplete immune responses in infected sites such as the intestine and respiratory tracts. The idea was, oral delivery of vaccines can induce intended immune responses in the intestinal mucosa, which is a major site of infection for viruses and bacteria. In addition to systemic generation of immunoglobulin G, mucosal immunization induces secretion of immunoglobulin A, which blocks viral and bacterial infections (Wang et al., 2012). Therefore, oral vaccination is an ideal strategy for preventing viral and bacterial infections because administration and immunization in both the mucosa and blood can be achieved. Despite the advantages, instability in the GIT and low permeability through the epithelial cellular membrane of therapeutic antigens significantly complicates the development of oral vaccines.

Buccal delivery of drug using hydrogels is an alternative strategy to oral delivery. The buccal mucosa consists of an epithelium layer about 40−50 cell layers thick, a lamina propria layer, a submucosa layer, and the mucus is secreted by salivary glands. The multilayered, nonkeratinized human buccal mucosal membrane is rich of underlying blood vessels and has relatively good drug permeability through both transcellular and paracellular routes (Shojaei, 1998). Buccal drug delivery is mostly used for the prolong drug effect in periodontal diseases, oral mucositis, and oral fungal infection. Acrylic-based hydrogels have been used extensively as mucoadhesive systems. They are well suited for bioadhesion because of their flexibility and nonabrasive characteristics in the partially swollen state, which reduce damage-causing attrition to the tissues in contact. Cross-linked polymeric devices may be rendered adhesive to the mucosa. The ensuing hydrogels exhibit mucoadhesive properties due to enhanced anchoring of the chains with the mucosa (Jain, 2008). Xu et al. reported genipin-crosslinked catechol−chitosan mucoadhesive hydrogels for the sustained release of lidocaine in the buccal cavity. They observed the release for 3 h and 1 ng/mL of lidocaine in rabbit serum, which was most likely due to the intimate contact provided by their mucoadhesive hydrogel (Xu et al., 2015).

Transdermal drug delivery provides lower levels of systemic drug exposure that reduces the side effects and avoids gastrointestinal and hepatic first pass metabolic degradation of the drug. However, the natural barrier properties of the skin make transdermal drug delivery a challenging task. Hydrogel-based formulations could enhance drug permeation while providing drug release in a controlled manner. Skin electroporation and low-frequency sonophoresis are external field−mediated transdermal transport phenomena to enhance skin permeabilization of molecules across epidermis. By this method passage of drugs through the skin in its transiently permeabilized state can occur rapidly, therefore it is possible that a transdermal patch or drug reservoir may provide a substantial barrier to the overall transdermal drug transport. In that view, Zhang et al. proposed an idea of giving low-frequency (20 kHz) sonophoresis to the drug-containing hydrogel. They concluded that relatively high rates of sonophoretic molecular transport across human skin are achievable when hydrogels are used as the ultrasound-coupling medium as long as a method is used to induce molecular mixing within the gel (Zhang et al., 1996). In a recent study, Carmona-Moran et al. (2016) studied using penetration enhancers (dimethyl sulfoxide, isopropyl alcohol, and propylene glycol) with gellan gum on the transport of NSAID diclofenac sodium in trans well diffusion medium and found that these system could be used for the efficient transport of drug molecules into the skin.

Microneedles (MNs) have been used to deliver the drugs to the dermis. Generally, MNs are composed of arrays of microprojections formed with different materials, generally ranging from 25 to 2000 μm in height with different tip shapes and tip intervals, being attached to a base support and they puncture the epidermis to reach the dermis. Hydrogel MNs are one kind of MNs that offers an advantage of higher amount of drug loading compared to solid MNs and drug-coated MNs (Hong et al., 2014). The mechanism of drug release is same as other hydrogels without fabricated into any device that they will diffuse, degrade, and release the drug molecule. Lee et al. demonstrated the protein delivery from FDA-approved ultra-low viscosity carboxymethyl cellulose and amylopectin-based MNs. The main advantage involved with this design is MNs itself could be dissolved once they are applied into skin. Thus after the insertion into the skin, MNs dissolve quickly and release the loaded drug in short time (Lee et al., 2008). In another study, Ling et al. also proposed the same type of dissolving MNs using chitosan, which contained insulin for the release. They observed the release in very short time about 5 min (Chen et al., 2013).

In advancement of MNs, researchers have developed stimuli-responsive hydrogels forming MNs for the efficient delivery of drugs. Hardy et al. used 2-hydroxyethyl methacrylate and ethylene glycol dimethacrylate to fabricate the MNs. Further they loaded ibuprofen into 3,5-dimethoxybenzoin conjugate, which is light sensitive and was further loaded into the MNs. They observed the release upto 160 h (Hardy et al., 2016). Donnelly et al. (2012) demonstrated a unique principle to fabricate MNs where they used cross-link density of the hydrogel system as the main principle to control the release of drug molecule.

Tables 13.1 and Table 13.2 shows some of the implants patented and available in the market.

Table 13.1 **Recent patens on stimuli-responsive hydrogels**

Patent no.	Pub. date	Inventors	Title	Description
US 9265836 B2 (Shih and Zentner, 2016)	Feb 23, 2016	C. Shih, G. M. Zentner	Biodegradable block copolymeric compositions for drug delivery	Inventors show copolymer drug carriers comprising A-B, A-B-A, or B-A-B block copolymers, wherein the A block is a biodegradable polyester or poly(ortho ester) and the B block is polyethylene glycol (PEG)
US 2013/0244972 A1 (Ben-Shalom et al., 2012)	Sep. 19, 2013	N. Ben-Shalom, Z. Nevo, A. Patchornik, D. Robinson	Injectable chitosan mixtures forming hydrogels	Inventors demonstrate the degree of acetylation to control the gelation of chitosan
US 8231890 (Cruise and Constant, 2012)	July 31, 2012	Cruise; Gregory M. Michael J.	Hydrogels that undergo volumetric expansion in response to changes in their environment and their methods of manufacture and use	Inventors explain the volumetric expansion of hydrogels triggered by pH or temperature as well as their manufacturing procedure, and use.
US/201200 27775A1 (Won and Cui, 2013)	February 2, 2012	Chee-Youb Won	Absorbable PEG-based hydrogels	Inventors display the fabrication of PEG-based hydrogels from multiarm PEG−vinylsulfone comprising about 3−8 arms and a multiarm PEG-R-sulfhydryl comprising 3−8 arms. Here R is designated as an ester linkage such as carboxylate ester, lactate ester, and isobutyrate ether. Further they also show the sustained action of protein and peptide from the PEG-based hydrogel.
EP 1 720 932 B1 (Lee et al., 2010)	January 23, 2013	Lee, Doo-Sung; Shim, Woo-Sun; Bae, You-Han; You, Je-Sun; Kim, Min-Sang	pH- and temperature-sensitive hydrogels	Inventors exhibit the pH- and temperature-sensitive hydrogels composed of block copolymer PLGA−PCL−PEG
20090238815 A1 (Udipi et al., 2008)	September 24, 2009	U. Kishore; T. Julie; G. Ya.	Nondegradable hydrogels for medical device application	Inventors demonstrate the nondegradable PEG hydrogels, their use in medical devices

Table 13.2 Drug-loaded implants available in the market

Brand name	Drug	Polymer	Implantation route	Duration	Application	References
OZURDEX	Dexamethasone	PLGA	Intravitreal	6 months	Macular edema	Roy and Hegde (2013) and Dugel et al. (2015)
Vitrasert	Ganciclovir	Poly(vinyl alcohol) and poly(ethylene vinyl acetate)	Intravitreal	32 weeks	CMV^2 retinitis	Rupenthal (2017) and Chang and Dunn (2005)
ZOLADEX	Gosere lin acetate	PLA-PLGA	Subcutaneous	3 months	Prostate cancer, breast cancer or endometriosis (noncancerous condition)	Mitchell (2004)
SUPPRELIN LA	Histrelin acetate	2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, trimethylolpropane trimethacrylate, benzoin methyl ether, Perkadox-16, and Triton X-100	Subcutaneous	1 year	Central precocious puberty	Thornton (2012) and Silverman (2012)
Testopel Pellets	Testosterone	Polyvinylpyrrolidone	Subcutaneous	3−6 months	Deficiency or absence of endogenous testosterone	Roberson and Kosko (2013)

13.5 Future prospects and conclusions

Current research of hydrogels for drug delivery focuses on delivery of multiple drugs from a single system and stimuli responsive release with a high level of control. Although the release of multiple drugs has been demonstrated, obtaining release of distinct molecules at different rates still remains a challenge. The potential application of such device can be used for the tissue repair, where tissue natural regeneration process involves the sequential signaling of several growth factors. Therefore, the development of broadly useful systems that provide reliable, robust stimuli responses will probably necessitate other novel strategies to release the drug at controlled rate.

Advances in polymeric biomaterials have broadened the scope of hydrogels designed for controlled drug delivery. The impact of hydrogels for controlled drug delivery is expected to increase a lot in the coming years by the increased fundamental understanding and target applications and to continue to improve human health.

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Antimicrobial polymeric gels

14

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14.1 Introduction

Certain biopolymers, in last few years, have shown a strong positive action as antimicrobials. These not only act on individual microbial cells but also on biofilms, which are becoming a menace in antimicrobial treatments. Among the biopolymers, cationic polymers have emerged as great and effective antimicrobial agents. In a nutshell, cationic polymers display the antimicrobial activity by disrupting the cell wall and membrane and release of intracellular materials that kill the target cell. It is also suggested that these biopolymers resist or inhibit further microbial growth. The most common polymeric structures used as antimicrobial strategy are films, beads, and nanoparticles (Chen et al., 2014). All these structures display a property of being formulated as hydrogel. By definition, hydrogels are the polymeric network structure that can imbibe and retain fraction of water (or liquid) more than its own weight, when placed in any aqueous media. Thus, all the hydrophilic polymers can be given the form of hydrogel. To form hydrogels, cross-linking of these polymers is a must. Cross-linking prevents dissolution of hydrophilic polymers and gives them a network structure. Because hydrogels are under extensive research to be used on biological platforms, it is extremely important that the polymers have great biocompatibility and biodegradability. It is also very important that the degradation product(s) has less or no toxicity and immunogenicity (Akhtar et al., 2016; Ahmed, 2015). Talking about the basic classification of hydrogels, they are classified according to their cross-linking behavior. If covalent bonds are involved in the cross-linking reaction, the hydrogels are termed as permanent hydrogels (polyhydroxyethylmethacrylate [pHEMA] and polymethylmethacrylate [pMMA]), whereas they are called physical hydrogels if noncovalent or physical interactions such as hydrogen bonds and ionic bonds are present in the formation. The water absorption in the hydrogel is primarily due to the presence of hydrophilic functional groups such as carboxyl (−COOH), amino ($-NH_2$), and hydroxyl (−OH) groups in the polymers. It is a matter of fact that a higher number of hydrophilic groups result in greater water-holding capacity of the resulting hydrogel while the same reduces on increasing the cross-linking (Buwalda et al., 2014; Pal et al., 2009). Some of the most widely used hydrophilic polymers are polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), poly acrylic acid, polyethylene glycol (PEG), alginate, and carboxymethylcellulose (CMC). Chitosan in its native form is not water soluble but soluble in acidic solvents while its modified forms, such as chitosan

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oligosaccharide and carboxymethyl chitosan, are water soluble (Caló and Khutoryanskiy, 2015; Liu et al., 2000; Jeon et al., 2001).

Many polymers have displayed significant innate antimicrobial activities. As stated in previous statement, antibiotic resistance has emerged as one of the most serious causes of concern globally (Ventola, 2015). Second to the antimicrobial resistance (AR), biofouling is another cause of concern globally and has a huge impact on medical and marine devices. One of the major reasons for AR is overprescription and misuse of antibiotics globally that renders the pathogen resistant. Antimicrobial peptides (AMPs) and cationic polymers have emerged as two great arsenals as substitutes to the conventional antibiotics in the war against global antibiotic resistance. These antimicrobial polymers act by disrupting the cell wall and cell membrane causing the cell death. Among anionic and cationic polymers, the cationic polymers poses innate antimicrobial properties as they can easily interact and disrupt the microbial cell membrane (Veiga and Schneider, 2013). The AMPs are polycationic in nature and can easily disrupt the negatively charged membranes of bacterial cell due to an electrostatic interaction mechanism. Schneider et al. synthesized the first synthetic AMP 20 amino acid residues long rich in lysine known as MAX1 that displayed significant activity against both gram-positive and gram-negative bacteria such as *Streptococcus aureus* and *Escherichia coli*. The team developed a second arginine-rich polypeptide known as MARG1. The MARG1 was found to be highly effective against one of the most drug-resistant bacteria Methicillin-resistant *S. aureus*. These AMPs were found to be selectively targeting microbial cells while having no effect on mammalian cells (Veiga and Schneider, 2013). Another most researched cationic biopolymers for its innate antimicrobial properties are chitosan. Chitosan is a deacetylated product of the parent homopolymer chitin. Chitosan is a polymer having *N*-acetyl-glucosamine and D-glucosamine as the repeating random units. Chitosan is highly biocompatible and biodegradable with negligible toxic effect on mammalian cells (Kumar et al., 2016). Modified chitosan has largely displayed efficacy against wide variety of microorganisms. Chitosan has also been reported to bind to the DNA of pathogens to inhibit their RNA synthesis.

Biofouling, in simple terms, is the collection of microbes or their proteins on the surfaces of certain equipment that damages the functioning of those equipment. The most severe damaging effects are seen in medical and marine devices. The microorganisms develop biofilms that are difficult to disrupt. Medical biofouling is a serious threat globally. This occurs in medical devices such as prosthetic implants, catheters, dental devices, etc. Medical biofouling often leads to surgical replacement of the implant that results more health and financial load to the patient (Bixler and Bhushan, 2012). Modification of medical device surfaces with antimicrobial polymers repels and neutralizes the microbes. PEG is currently considered as one of the best hydrophilic antibiofouling polymers for medical devices. Apart from PEG other widely used polymers include polyamides, polyurethanes, and chitosan (Harding and Reynolds, 2014). Thus, this chapter would try to bring various information about antimicrobial polymers and their few applications (Fig. 14.1).

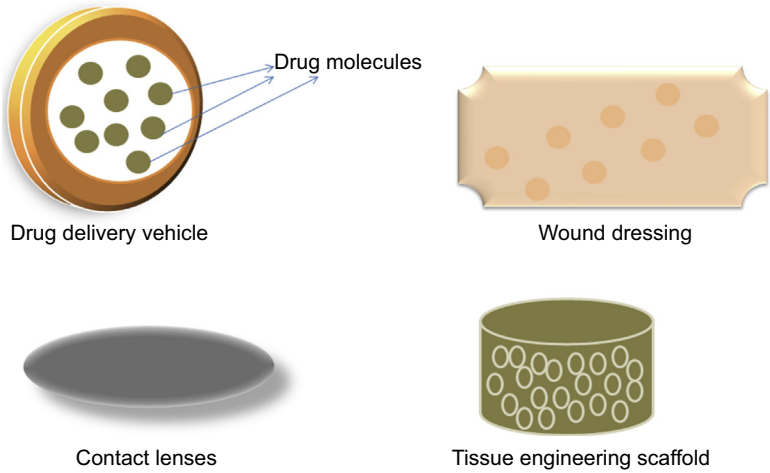

Figure 14.1 A few commercial applications of polymeric hydrogels.

14.2 Definition of "gel"

A gel, as defined by Almdal et al. way back in 1993, is any soft or semisolid material composed of two or more components one of which is a liquid. In the same article, Almdal reported the definition of gel (by P.H. Hermans) as

> "A coherent colloidal system of at least two components with mechanical characteristics more similar to the solid where dispersed component and dispersion medium are equally and continuously extended." (Almdal et al., 1993a,b). Another definition of gel as mentioned in Encyclopedia of Polymer Science and Engineering is
> "A crosslinked network of polymer(s) which can easily show swelling in aqueous medium" (Almdal et al., 1993a,b). In biomedical applications, gels are generally of two types namely hydrogel and organogel.

14.2.1 Hydrogels

As mentioned in the previous section, hydrogels are the cross-linked network of polymers that can imbibe substantial large amount of water as compared to its dry weight. This water-holding characteristic of hydrogel is due to hydrophilic polymers, which are the primary component(s) of the hydrogel (Pal et al., 2009). Hydrogels can virtually be composed of any water soluble or hydrophilic polymer and can be given the form of nanoparticle, macrobeads, films, and coatings. The porosity of hydrogels can be controlled by controlling the cross-linking density (Hoare and Kohane, 2008).

14.2.2 Organogel

On the other hand, organogels are the polymeric gel network with organic continuous phase, as opposed to the aqueous phase in hydrogel. In an organogel network, the polymers immobilize the organic phase or solvent. Several organogelators such as lecithin, glyceryl fatty acid esters, and sorbitan monostearate have been used in the formation of organogels for different biomedical applications. Among the biomedical applications, organogels are very well suited for transdermal drug delivery because many components of organogels such as fatty acids, surfactants, essential oils, and terpenoids are effective permeation enhancers (Vintiloiu and Leroux, 2008). Researchers have recently developed lanolin and sorbitan−sunflower oil−based organogels for drug delivery (Sagiri et al., 2013, 2014).

14.3 Introduction to hydrogel

14.3.1 Definition

Hydrogels, as mentioned earlier in previous sections, are the three-dimensional cross-linked hydrophilic polymers that can imbibe substantial amount of water or aqueous medium. These hydrogels can be structured into a variety of forms such as nanoparticles, macrobeads, coatings, and films and can be used for applications such as tissue engineering, drug delivery, and device coatings. The porosity of the hydrogels is useful in imbibing aqueous media and also releases of the components encapsulated (Hoare and Kohane, 2008).

14.3.2 Classification of hydrogels

Hydrogels have been classified on various bases. A few classifications of hydrogels are as follows:

On the basis of source: Hydrogels are natural if the constituent polymers are from natural source (chitosan) while they are synthetic when the constituent polymers are synthetic (PEG).
On the basis of polymeric composition: Homopolymeric hydrogels are formed of a single polymer with same repeating monomers. They may be cross-linked too. Copolymeric hydrogels have multimonomeric polymer(s) with at least one hydrophilic monomeric unit in the chain. Multipolymeric interpenetrating networks are composed of two or more polymers, which may or may not be cross-linked.
On the basis of cross-linking nature: Hydrogels are said to be chemical hydrogels when the cross-linking involves chemical cross-linkers that result in strong covalent bonds, whereas physical hydrogels have chain entanglements or physical interactions such as ionic bonds and hydrogen bonds.
On the basis of net electrical charge: Hydrogels can be nonionic, ionic, amphoteric, or zwitterionic in nature (Ahmed, 2015).

14.3.3 Synthesis and properties

In basic terms, hydrogels are a random cross-linked network of hydrophilic polymers. Generally, the three basic components for the formation of hydrogels are monomer (or polymers), initiator, and cross-linker. Bulk polymerization involves monomers and monomer-interacting initiators. The bulk polymerization technique creates glass appearance polymer that is usually hard but swells and imbibe water in aqueous media and becomes soft and flexible. Solution polymerization or cross-linking method involves use of a suitable cross-linker that provides extrastability to the polymer. A solvent such as water, ethanol, or a mixture of both is used as synthesis solvent and also to minimize the heat generated during the process of polymerization. In the dispersion polymerization, the polymers are obtained as powder or microbeads. Because water-in-oil solvent is used in this method, it is normally referred to as inverse suspension (Ahmed, 2015).

While physical hydrogels have mild or nontunable physical and mechanical properties, chemical hydrogels have tunable properties and have high in vivo residence time. The applications of hydrogels depend on their properties. The basic properties, such as swelling, permeability, and mechanical properties, remain theoretically similar but they do differ on the basis of constituting polymer(s) and cross-linker (if any). Physical hydrogels generally imbibe a large amount of water and tend to disintegrate gradually due to large pressure developed inside, whereas cross-linked hydrogels tend to pull the polymeric chain inward and resist bursting or disintegration of hydrogel rapidly. The permeability of hydrogels is significantly good and this enables them to release any molecule of interest encapsulated in them (Bajpai, 2001). Apart from the properties such as swelling or permeation, biological properties are extremely important for hydrogels to be used in biological system. Biocompatibility is the primary concern that relates to the interaction between the biological system and the biomaterial used. Not only the primary hydrogel system should be biocompatible but also even their degradation product should not result in any immunogenic reaction and should be easily eliminated through the natural elimination system of the body. For interventional application, mechanical properties such as tensile strength and degradation rate should also be optimized (Kamath and Park, 1993).

14.3.4 Biomedical applications

The biomedical application of hydrogels can be traced back to 1960s when Wichterle and Lim developed hydrogel network from cross-linked pHEMA that was used for removable soft contact lenses. The two most utilized synthetic polymers for biomedical applications were pHEMA and pMMA (Caló and Khutoryanskiy, 2015). Current applications of hydrogels include tissue engineering scaffold, wound dressing, drug delivery vehicles, contact lenses, and hygiene products. The first commercial biomedical application of polymeric hydrogels was shown by Wichterle and Lim in

the form of contact lens. The product was later acquired by the famous company Bausch & Lomb, which started selling the lenses. But a pictorial report mentioned in a recent article by Calo et al. mentioned that by 2012, the greater proportion in the lens market was acquired by silicone hydrogels. Shifting from contact lenses discussion, wound is a pathophysiological condition, which is characterized by a physical break or trauma in the skin and the layers beneath that result in conditions such as necrosis and edema due to microbial colonization. In context of extensive management of wounds, polymeric hydrogel dressings are being seen as useful alternative to conventional dressings. Hydrogel films/patches are particularly useful in moist wounds as they can absorb moist exudates and also release any antimicrobial agent encapsulated in them. Hydrogels in "gel" form are also useful for dry wounds because they do not further need moisture to become gel and can release drugs in their commercial form. The high water content of the hydrogels allows oxygen transport to the wounds such as pressure ulcers and surgical wounds. Hydrogels can easily contain iodine and silver, which are potent antimicrobials for superficial necrotic abscessed wounds that harbor *Pseudomonas aeruginosa* and *Staphylococcus aureus* as the primary inhabitants that produce biofilms. A few examples for commercial hydrogel wound dressings are Granugel (from ConvaTec), WoundTab (from First Water), and Purilon gel (from Coloplast). Drug delivery is another highly researched area where hydrogels are widely utilized. The unique porosity and water imbibing property of hydrogels make them beneficial for drug loading and release. The release of drugs in hydrogels can be controlled by means of stimulus, diffusion, and swelling. Hydrogels are one of the key elements in the scaffold structure for tissue engineering constructs. Because of their biocompatibility and biodegradability, several polymers such as chitosan, alginates, CMC, hyaluronic acid, polyethylene oxide and PVA have been used as hydrogel structures to construct scaffolds on which the cells would be seeded and proliferate. These hydrogel scaffolds basically mimics the extracellular matrix of the natural organ (Caló and Khutoryanskiy, 2015).

When pathogenic microorganisms and their products stick and colonize on the medical devices or when the aquatic organisms such as barnacles and microbes stick on to the in-water surfaces, they gradually become a severe health and environmental problem. This unwanted phenomenon is termed as *Biofouling*. Apart from the earlier copper plate or tributyltin paint usage, polymers have gained recent attraction as antimicrobial coatings on medical and aquatic devices. In an extensive review, Murosaki et al. have described a range of hydrogel polymers as antibiofouling coatings primarily for marine fouling. In the article, PVA and polysaccharide hydrogels were reported to have excellent antibiofouling effect against barnacles. They reported that gels with sulfonic or hydroxyl groups display better antibiofouling effect. pHEMA hydrogels were found to be very effective against marine algae and diatoms in in vitro conditions (Murosaki et al., 2011). PEG has shown to be highly effective against diatoms and inhibit their attachment. It was also reported that polyvinylidene fluoride membranes with PEG coating were excellent in hindering fouling protein adsorption. Getting inspiration from natural antifouling nature of RBCs membrane, it was reported that polymers with zwitterionic chains show excellent antifouling effect (Krishnan et al., 2008). Polyoxazolines, which are

nontoxic hydrophilic polymers, have gained recent interest as antibiofouling polymer for medical devices. It was reported that a coating of copolymer of poly (L-lysine) and polyoxazoline on metal surface reduced the adsorption of serum proteins to negligible levels (Krishnan et al., 2008). Hydrophilic polymers are excellent antifouling entities because they inhibit attachment of fouling elements by forming a separating hydration layer between the target material and the fouling elements. On the other hand, hydrophobic materials resist the attachment of fouling elements by repelling them. A recent report stated that fluorinated hydrophobic coatings are biocompatible and even more with a secondary polymeric layer termed as slippery liquid infused porous surfaces that effectively inhibited the attachment and growth of pathogenic microbes such as *P. aeruginosa* and *S.aureus*. As mentioned above, zwitterionic polymers serve as excellent antifouling surfaces. Most used zwitterionic polymers for medical device coating research are poly(sulfobetaine) and poly(carboxybetaine) attached to methacrylate surface. These zwitterionic surfaces provide a nonspecific detachment of fouling elements and also provide a hydration layer. Attaching AMPs on the polymer surface is another very useful approach because AMPs are inherently biocompatible and microbes can develop minimal resistance against them. The AMPs also inhibit the formation of biofilms, which is a serious hazard for biomedical devices (Harding and Reynolds, 2014).

Thus, owing to diverse applications and modification possibilities of antimicrobial polymers, they can be said to be a real boon for biomedical and marine industries.

14.4 Chitosan as antibacterial and antifungal polymer

14.4.1 Chitosan as antibacterial polymer

Chitosan is a partial deacetylated derivative of the parent polymer chitin. It comprises random repeating units of *N*-acetyl-D-glucosamine and D-glucosamine. Due to presence of large number of amine groups ($-NH_2$), it is polycationic in nature. Owing to its excellent biocompatibility, it has a wide usability in biomedical research area (Kumar et al., 2016). In a seminal article by Sudarshan et al. dating back to 1992, the team demonstrated a significant bactericidal effect of chitosan lactate and chitosan glutamate against both gram-negative and gram-positive bacteria. They showed that one of the mechanisms by which chitosan derivative caused bactericidal effect is causing leakage of intracellular materials resulting in cell death. They finally concluded that water-soluble chitosan could have an excellent bactericidal effect (Sudarshan et al., 1992). In a seminal report about action of chitosan on bacterial cell, it was demonstrated that chitosan disrupts the cell wall and cell membrane leading to cellular leakage concluding in bacterial death. The activity is mainly due to electrostatic interaction between the positively charged amine group of chitosan and the negatively charged phospholipids of bacterial cell (Liu et al., 2004). In another report, it was concluded that the water-soluble chitosan has significantly better antibacterial activity and this effect increases with an increase in degree of

deacetylation (DDA). Another important structural-activity relationship drawn was that antibacterial activity of chitosan increased with an increase in number of amines ($-NH_2$). Another important antibacterial action noticed apart from cell leakage is the inhibition of translation of DNA that inhibits the protein synthesis directly (Liu et al., 2000). Among all the proposed mechanisms of antibacterial effect of chitosan, the best accepted is the interaction between the positively charged (cationic) chitosan and the negatively charged (anionic) bacterial cell membrane. The reason for this interaction is the presence of $-NH_2$ group and displacement of calcium ions that stabilize the microbial cell membrane. This electrostatic interaction results in disturbance of osmotic balance of the microorganisms and hydrolysis of microbial cell wall that results in leakage of intracellular materials such as electrolytes, nucleic acid, and glucose. Because this interaction mechanism that creates leakage is based on the number of amine groups, it again proves that increasing the number of amine groups increases the antibacterial activity of chitosan and its derivatives. In terms of comparison, chitosan is found to be more effective against gram-negative bacteria due to greater interaction between the positively charged chitosan and negatively charged outer lipopolysaccharide membrane of these bacterial, but this observational comparison remains controversial. Owing to the strong metal chelating activity of chitosan, the third mechanism is binding of certain essential metal ions around bacteria that inhibit some essential nutrient flow resulting in bacterial death. In terms of its molecular weight, low-molecular weight chitosan shows much better antibacterial effect (Goy et al., 2009; Raafat and Sahl, 2009; Andres et al., 2007). In a counterrevelation, Raafat et al. said that binding of chitosan with bacterial DNA is very unlikely because chitosan is unlikely to circumvent the cell wall and membrane and enters intracellular space (Raafat and Sahl, 2009). In another modification approach, a synthetic polyelectrolyte chitosan-γ-glutamic acid hydrogel displayed effective antimicrobial activity against *E. coli* and *S. aureus* (Tsao et al., 2010). Quaternization of chitosan also increases its antimicrobial property (Ng et al., 2014). A recent article reviewed in-depth mechanisms of antimicrobial activities of cationic polymers. Chen et al. reviewed that polycationic polymers displace calcium ions that stabilize the bacterial membrane. Loss of calcium ions results in loss of fluidity resulting in disruption in membrane structure (Chen et al., 2014). While visualizing antimicrobial effect for medical implants, chitosan and quaternary ammonium nanoparticles were used with pMMA bone cement. The result displayed additional and effective antimicrobial effect with no cytotoxic effects. But the major and critical adverse effect of quaternary ammonium cationic polymers is hemolysis. Thus, for further in vivo and clinical experiments, nonmodified simple cationic polymers can be chosen for their antimicrobial activities (Chen et al., 2014). In a very recent study using low-molecular weight (LMW) water-soluble β-chitosan, scientists have found a significant antibacterial action of these chitosans in patient-retrieved clinical drug susceptible bacterial strains. They compared the result with some positive control commercial antibiotics and β-chitosan was found to be better than some of these antibiotics. They also confirmed the membrane lytic effect in *E. coli*. The β-chitosan also displayed great antibacterial action in *P. aeruginosa*−infected mice models both symptomatically and histologically (Park et al., 2015). In a recent highly

elaborative review on antibacterial action of chitosan, it was reported that chitosan binds to the polyanionic teichoic acid of peptidoglycan layer of gram-positive bacteria through noncovalent interactions. The teichoic acid component of cell wall is very important for cell division, thus its blockage leads to cell death. In case of gram-negative bacteria, chitosan chelates the cations such as Ca^{2+} and Mg^{2+} that damage the cell wall integrity leading to cell damage. The second mechanism, as stated above, is the interaction with the anionic moieties of lipopolysaccharide layer of outer membrane resulting in cellular disruption and finally cellular leakage. Another mechanism suggestive of antibacterial activity of chitosan is the entry of chitosan in bacterial cells and binding with the bacterial DNA leading to inhibition of transcription and translation (Verlee et al., 2017). Thus, on the basis of cited examples, we can say that chitosan and certain derivatives have excellent antibacterial action, even of clinical and drug-resistant species.

14.4.2 Chitosan as antifungal polymer

Fungal contamination to agriculture products and human and animal infection has become a serious health threat globally. Though fungi are not becoming resistant as fast as bacteria, but we do not have a strong antifungal pipeline too. More than two decades back, Ghaouth et al. showed that chitosan was very effective in controlling the postharvest fungal growth. Chitosan was very effective against two tested fungi *Botrytis cinerea* and *Rhizopus stolonifer*. On one hand where chitosan resulted in cellular leakage of these fungi resulting in outflow of amino acid and proteins, it also displayed significant morphological change in *R. stolonifer*. They also demonstrated that the antifungal activity was positively correlated to the DDA of chitosan. Another important result from this experiment was the activation of fungal chitin deacetylase due to chitosan action that resulted in disturbance of fungal wall elasticity (El Ghaouth et al., 1992). Roller et al. also demonstrated that chitosan glutamate was very effective against *Zygosaccharomyces bailii* (at concentration of 0.1−0.4 g/L) and *Mucor racemosus* (at concentration of 1 g/L), whereas the strain that was found to be resistant was *Saccharomycodes ludwigii* that required a concentration of 5 g/L for complete deactivation. The experiment was conducted to see the effect of chitosan glutamate on common fungi associated with spoilage of apple juice (Roller and Covill, 1999). Almost a decade ago, Chien et al. tested two chitosan (low-molecular and high-molecular weight) with almost 94% DDA as a protective layer on a local citrus fruit to protect them postharvest against three species of fungi namely *Penicillium digitatum*, *Penicillium italicum*, *Botrydiplodia lecanidion*, and *B. cinerea*. They found that fruits coated with chitosan were in significantly better condition (less decayed) than the uncoated control fruits even after 42 days of storage of both. They even concluded that LMW chitosan was much effective against these fungi as compared with the high-molecular weight chitosan. The maximum fungal growth inhibition witnessed by them was around 90% (Chien and Chou, 2006). Tikohonov et al. also demonstrated that LMW chitosan and its succinoyl derivatives were highly effective against fungal species *Candida krusei* and *Fusarium oxysporum*. The results were highly impressive for

the fact that LMW chitosan significantly inhibited growth of fungal colony and spore germination at a concentration as low as 0.01% (w/v). A succinoyl substitution degree of 9% was most effective against all the bacteria and fungus tested in the experiment (Tikhonov et al., 2006). A similar highly effective antifungal activity of LMW chitosan was demonstrated by Park et al. (2008) a decade ago, with evidence of membrane disruption and leakage resulting in fungal cell death. In contrast to the effect of molecular weight discussed above, a contrasting r = finding was reported very recently by Gabriel et al. They reported that the antifungal effect of diethylaminoethyl chitosan (DEAE chitosan) against *Aspergillus flavus* increased with an increase in molecular weight with best activity at medium weight (200−500 kDa). The attachment of DEAE group also increased the water solubility of chitosan (Gabriel et al., 2015). In two seminal articles on the action of chitosan on fungi, Guerrero et al. demonstrated that chitosan permeabilizes the fungal cell membrane and causes leakage of the intracellular elements that result in fungal cell death. They demonstrated that entry of chitosan is energy (ATP) dependent and does not occur by simple endocytosis. They used the model organism *Neurospora crassa*. The team also demonstrated that chitosan lysed the fungal spores and that different parts of fungal growth (conidia, germ tubes, and hyphae) are affected differently by same chitosan. Another very significant finding by the team was that the cell membrane of chitosan-sensitive fungi had higher concentration of polyunsaturated fatty acids (primarily linolenic acid) than the chitosan-resistant fungi. They also came to a finding that attachment of chitosan to the negatively charged membrane phospholipids leads to loss of membrane fluidity that favors the entry of chitosan (Palma-Guerrero et al., 2009, 2010). In another recent report, quaternized chitosan derivatives were tested for their antifungal effect against *Microsporum gypseum*, *Trichophyton rubrum*, and *Trichophyton mentagrophyte* at a pH of 7.2. Quaternized chitosan displayed significant antifungal activity against the said species with minimum inhibitory concentration ranging between 125 and 1000 μg/mL and minimal fungicidal concentration between 500 and 4000 μg/mL. This group demonstrated that the antifungal activity of the quaternized chitosan tested increased with an increase in molecular weight, degree of quaternization, and presence of hydrophobic moiety against a few fungal species. All these fungal species were pathogenic to humans and animals (Sajomsang et al., 2012). In a very recent elaborative review of mechanism of action of chitosan and its derivatives, it was reported that the first step in antifungal action of chitosan is the entry of chitosan leading to cellular leakage. Once the chitosan enters the fungal cell, it can bind to DNA/RNA resulting in inhibition of replication, transcription, and translation (Verlee et al., 2017).

14.5 Antimicrobial polymeric gel

14.5.1 Antibiofouling agent

As stated in previous section, biofouling is referred to as undesirable attachment of microbes, organisms, and their cellular products on surfaces such as marine

equipment and medical implants that damage the devices and pose a serious threat to environment and health. The best sought after approach today is the coating of such susceptible devices with antibiofouling polymers or the polymers that resist the attachment of such potentially dangerous microbes, organisms, and their products. Several classes of polymers such as polysaccharides, polyacrylates, phospholipid mimics, and PEG have been tested for their effective antibiofouling effect. In a review to general characteristics of antibiofouling polymers, it was suggested that such polymers should be hydrophilic, electrically neutral and consist of hydrogen bond acceptors and not donors. Apart from the various polymers and derivatives given in previous section (see Section 14.3.4), unique synthetic peptides known as peptoids have been synthesized as antibiofouling polymers. Peptoids consist of N-substituted glycine oligomer attached to a short functional peptide chain responsible for surface adhesion. These are, thus, synthetic peptide mimics that have side chain substitution on the amide nitrogen rather than on the α-carbon atom. The first peptoid, PMP1 (peptidomimetic polymer), was synthesized with methoxyethyl side chains making the peptoid highly hydrophilic structural similarity to PEG. Because of high biocompatibility and low immunogenicity, peptoids have been proposed as coating for medical implants as well (Dalsin and Messersmith, 2005). Biocompatible hydrogels, whether or not having innate antimicrobial activity, can be loaded with known antimicrobials to form structures such as device coatings and injectable hydrogels that can release the antimicrobial compound in a controlled manner. Hydrogel properties can be tuned in a way that they degrade slowly and can release drugs continuously. Combining such known commercial antimicrobial drugs with polymers having innate antimicrobial activity can prove to be a double boon as an antibiofouling coating on medical devices (Gonzalez-Henriquez et al., 2017). Even if rational use and effective availability of antibiotics have significantly reduced the surgical site infection in orthopedic implant surgeries, periprosthetic joint infection can still have serious and often fatal implications. Implant-related infections are one of the most disturbing things for an orthopedic surgeon. Though the patients receiving an orthopedic implant are administered the required dose of antibiotic timely, the problem associated with the systemic administration of antibiotics is proper maintenance of time gap, low drug amount reaching the surgical joint, and development of biofilm. Therefore, to overcome such serious complications, the implant surface should be modified in a way to resist the microbial infection on the surface with minimal systemic antimicrobial administration. The surface coating should be highly biocompatible and can have very slow or no biodegradation (as per the requirement). At the same time, such antifouling coatings should not interfere with the restorative mechanism of osteointegration. Certain polymer coatings such as PEG and polymethacrylate can be used as surface coatings on metallic orthopedic implants. Incorporation of chitosan and silver (having innate antimicrobial activity) nanoparticles on the surface can be another excellent alternative to implant surface modification (Romanò et al., 2015). Some of the approaches in pretreatment of implant surfaces are antiadhesive coatings, innate antimicrobial coatings, and combination of antimicrobial polymers with antimicrobial drugs (Gallo et al., 2014). Polydimethylsiloxane (PDMS) has been a widely used material for biomedical

devices such as contact lenses, pacemaker capsules, implants, and catheters but suffer a lot from biofouling. In a recent review, several approaches are discussed to coat PDMS devices that could prevent biofouling. Some of these approaches are coating with polyethylene oxide (PEO) and PEG, polyzwitterion-based coatings, polysaccharide-based coatings, polyhydroxy polymer coatings (e.g., PVA), fluoro containing coatings, and amide containing coatings (e.g., PVP) (Zhang and Chiao, 2015). Epsilon-poly-L-lysine (EPL), a natural AMP produced by *Streptomyces albulus* displays a wide range and strong antimicrobial effect against both bacteria and fungi. EPL kills microbes by surface disruption, therefore, resistance against it difficult to develop. Recently, a group reported a synthetic hydrogel made from EPL-methacrylamide (EPL-MA) combination showing wide spectrum antimicrobial action. The combination hydrogel was highly biocompatible with negligible hemolytic activity. EPL-MA hydrogel was easily tunable to film form so that it can be used effectively on the surfaces of surgical implants (Zhou et al., 2011).

14.6 Conclusion and discussion

Polymers are highly useful chemical entities, especially for biological purposes. Those polymers that can for gel structures are even more useful because they are tunable to provide properties of interest. Hydrogels, water-absorbing hydrophilic polymer network, are highly useful in biomedical industry. They can be used for applications such as drug delivery, tissue engineering, biosensors, and medical implants. Certain polymers with antimicrobial properties are finding a wide range of applications in biomedical industry. Chitosan, the seminatural polysaccharide polymer, has excellent innate antimicrobial properties that make it an excellent choice for applications such as tissue engineering, injectable tunable hydrogels, implant materials, and more. Chitosan derivatives such as quaternized chitosan have also been tested for more efficient antimicrobial action. The antimicrobial action of polymers depends on their physical and chemical properties. On one hand where chitosan with high DDA has shown better antibacterial effect, modification with more positive charge on polymer chain renders it with much better antimicrobial effects. Polymeric hydrogels have been effectively used for their drug loading and dispensing actions. They can be provided a variety of shapes such as beads and films for different applications. Hydrogel coatings can provide an excellent option as coatings on surgical devices to resist biofouling on the implant surfaces. Such polymers can have innate antimicrobial activities or can encapsulate and release required drugs in a controlled manner (Rather et al., 2016). In terms of resisting marine biofouling too, hydrogel polymers have been highly beneficial. In marine environment, tunable polymers can not only resist microbes but also marine organisms that are responsible for fouling. Antimicrobial polymers can be highly effective in being a component or whole in the scaffold for tissue engineering too. Such tissue engineering scaffold seeded with required cells and molecular factors when implanted can effectively resist the accumulation and growth of microbes at the surgical implant site.

Therefore, availability of natural, seminatural, and synthetic antimicrobial polymers with considerable tunable property grant these polymers wide applicability, especially in biomedical field of research. Because the sources and synthesis of such polymers are limitless, these can become a boon for mankind.

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Polymeric gels for diagnostics applications

15

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15.1 Ultrasound polymeric gels

15.1.1 Basic principles and indications for use

Ultrasounds are widely used in medicine both in medical diagnostics and therapy. For ultrasound therapy to be effective, they must penetrate into tissues efficiently.

All materials, including tissues, exhibit acoustic impedance in relation to ultrasounds. This impedance depends, inter alia, on the density of the material (tissue) and the elasticity of the tissue (material). Ultrasound transmission is maximized if the acoustic impedance of two centers is identical. In practice, however, this situation is very rare. In the case of medical use of ultrasounds, whether for therapy or medical diagnostics, ultrasounds are generated by a generator in the head that is in contact with the tissue. There is an air layer between the head and the tissue, which almost completely reflects ultrasounds. To reduce the reflection coefficient, coupling agents are used, which are generally hydrogels—Fig. 15.1.

Thus the coupling agents are substances that are intended to transmit ultrasound energy into tissues. The key parameters of coupling gels are acoustic properties, viscoelasticity, ease of use, air displacement, and price (Lutz and Buscarini, 2013).

Acoustic properties determine the extent to which ultrasound energy generated by the head gets weakened. The higher the ultrasound energy penetrates tissues, the better. Viscoelasticity, in turn, determines the amount of coupling agents to be used and the ease of use. The surface to which the head is applied is not smooth and coupling agents are supposed to give the proper slide. Due to the great popularity of both ultrasound diagnostics and therapy, the cost of coupling gels is of great importance (Klucinec, 1996).

15.1.2 Composition

Water, glycerine, or alcohol can be used as coupling agents. The typical composition of coupling hydrogels is presented in Table 15.1. These compounds, however, have adverse application properties and their acoustic, thermal, and viscoelastic properties are not conducive to using them.

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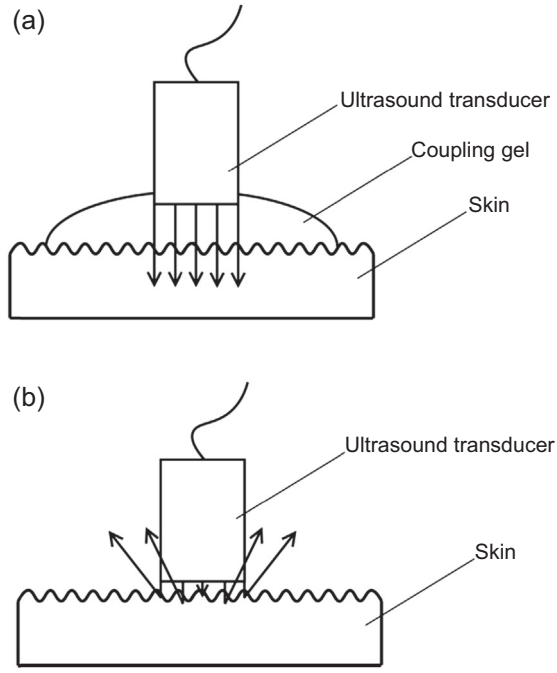

Figure 15.1 Using a gel, which has the acoustic impedance very close to water and tissue, causes drastically reducing reflection on the air−tissue interface (a). Without the coupling media, ultrasound beam strikes the air−tissue interface and almost the entire energy of the beam is reflected (b).

Table 15.1 The typical composition of coupling hydrogels

Composition for 500 g of gel	Amount (g)
Carbomer	10
Ethylenediaminetetraacetic acid	0.25
Propylene glycol	75
Trolamine	12.5
Water	up to 500.0

As a consequence, gels are used most often as coupling agents. A distinction can be made between hydrogels and lipogels (oleogels). Lipogels are usually composed of lipophilic substances such as mineral, synthetic, or plant oils. Their use is limited because they give the impression of viscosity on the skin and are difficult to remove. Hydrogels, on the other hand, do not have these disadvantages and can be easily removed from the skin without leaving any residue or greasy stains on clothes

(Lautenschläger, 2008). In addition, hydrogels, unlike lipogels, have no negative effect on the plastic or rubber parts of the head (transducer) (Lutz and Buscarini, 2013).

The procedure for preparing such a gel is as follows: dissolve ethylenediaminetetraacetic acid in 400 g of water and add propylene glycol. Then add the carbomer and mix with a high-speed stirrer to form a hydrogel without air bubbles. In the last step, make up to 500 g with water (Lutz and Buscarini, 2013).

Hydrogels for diagnostic purposes are designed for short contact with tissues and therefore belong to a group of products that can be defined as "rinse-off characteristics." Due to the high water content, hydrogels often contain preservatives such as MDGN (1,2-dibromo-2,4-dicyanobutane). However, preservatives, through the skin sonification, can bypass the skin barrier and cause undesirable effects.

Hydrogels as couplers have proven their effectiveness in numerous studies (Bosch et al., 2000). Hydrogels, other than the most common ones, i.e., carbomer and propylene glycol, may also include polymers such as polyacrylamide (PAA). PAA-based ultrasonic hydrogels can contain a lot of water—even over 90%, which makes them easy and cheap to produce. In addition, their physicochemical properties, such as viscoelasticity, can be easily modified by changing the concentration of acrylamide monomer units. PAA is also characterized by very high biocompatibility (Prokop et al., 2003).

Despite the fact that the common ultrasound gels are not expensive, their cost and availability can pose challenges in developing countries. As a result, alternative solutions based on other polymers, most often of natural origin, are proposed. One such example is maize starch (Binkowski et al., 2014). Gels based on natural ingredients neither imply worse image quality nor they pose a threat in the context of skin irritation or risk of infection (Binkowski et al., 2014).

15.1.3 Viscoelastic and rheological properties

Viscoelasticity is the property that determines the resistance of the material to the applied force.

Viscoelastic properties are described by means of four main rheological parameters: G* (measures overall viscoelastic properties or "hardness"), G' (measures elastic properties), G'' (measures viscous properties), and tan δ (measures the ratio between viscous and elastic properties). The total energy needed to deform material using shear stress is referred to as G*, the "complex modulus." The energy fraction of G* stored by the gel during deformation and used to recover the original shape afterward is represented by G', the "storage/elastic modulus." G' measures the elastic behavior of a gel or to what extent it can recover its shape after shear deformation. The energy fraction of G* lost on shear deformation through internal friction is referred to as G'', the "loss/viscous modulus." Tan δ represents the material elasticity. It is a measure of the ratio of viscous to elastic components of G*, defined as $\tan\delta = G'/G''$. By means of tan δ, it is possible to determine whether the material is mainly elastic ($\tan\delta < 1$), exhibiting a gellike behavior (e.g., a block of gelatine), or whether it is mainly viscous ($\tan\delta > 1$), behaving more like a viscous liquid (e.g., honey) (Pierre et al., 2015).

The rheological properties of gels affect their practical application. Gels should not be too rigid because it would be difficult to take them out of the packaging or too

"fluid" as they would spontaneously emerge from the packaging and "spill" over tissues. In this case, the air would get between the head and the tissue, and it would reflect nearly 100% of ultrasounds. As a result, they would not reach the tissue.

Manufacturers of ultrasound gels do not declare a specific value of particular viscoelastic parameters. This parameter largely depends on the temperature and frequency of sonification. Only gels with lower and higher viscosity are distinguished. Gels with higher viscosity are more comfortable to use but are more prone to air bubbles, resulting in the formation of artifacts in ultrasound images (Poltawski and Watson, 2007).

15.1.4 Acoustic and thermal behavior

Ultrasound gels are expected to transmit ultrasounds to the greatest possible extent. Nevertheless, during the passage of ultrasound waves through the gel, many physical processes may interfere with their flow, thereby reducing its transmission properties. Suppression of ultrasound transmission may occur during ultrasound absorption, ultrasound energy conversion to heat, and diffusion in a heterogeneous gel (Poltawski and Watson, 2007).

Importantly, the same gel can differently inhibit the transmission of ultrasounds depending on their frequency. Thus, the comparison of the acoustic properties of ultrasound gels should include a relatively wide frequency range.

The acoustic impedance of human skin is about $1.5-1.7 \times 10^6$ kg/m s (Payne, 1991). In turn, the acoustic impedance of water (20°C) is 1.48×10^6 kg/m^2 s.

Due to the different composition of ultrasound gels, their acoustic impedance may vary. However, the acoustic impedance of gels is usually given in relation to the acoustic impedance of water. The following results have been obtained: Docker et al. (1982): 112% at 2 MHz, Reid and Cummings (1977): 123% at 870 kHz, Benson and McElnay (1988): 80% at 1.5 MHz and 98% at 3 MHz. Poltawski and Watson (2007) obtained the following results for the following gels: KY 108%, EMS 105%, Aquasonic 104%, JPM 100% Physiomed 98%, SKF 95% Biofreeze 89%. In turn, Casarotto et al. (2004) found that the acoustic barrier (106 kg/m^2 s) is 1.48 for water, 1.47 for gel, and 1.26 for mineral oil.

In summary, the range of acoustic impedance for the most commonly used gels is very close to the acoustic impedance of water and does not exceed ±15% of the acoustic impedance of water.

Ultrasounds cause heating of both the tissue and the coupling gel. The temperature increase during a standard diagnostic procedure is usually low. Merrick et al. (2002) found that during the skin sonification using a standard ultrasound gel at the frequency of 1 MHz/cm^2, power of 1.5 W/cm^2, and head surface of 5 cm, there was no statistically significant increase in the ultrasound gel temperature. In the study published in Draper et al. (1995), it was found that the temperature of the ultrasound gel increased by 3.2°C after applying sonication: 1 MHz frequency, power of 1.5 W/cm^2, sonification time of 10 min.

In turn, the studies published in Casarotto et al. (2004) indicated that the head temperature varies depending on the coupling medium used and the thickness of the medium layer. For the layer thickness of 0.3 mm, the increase in the head temperature

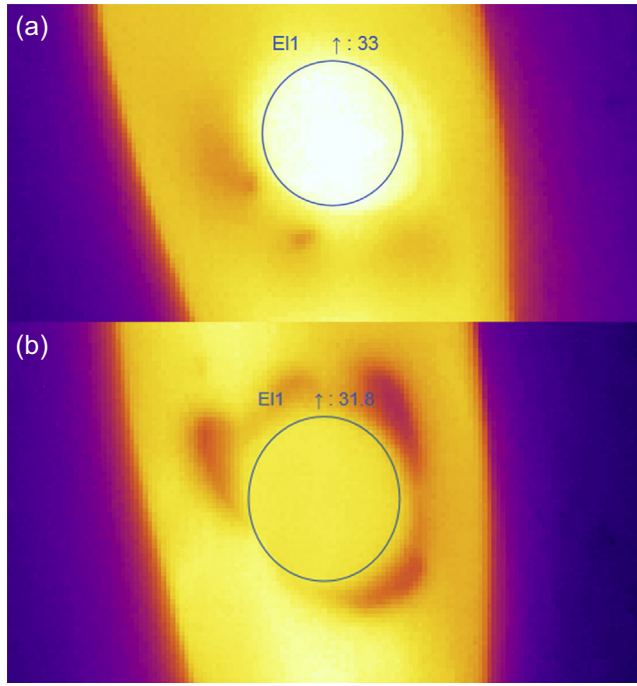

Figure 15.2 Skin temperature after sonification at two different coupling media: (a) water $\langle \text{ROI } T_{max} = 33°C \rangle$; (b) ultrasound gel $\langle \text{ROI } T_{max} = 31.8°C \rangle$.

was $10.54 \pm 0.05°C$ for water, $10.42 \pm 0.05°C$ for gel, and $13.03 \pm 0.05°C$ for mineral oil. In turn, for the coupling medium layer of 0.5 mm, the values were $11.19 \pm 0.05°C$, $11.06 \pm 0.05°C$, and $12.09 \pm 0.05°C$ for the water, gel, and mineral oil, respectively. Fig. 15.2 shows an exemplary difference in temperature induction with two different coupling media: water and gel. The highest temperature achieved in the oil medium can be explained by the lowest thermal conductivity. However, in the cited studies no gel temperature is indicated, so the results obtained cannot be translated directly into the thermal properties of the coupling media.

In conclusion, given the above data, it is to be assumed that the standard diagnostic procedure lasting 5 min does not cause a significant increase in the temperature of the coupling medium and thus will not negatively affect the skin surface.

15.1.5 Biocompatibility and side effects of application

Adverse skin reactions with ultrasound gels rarely occur and are mostly related to allergic contact dermatitis or contact urticarial (Villa et al., 1998; Gonzalo-Garijo et al., 2011; Chasset et al., 2016). In spite of the tremendous amount of procedures performed with ultrasound gels, only 14 cases of intolerance to them have been identified in the scientific literature so far (Chasset et al., 2016; Zlatanova and Mirzabekov, 2001). It should be noted that the most common components causing allergic reactions

are as follows: preservatives (primarily phenoxyethanol, parabens, and imidazolidinyl urea), propylene glycol, triethanolamine. In other cases, the gel component causing undesirable effects could not be identified.

In conclusion, ultrasound gels are characterized by excellent biocompatibility and safety. Scientific literature points to only isolated cases of intolerance to ultrasound gels manifested by allergic contact dermatitis or contact urticarial.

15.2 Hydrogel microarrays in molecular diagnostics

15.2.1 Basic principles and classification

Biochips are small platforms with spatially arrayed macromolecules (or pieces thereof) that enable to collect and analyze large amounts of biological information. The technology is based on specific molecular recognition interactions between the arrayed macromolecules and the test molecule. The interactions between the two complementary strands of a double-helical DNA molecule, between a single-stranded DNA stretch and the messenger RNA copied from it during transcription, between an antigen and an antibody, and between small ligands and their nucleic acid or protein partners are all examples of such recognition reactions (Zlatanova and Mirzabekov, 2001). These interactions require a favorable environment to occur, and hydrogels appear to be an ideal environment.

Hydrogel-based technologies designed for a range of biotechnology applications, including prenatal diagnostics (Lo et al., 2014), cardiac diseases (Moric-Janiszewska and Hibner, 2013), cancer diagnosis (Tiwari, 2012; D'Angelo et al., 2014; Darweesh et al., 2014; Minca et al., 2014), tissue engineering (Song et al., 2013; SanMartin et al., 2014; Jia et al., 2016), drug analysis (Hou et al., 2014; Tu et al., 2014; Meli et al., 2012; Levy, 2003), have developed to a great extent in recent years.

Due to their hydrophilic, biofriendly, and highly tunable nature, hydrogels are versatile materials, and thus they can be applied in this varied range of contexts.

Hydrogels are made of cross-linked hydrophilic polymer chains and can be easily functionalized with various biological entities, e.g., nucleic acids or proteins (D'Angelo et al., 2014). Therefore, hydrogels can be engineered to capture and detect clinically relevant analytes, including, but not limited to, proteins, DNA, mRNA, and microRNA. In addition, due to their solution-like environment, chemical tunability and nonfouling nature in biologically complex fluids (e.g., serum), hydrogels are ideal for diagnostic applications. To allow the diffusion and reaction of large biomolecules, the three-dimensional (3D) scaffold can be porosity-tuned, while remaining structurally stable under harsh mixing or flow conditions (Le Goff et al., 2015).

It is mainly the bulk structure of hydrogels that determines their suitability as biomedical materials and their performance in a particular application. The network structure of hydrogels is characterized by parameters such as the polymer volume fraction in the swollen state, the molecular weight of the polymer chain between two neighboring cross-linking points and the corresponding mesh size. The polymer

volume fraction in the swollen state is a measure of the amount of fluid imbibed and retained by the hydrogel. The molecular weight between two consecutive cross-links, being either chemical or physical in nature, is a measure of the degree of cross-linking of the polymer. It is noteworthy that because of the random nature of the polymerization process itself, only average values can be calculated. The correlation length or distance between two adjacent cross-links, n, is a measure of the space available between macromolecular chains (e.g., for drug diffusion). However, it can be also reported as an average value. It is possible to determine these interrelated parameters theoretically or by means of various experimental techniques (Peppas et al., 2006).

Numerous hydrogel chemical compositions have been explored for DNA, RNA, or protein microarrays, in particular polyethylene glycol (PEG) (Gasparian et al., 2015; Lin et al., 2014; Zasedateleva et al., 2014; Roy et al., 2016), PAA (Narla and Sun, 2012; Gumuscu et al., 2015; Cheng et al., 2009), and alginate derivatives (Andersen et al., 2015; Ge et al., 2013). An emerging method allowing for cell immobilization involves their entrapment within sol−gel-derived silica materials (Wang et al., 2013; Jang et al., 2015; Ghafari and Hanley, 2012).

15.2.2 Hydrogel in microarray bioprinting

Bioprinting is particularly popular in drug analysis. Drug analysis is a long-term and costly process. To identify promising therapeutic agents, their effects on human cells must be determined. Traditionally, it is done with the use of laboratory animals or in 96-well monolayer culture plates. The solution that is particularly preferred, especially in earlier stages of research, involves using human immobilized cells. In spite of numerous advantages of such a solution, the conditions do not correspond to the real ones, and as a consequence false negative or false positive results are obtained. Therefore, methods and technologies that will solve this problem are sought. One such method is microarray bioprinting (Datar and Joshi, 2015; Xu et al., 2011). Other methods include, inter alia, 3D scaffolds (Lee and Cho, 2015; Li et al., 2014), and microbeads (Zem et al., 2006).

Microarray bioprinting consists in printing cells encapsulated in hydrogels in a spatially addressable manner by means of automated liquid dispensing robots such as microarray spotters (Datar and Joshi, 2015).

The factors determining the choice of an optimum hydrogel for bioprinting are as follows: gelation mechanism, compatibility with surface, porosity and interconnectivity, biocompatibility, and mechanical properties. Nevertheless, special attention should be paid to the gelling mechanism, which can affect all other parameters (Datar and Joshi, 2015; Li et al., 2016). Gelation can be induced, inter alia, by photopolymerization, biocatalysis, or pH change (Datar and Joshi, 2015).

Biocompatibility is the key factor. Scaffold materials must be compatible with the encapsulated cells and the recipient's body. Hydrogels have special characteristics in this respect. They have a spatial, 3D structure so they can consume large amounts of water, and a polymer network is expected to form from the physical junctions between hydrogel macromolecules (Li et al., 2016).

Porosity and interconnectivity have a major role in the growth of cells and tissues. Pore size, shape, and distribution directly affect cell adhesion to the scaffold. A large number of interconnected pores facilitate the access of oxygen and nutrients and facilitate the removal of metabolic products (Li et al., 2016).

Hydrogels used in microarray sensing can be divided into natural and synthetic ones. Natural hydrogels include collagen, fibrin, alginate and hyaluronic acid (HA), agarose, and chitosan (Peppas et al., 2006; Datar and Joshi, 2015). Among the synthetic hydrogels particularly popular are PEG, poly(hydroxyethyl methacrylate), poly(vinyl alcohol) (PVA), and PuraMatix (Peppas et al., 2006; Datar and Joshi, 2015). Natural components of hydrogels enable efficient growth of cells because they contain many cell-signaling domains present in the vivo extracellular matrix (ECM) (Peppas et al., 2006). However, they have unfavorable mechanical properties, and as a consequence, hydrogels that contain naturally occurring polymers such as HA and collagen have to be chemically premodified to improve their chemical properties (Datar and Joshi, 2015).

Collagen used in hydrogels has poor mechanical properties and usually requires improvement. Collagen type I is the most common, whereas type IV is less often used (Peppas et al., 2006; Datar and Joshi, 2015). The disadvantage of collagen is also its susceptibility to enzymatic degradation (Walters and Stegemann, 2014).

Fibrin belongs to the group of glycoproteins and has advantages and disadvantages similar to collagen, especially mechanical properties that can be improved by adding Ca^{2+} ions. Fibrin is also sensitive to proteolytic enzymes and should therefore be protected by the addition of inhibitors such as aprotinin (Datar and Joshi, 2015).

Alginate is a linear polysaccharide. The cross-linking density of alginate gels is a function of the monomer units and the molecular weight of the polymer. Alginate gel degradation process is slow as the mechanical properties of gels are altered with time (Peppas et al., 2006). Alginate promotes efficient diffusion of nutrients and oxygen to cells (Datar and Joshi, 2015). Nevertheless, it can cause an inflammatory response in the presence of immune system cells, which limits its application (Datar and Joshi, 2015).

Agarose is a polysaccharide derived from marine algae. It has similar properties and application to alginate. Its main advantage is low toxicity and high biocompatibility (Peppas et al., 2006).

HA, a naturally occurring glycosaminoglycan and one of the main components of the ECM, is getting more and more common in biomedical applications because of its ability to serve as a blank slate and its biological activity. In particular, it is increasingly used to build hydrogel scaffolds for tissue engineering, which may in turn be applied for localized drug and DNA delivery purposes (Lam et al., 2014).

Chitosan is an amino polysaccharide copolymer of 1,4-D-glucosamines and *N*-acetyl glucosamines derived from chitin (Debnath et al., 2015). Chitosan is a natural cationic polymer that is capable of binding negative charged siRNAs through an electrostatic interaction to form complexes spontaneously in slightly acidic aqueous milieu. It has been subjected to detailed testing to check its drug, plasmid, and siRNA delivery ability. Due to its low toxicity, low immunogenicity, low cost,

and good biocompatibility, it has been considered as a suitable transfection vehicle (Ma et al., 2014).

Hydrogels based on synthetic components such as PEG or PVA are nontoxic, nonimmunogenic and have a Food and Drug Administration approval.

PEG is one of the most commonly used components of hydrogels in microarrays because it is easily dissolved in water, nontoxic, and nondegradable under the influence of cellular enzymes. In addition, PEG gelation is easy to control—most often by UV irradiation or a simple redox reaction (Datar and Joshi, 2015). The physical properties of PEG-based hydrogels can be easily controlled by changing the molecular weight of the PEG molecules. Moreover, the transparent nature of PEG hydrogels makes them suitable for a variety of detection schemes in biosensing applications (Hong et al., 2017).

15.3 Electrophoresis gels

The enormous popularity of molecular electrophoretic techniques used in all life sciences and basic sciences requires the provision of current and complete methodological information, information on possible, and new applications.

Electrophoresis is used to separate and visualize such biomolecules as polypeptides and nucleic acids as well as smaller peptides. An example of Western blotting acrylamide gel is presented in Fig. 15.3. There are several types of electrophoresis, which differ in terms of the composition of electrophoresis gels, separation conditions, and applications.

Gel electrophoresis, unlike capillary electrophoresis, is carried out in a gel obtained by polymerization of selected substances. The gel acts as a molecular sieve, in which large molecules, having less freedom of movement in the gel structure, migrate more slowly than smaller molecules (Kuhr, 1990).

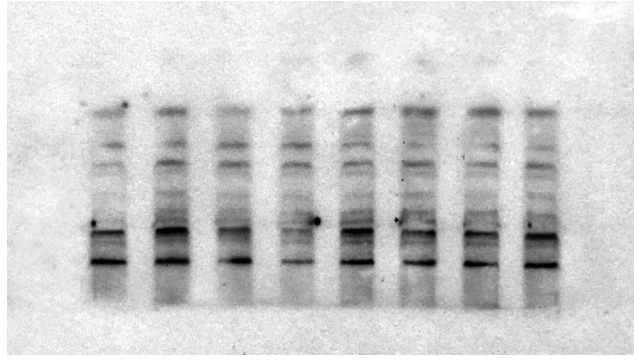

Figure 15.3 An example of Western blotting acrylamide gel—cPLA$_2$ protein distribution—cytosolic form of phospholipase A$_2$ protein. Gel composition: Arylacide/bis 30%T/2.67%C; 1.5 M Tris-HCL pH8.8; 0.5 M Tris-HCL pH 6.8; 10% sodium dodecyl sulfate; H_2O.

15.3.1 Basic principles of gel electrophoresis

Electrophoresis is an electrokinetic phenomenon in which some selected macromolecules, endowed with an unbalanced electric charge, move when exposed to an electric field. The movement of an electrically charged macromolecule is mainly dependent on its charge, shape, size, and environmental resistance (environmental viscosity). Using the aforementioned relationships, individual macromolecules can be separated quite fast with relatively simple equipment and at relatively low cost (Laemmli, 1970).

Electrophoretic separation can be carried out directly in electrolyte volume. Such a solution is often used in capillary electrophoresis or in electrophoretic media filled with a suitable electrolyte. In the latter case, the electrophoretic medium (blotting paper, cellulose nitrate, agarose, PAA, and others) not only stabilizes the electrolyte but also often contributes to better separation of macromolecules. In particular, the use of porous media (agarose, PAA), which exhibit molecular sieving properties, enhances the separation effect by additional fractionation (Buszewski et al., 2012).

15.3.2 Agarose and polyacrylamide matrices

Agarose and PAA gels are spatially cross-linked and although they occupy only about 0.5%−1.0% of the total volume of the medium (the rest is occupied by electrolytes), the porosity is comparable to the size of typical macroions, and as a result, the bigger ones are more resistant to movement in an electric field. The effect of separation resulting from the different mobility of macroions due to their size and hydration coincides with the effect of separation resulting from the resistance to motion with respect to the medium. Because both effects work in the same direction, separation of macroions in a porous medium is very good. Even better separation results can be obtained in porous media with increasing (along with migration distance) cross-linking density. Then we talk about gradient media (gels). In such media, large protein macromolecules or their complexes, as well as small peptides, are relatively well separated. Acrylamide enables to prepare media with very dense cross-linking and small pores, which allows for separation of protein macromolecules with masses of 5−300K or polynucleotides of 5−2000 base pairs. Agarose media are characterized by significantly poorer cross-linking and larger pores. This allows for the separation of much larger protein macromolecules, for example, multimeric molecules of Von Willebrand factor (1−10 M) or IgM immunoglobulin (1 M), and much larger fragments of nucleic acids (50−30,000 bp). When looking for a new type of media, it should be always remembered that it must be electrically neutral. The occurrence of the medium-related electric charge leads to uncontrolled interactions between macroions and this charge, followed by changes in the speed of their migration until they stop completely. At the same time, the existence of such an electric charge contributes to the migration of water molecules toward the cathode. This process is called electroendoosmosis. In general, the existence of a surface-related electric charge leads to a significant reduction in the resolution of electrophoresis (Viglasky, 2013; Arndt et al., 2012; Stellwagen, 2009; Voytas, 2001; Upcroft and Upcroft, 1993).

Agarose gels are prepared from agarose, a polysaccharide derived from agar. Agarose in commercial form is white or slightly yellowish powder. It dissolves very easily in boiling water and remains in liquid state up to about 40°C. Below this temperature, it solidifies into a porous gel. After solidification, it remains in this form even at elevated temperatures reaching several tens of degrees Celsius. At boiling point, it melts again. Porosity can be adjusted using agarose at various concentrations. The higher the concentration of agarose, the richer the cross-linking, and the finer the pores. Gels of agarose concentrations of about 0.4%−4.0% are typically prepared, and they are used in horizontal electrophoresis apparatuses. The advantage of agarose gels is the ease and speed of their preparation and the ability to separate large macromolecules. The disadvantage is their poor mechanical strength and the difficulty of fixing them after the separation. Dried gels fall apart under very small forces (Stellwagen, 2009; Voytas, 2001; Upcroft and Upcroft, 1993; Michalk et al., 2012).

PAA gels are prepared from a solution of acrylamide monomers and a cross-linker. It should always be remembered that monomeric acrylamide is a very potent neurotoxin, and even after the polymerization process, it presents a serious health risk due to free monomer residues in the gel volume. *N*,*N*′-methylbisacrylamide (bis-acrylamide) is most commonly used as the cross-linker. The polymerization reaction, which is essentially a free-radical polymerization reaction, can be initiated chemically or photochemically.

In the case of chemical initiation of the process, ammonium persulfate in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) catalyst is most commonly used. The photochemical initiation of the polymerization process takes place in the presence of riboflavin under longwave UV light exposure and is catalyzed by TEMED. Due to the release of significant amounts of heat during the polymerization of acrylamide, proper dosing of the initiators and catalysts must be followed so that the polymerization time is not shorter than 30 min. In specific cases when the acrylamide content exceeds 15%, effective dissipation of the resulting heat must be ensured by placing the cassette with the polymerization gel in the water bath. Cross-linking density and pore size can be adjusted by appropriate selection of acrylamide and bis-acrylamide concentration. The properties of the gel are described by two parameters. The total concentration of acrylamide is generally mentioned: T [%] = ((acrylamide + bis-acrylamide) [g]/volume [mL]) × 100. An additional parameter is the total weight ratio of the cross-linking agent to the sum of the acrylamide and the cross-linking agent: C [%] = (bis-acrylamide [g]/(acrylamide + bis-acrylamide) [g]) × 100. As the T value increases, the average pore size decreases. On the other hand, the minimum pore size, at the set T value, is obtained for C = 5%. Above and below this value, the pore size increases. If the subject of separation is a mixture of proteins with highly differentiated masses, it is advisable to use a gradient gel. It is a gel with growing, along with the distance of migration, cross-linking density. At its entrance the pore sizes are large and allow for migration of both large and much smaller macromolecules. In this area, large macromolecules are separated from small ones and there occurs differentiation of motility of large macromolecules. As the migration distance increases, cross-linking increases and pore sizes decrease. Large macromolecules no longer enter this area and further

separation of medium and small molecules occurs. With a further increase in cross-linking density, the pore sizes become very small and allow for the migration and separation of only the smallest macromolecules (Upcroft and Upcroft, 1993; Michalk et al., 2012; Westermeier, 2011; Gallagher, 2012).

15.3.3 Polymeric gels for pulsed-field gel electrophoresis

Electrophoresis in the pulsed field is used to separate large DNA molecules—from 2104 to 107 bp or from 20 kb to 10 Mb. In this way, all chromosomes, e.g., yeast ones, can be separated using PAA gels (described earlier). The electric field is switched on and off in short intervals. When the electric field is on, the molecules migrate according to their size, and when it is off, they tend to relax and collapse into random loops. The time required for relaxation is directly proportional to the length of the molecule. Then the direction of the electric field is changed by 90 or 180 degrees in relation to the previous one. Longer molecules start to move more slowly than shorter ones. Repeated changes in the field direction gradually cause separation (Antonishyn et al., 2000; Price et al., 2002).

15.4 Allergy diagnostic gels in immunoassay tests

Allergic diseases include a group of diseases whose pathophysiology may refer to any component of nonspecific and specific immune responses. If an allergic disease is suspected, a number of tests should be undertaken to make the correct diagnosis and eliminate the allergens. The basic allergy tests include skin prick tests, patch tests, provocative tests, and blood tests, based on the determination of the level of IgE antibodies and the number of eosinophils. Their aim is to identify a substance that the patient's body reacts incorrectly to, in contrast to healthy people. In the first place, screening tests should be carried out, followed by specialist tests, e.g., using agarose gels, which are less accessible and more expensive (Cooper et al., 2003).

15.4.1 Evaluation of the complement system

A complement is a system of several dozen proteins that play an important role mainly in the nonspecific humoral immune response. Its main functions include participation in opsonization and removal of pathogens, histamine reaction, and subsequent inflammation. Activation of H1 receptor, which is responsible for the occurrence of hypersensitivity symptoms, plays a significant role in the allergic response of the body. The study of the alternative pathway of complement hemolytic activity (AH 50, alternative serum hemolytic assay) uses the radial immunodiffusion technique. The patient's serum is applied to an agarose gel containing chicken red blood cells. The alternative complement pathway, whose activity is proportional to the area of lysed blood cells, is activated (Bonilla et al., 2005).

Tests using the gradient of a chemotactic factor determine leukocyte chemotaxis. This method measures the spontaneous migration of nonpolarized leukocytes on the

agarose gel towards the chemotactic factor (fMLP—formyl-methionyl-leucyl-phenylalanine, C5a—complement component, cytokine—IL-8) (Dembińska-Kieć and Naskalski, 2010).

Taking into account the potential contribution of respiratory tract infections to inducing exacerbations of allergic reactions, including spastic changes and asthma, an additional examination should be a virological test. Rhinoviruses are identified through the use of polymerase chain reaction (PCR) using three primers directed to untranslated regions in the picornavirus genome, which were identified by the bioinformatic analysis of the sequence (Johnston et al., 1993). The genome of rhinoviruses is composed of a single strand of RNA, with a positive polarity, sized from 7.2 to 9.0 thousand base pairs. The virion of these viruses is composed of 60 protomers packed into an icosahedral structure with a diameter of about 27−30 nm. The isolated RNA is a template for the reverse transcription reaction. The amplification products are separated by electrophoresis in a 2% agarose gel. The identification is carried out both by comparing the size of the PCR product and sequencing. Agarose is a polysaccharide that is a polymer of galactose derivatives, obtained by purification from edible agar. Agarose is easily soluble in water, reversibly forming a gel at room temperature. The temperature of gel−sol transition (colloquially agarose melting) is higher than the solidification temperature. RNA electrophoresis in an agarose gel is a standard method for separating, identifying, or purifying RNA fragments. The advantages of this method include its simplicity and the possibility of direct localization of RNA fragments in the gel using an intercalating dye—ethidium bromide. Larger molecules migrate more slowly than small ones due to greater resistance to movement and greater difficulty in penetrating the pores of the gel that acts as a molecular sieve. The rate of migration is inversely proportional to the decimal logarithm of the number of base pairs. By increasing the agarose concentration, the rate of gel migration is slowed down (Dembińska-Kieć and Naskalski, 2010; Johnston et al., 1993).

Electrophoresis of serum proteins (proteinograms) is the basic laboratory test enabling the detection of dysproteinemia, providing answers to often difficult diagnostic problems, including allergic reactions (O'Connell et al., 2005). The level of total protein in the blood serum varies within the limits of 6.6−8.7 g/dL and may change depending on the human health. In addition, to quantitative changes in total protein, there may be changes in the proportions of individual proteins. The proteinogram usually allows to obtain six protein fractions. Among them, there is the largest homogeneous fraction of albumin and the remaining fractions representing globulins: α-1, α-2, β-1, β-2, and γ-globulin. Individual fractions consist of various specific proteins with similar charge and electrophoretic mobility, but with different structure and biological functions (Giot, 2010).

There are data indicating an increase in the activity and migration of neutrophils during allergic processes (bronchial asthma) (Bafadhel et al., 2012; Chung et al., 2014; Radeke et al., 2005). Agarose is impermeable to cells. Cells migrate toward a higher concentration of chemotactic factor introduced by diffusion into the agarose. A suspension of test cells is poured into suitably cut wells of a petri dish (nutrient medium with 0.75% agarose), chemoattractants into others. After 2.5-h incubation, at 37°C and in 5% CO_2i, the dishes are fixed with formalin. After removing the agarose

and staining the cells stuck to the glass, the range (in mm) of cell migration toward the chemotactic factor and spontaneous migration to the control medium is measured and the chemotactic index is calculated (Barkefors et al., 2008).

Type I allergy is characterized by an elevated level of IgE antibodies in the patient's serum. These antibodies are specific for a sensitizing antigen.

Measurement of the concentration of specific IgE antibodies in the blood is a standard allergy detection test that also allows for the assessment of the sensitization degree (Chung et al., 2014; Radeke et al., 2005; Barkefors et al., 2008).

15.5 Polymeric gels for endoscopic purposes

The first attempt to depict the interior of the human body, using a primitive endoscope, was described by Bozzini in 1805. The medical device he constructed was illuminated with a candle light reflected in the mirror and used to examine the interior of the urethra, bladder, and vagina (Berci and Forde, 2000).

In today's medicine, endoscopic examination, as an endoscopy of the body's pipelines without breaking the continuity of tissues, consists in inserting an endoscope probe inside the patient's body (part of the endoscope containing the optical fiber for illumination of the examined field, a fiber-optic scope—transmitting the image from the interior of the examined organ, and a suction channel used to introduce selected tools for both material collection, as well as for examination, and surgery) (Berci and Forde, 2000). Endoscopic examinations are divided into gastroscopy, bronchoscopy, colonoscopy, sigmoidoscopy, rectoscopy. In the case of endoscopy, local anesthesia, deep sedation with analgesia, or full anesthesia are used (depending on the diagnostic procedure). In the case of local anesthesia, surface anesthesia of the mucous membrane is used—the use of drugs in the form of aerosol or gel applied to the mucosa at the site of the examination. An example can be covering the inserted colonoscope, sigmoidoscope, or rectoscope with a gel layer containing an anesthetic substance. Some endoscopic gels also have antiseptic and antiadhesive effects. Their use allows for a smooth, sterile, and possibly painless way of placing an instrument or catheter into the patient's body cavities (Berci and Forde, 2000).

A lubricant based on hydroxyethylcellulose, propylene glycol, with an additional anesthetic effect (Modlin et al., 2004).

The gel contains a local anesthetic (lidocaine—2%) and antiseptic substance (chlorhexidine gluconate—0.25%). It is also often used for the insertion of catheters, intrauterine devices, nasopharyngeal tubes, as well as cystoscopy, hysteroscopy, proctoscopy, sigmoidoscopy, colonoscopy, and other diagnostic procedures.

Local anesthesia with an endoscopic gel makes the performed procedure as comfortable as possible for the patient. In the first phase of the gel effect, the patient may feel a slight tingling sensation. This sensation, however, is short-lived and ceases after the activation of local anesthetic substances. Typical composition of endoscopic gel is presented in Table 15.2.

The anesthetic effect of the gel lasts an average of 30 min. The most common form of endoscopic gels in a sterile, single use can be syringes with an applicator.

Table 15.2 The typical composition of endoscopic purposes gel

Composition for 100 g of gel	Amount (g)
Concentrated solution of chlorhexidine gluconate	0.25
Methylhydroxybenzoate	0.060
Propylhydroxybenzoate	0.025
Lidocaine hydrochloride (local anesthesia)	2.0
Hydroxyethylcellulose or propylene glycol	q.s.
Purified water	up to 100.00

q.s., quantum satis.

The prefilled syringes are available in various sizes, meeting the needs of specific clinical procedures and limiting the amount of unnecessary waste.

If it is necessary to reapply the gel (further diagnostics), it is recommended to maintain a 3-h interval between the next application of the gel and to prevent the buildup of lidocaine in the blood. The half-life of lidocaine in the blood is 3 hours.

The described example is a Class III medical product due to its basic lubrication function. Its anesthetic and antiseptic effect is auxiliary. The gel provides optimal hydration, local anesthesia reduces pain, and antiseptic properties reduce the risk of infection.

15.5.1 Gel based on hyaluronic acid

It is a sterile, transparent, high viscosity gel consisting of 100% pure HA. Due to its physicochemical character, it perfectly adheres to the surface of the tissues and walls of the cavity subjected to endoscopy, creating a barrier separating the tissues during the regeneration phase after the surgery at the site where there is a high risk of postoperative adhesions. Its use is indicated in the prevention of the formation of adhesions after surgical procedures in the abdominal cavity and pelvis (e.g., removal of fibroids, tubal surgery, endometriosis), after Caesarean deliveries, after the procedure of intrauterine adhesion removal, to prevent their reformation. The treatment gel remains in the application area for about a day and then is completely absorbed.

It is common knowledge that HA is a substance naturally occurring in the human body, which when administered in a low-molecular weight form (it penetrates into the large intestine through the mucosal layer of the mucous membrane) fills intercellular spaces, thus preventing the leukocytes from penetrating into the intracellular space of the intestinal wall and their embedding in the mucous membrane layer. By limiting the presence of leukocytes (white blood cells) in the intestinal mucosa, the pathologically occurring inflammatory processes are suppressed. The administration of low-molecular weight HA also reduces (during the healing process) the fibrogenic effect during the renewal of the intestinal wall, which limits the formation of scarred stenosis of the intestine. High-molecular weight HA after being applied in the form of an ingot

is embedded on the mucus layer covering the intestinal wall. Thus, the mucus layer that lines its walls is strengthened. In addition, high-molecular weight hyaluronan causes an increase in viscosity of the mucus, which in turn reduces the risk of toxin penetration through the intestinal wall. High-molecular weight HA also limits and blocks the movement of leukocytes directed to the damaged mucous membrane covering the wall of the large intestine, which cannot pass through the mucus layer strengthened with HA (Fiorino et al., 2014; Di Simone et al., 2012).

15.6 Conclusions

Polymeric gels have great potential in the future biomedical applications. Especially "smart" polymeric gels combined with new detection techniques and methods can address new directions for target detections on gel particle arrays.

The development of polymeric gels is primarily due to the availability of new materials: new synthetic polymers and the modification of natural polymers. Thus, they can be "programmed" for optimal parameters: density, viscosity, pore size, etc.

In turn, polymeric gels used in ultrasonography or endoscopy will become more and more biocompatible and multifunctional.

Incorporation nanomaterials and therapeutic agents into polymeric gels will provide new methods of drug delivery for medical applications.

With wide options for selecting suitable hydrogels, they provide great control over material properties and thus enable to selecting optimal solution on specific biomedical application.

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Rapid prototyping for polymeric gels

16

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16.1 Introduction to rapid prototyping

Rapid prototyping (RP), a process described by Charles Hull in 1986 is a means to join material layer-by-layer to form the desired model. Rapid prototype technology refers to a class of advanced manufacturing technologies based on an additive process of constructing complex geometry in a layer-by-layer fashion as per the computer program. A leading characteristics of RP is the free-form fabrication, i.e., in all the additive-based RP techniques, firstly the computer-aided design (CAD) information is made using computer-aided manufacturing (CAM) software, which is converted to an STL type file format. This format is basically a conversion of CAD data into a series of digital cross-sectional layers for having a polygonal representation of the surface of the geometry. The three-dimensional (3D) CAD model is automatically sliced by the use of native software. This sliced two-dimensional (2D) layers are then made as solid model with the help of various RP techniques Fig 16.1. The layers are then produced either by bonding of particles with help of laser beam or layer-by-layer photopolymerization or solidification of molted filaments, hence printing the solid model of the geometry as shown in the desktop screen (Gupta et al., 2018).

The 3D model can be either grafted manually, or, it can be developed in form of customized CAD model by data obtained through CT and MRI images. For the latter, the required implant area to be prototyped is scanned by CT or MRI, and the obtained data are then imported to CAD software. A biodegradable, absorptive, and biocompatible scaffold is prepared from the information being given to the RP system (Gupta et al., 2018).

RP technology helps in reduction of waste with sufficient accuracy in model development. A new RP technique for the fabrication of scaffold in tissue engineering was developed in the year 2000 at the Freiburg Materials Research Center. The geometry developed from this technique can either be a biomedical device that absolutely serves as implants to be placed at patient's tissue defect, tissue implant prostheses, scaffolds, or as tissue/organ microstructure. All of this design will be of desired dimensions and perfectly interconnected. The scaffold generated out of RP will be porous and resorbable, hence it can stimulate cell activity and induce tissue formation due to proper physical, biological, and mechanical cues. The scaffolds produced have a controlled porosity making it suitable for cell-seeding cues (Gupta et al., 2018).

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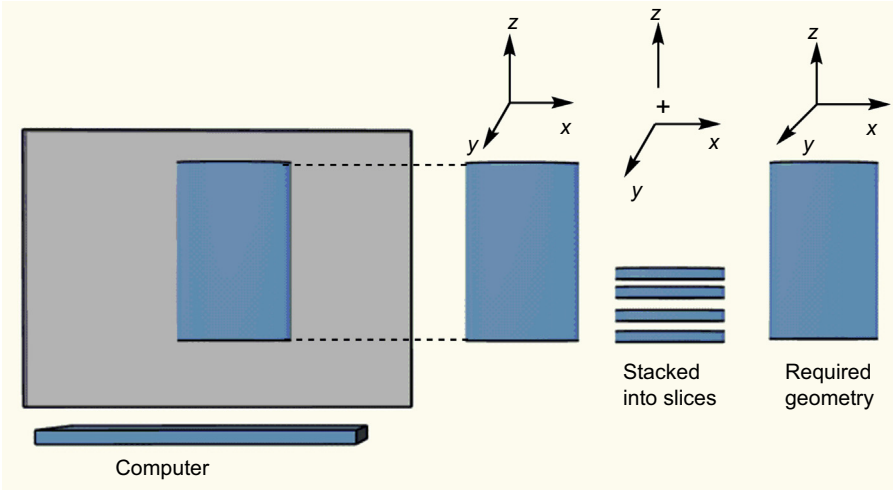

Figure 16.1 Functional principle of three-dimensional (3D) printing: The 3D model of the geometry to be printed is shown in PC screen, which is then stacked along different layers. Then superposition of 2D slices by layer-by-layer printing of series of layers along *z* axis to get desired 3D model (Gupta et al., 2018).

The conventional RP techniques mainly employ synthetic, ceramic, and natural biomaterials for the development of scaffolds that are used in tissue engineering. At present, hydrogel or bioink-based scaffolds are in recent demand as biomaterial for RP because it leads to the creation of geometry with defined shape and pore size. This RP of liquid-based ink will lead to a rapid advancement in computer-based tissue engineering and guided tissue repair, by RP of liquid-based ink containing components that are biologically active such as cells and growth factors (Cheah et al., 2003).

16.2 Rapid prototyping technique for polymeric gel printing

Recent advancement of RP technologies in the field of medicine has enabled the production of patient-specific biological substitutes (Fig. 16.2). This utilization of RP has rapidly developed the field of tissue engineering (Fig. 16.3). Tissue engineering aims to combine the cells, biomaterials, and biochemical and physiochemical growth factors to realize the repairing and regeneration of tissue-engineered constructs. There are a variety of contexts of tissue engineering.

16.2.1 Three-dimensional printing technology

The RP technique named as 3D printing technology is an additive process fundamentally assisted on CAD and CAM. A product having complex geometry is expected by

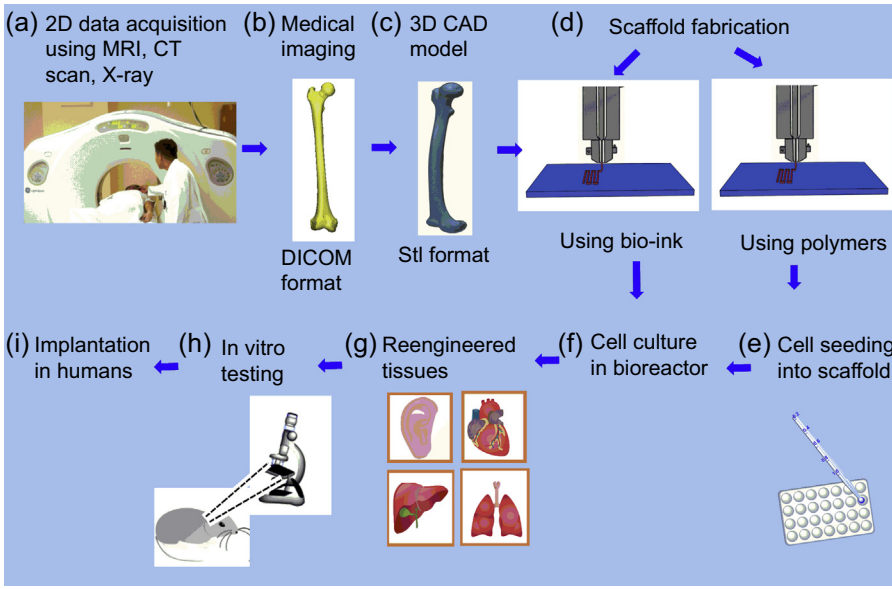

Figure 16.2 Step-by-step description of the three-dimensional (3D) bioprinting process: (a) acquiring two-dimensional (2D) data from noninvasive imaging techniques; (b) medical image in DICOM format; (c) converting DICOM format into (STL) format using MIMICS software; (d) scaffold fabrication using bioink and polymers; (e) seeding of polymer-based cells into scaffold; (f) culturing the cell seeded scaffold in bioreactor; (g) engineered tissue created; (h) In vitro testing in mice; (i) implantation in human being (Gupta et al., 2018).

the use of 3D printing as a rapid tooling and manufacturing unit. Various RP techniques (stereolithography [SLA], fused deposition molding [FDM], selective laser sintering [SLS]) are used to generate models of complex tissue structure. In the 3D printing technology, a 2D pattern is printed first having a certain thickness using suitable biomaterials and then the new layers formed are piled up over it successively. This additive process allows printing precisely architecture with controlled external shape, interconnectivity, and internal pore geometry. And with the advancement in medical imaging system, the 3D printing technology is capable to generate patient-specific implants of appropriate geometrical shape and size of the damaged part (Park et al., 2017).

These advantages enhanced the application of 3D printing technology in a variety of industries such as biomedical, aerospace, food, automotive, building, and construction industry (Attaran, 2017). Further the various technologies of 3D printing method based on the energy source and type of material used are highlighted.

16.2.1.1 Fused deposition modeling

FDM printing can be directly and indirectly used in RP. The direct RP applications use the FDM printed parts that are treated with cells directly. Meanwhile the indirect RP

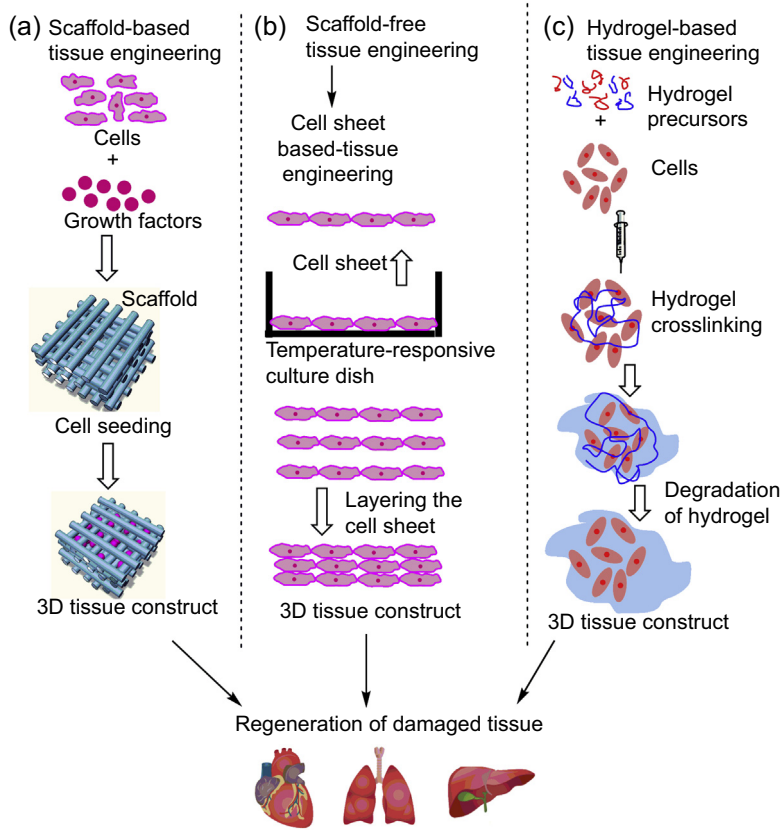

Figure 16.3 Overview of rapid prototyping in tissue engineering for polymeric gel printing three-dimensional (3D) tissue construct using (a) scaffold that mimics the ECM, (b) scaffold-free cell sheet technology, and (c) hydrogel-based ink.

application produces the mold from FDM-printed parts. FDM is a standard process to fabricate geometry using thermoplastics such as PVC, ABS, and PLA, as they have low melting temperature. The path in which nozzle deposits the binder is the layer-filling strategy involved in process such as FDM. The layer-filling strategy determines the bonding of build material between intralayer bonding and interlayer bonding. This bonding of materials affects the mechanical properties of the geometry produced. The shorter the path followed to deposit material, more will be the bonding between molten filament as less time is required by filament to solidify, whereas longer path take more deposit time and may lead to overlap or voids.

In FDM-based printing process, deposition process involves the build materials in the form of filament, which is exposed to heat and extruded from a temperature-controlled nozzle in a semiliquid state. This filament in semiliquid state is extruded in ultrathin layers from the extrusion head, which moves along x and y axes to print the 2D layer pattern of the object. Then the platform lowers along z axis to form

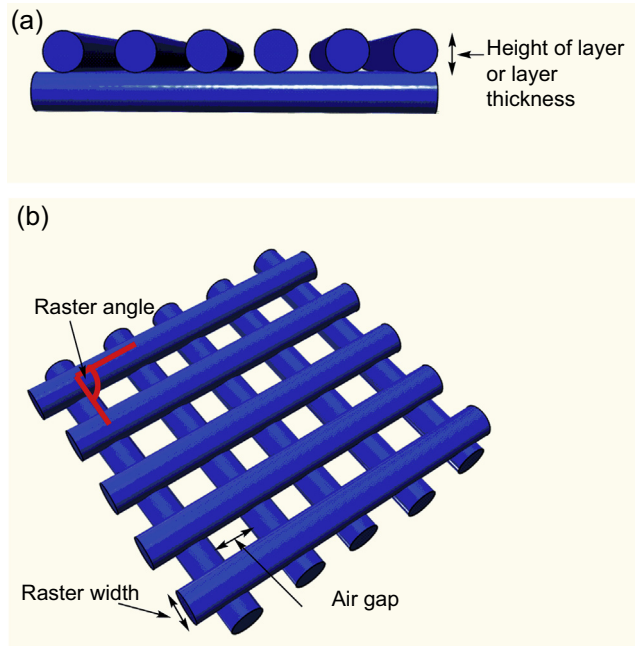

Figure 16.4 General structure of fused deposition molding—printed part showing the various process parameters: (a) layer of thickness, (b) raster orientation, raster width, and air gap.

the new layer, which is glued on to the top of previous layer. The build material is extruded, form layers successively, solidifies after cooling, and then fused together by bonding with adjoining material to form the 3D geometry desired. The deposition method used follows raster path as in Fig. 16.4. The number of direction change to build a rectangle of length L and breadth B, i.e., number of direction change for raster path can be calculated as (Kulkarni and Dutta, 1999):

$$N_{\text{raster}} = \sqrt{2}\left(\left(\frac{L}{W} - 1\right) + \left(\frac{B}{W} - 1\right)\right) \tag{16.1}$$

where W is the width of nozzle.

The quality of the out-coming printed parts are controlled by various printing parameters, such as thickness of layer, air gap, orientation of printing, raster width, and raster angle (Wu et al., 2015). These process parameters have a considerable effect on the final product. Raster width is the width of the deposited layer of pattern, whereas raster angle is the different angle at which the layer of pattern fills the inner portion. There is a particular relationship between raster angle and the mechanical properties of the printed parts. Till now more focus is emphasized only on theoretical relations between the process parameters. A proper mathematical relation or data driven modeling is still required.

These RP techniques allow the production of complex geometries with mechanically stable thermoplastics. Also it allows printing of different materials simultaneously with provision of multiple extrusion nozzle. But the printable material is only limited to thermoplastic polymers having suitable melt viscosity. High-viscosity material provides support to structure and low-viscosity material is desired for better extrusion. Hence, viscosity must be such so as to allow extrusion and also provide structural support. And sometimes it is difficult to have a uniform dispersion of the layers.

The rate at which the filament in solid form becomes fluidic in the melt flow channel is called as volumetric flow rate of material and also helps to define the inlet velocity of filament. During printing of scaffold, this parameter like volumetric flow rate of material to the extrusion head, extrusion head temperature affects the melt flow of filament. This polymeric filament melt is not in pure viscous liquid state and its property is referred as viscoelastic behavior or viscoelasticity (between ideal Newtonian [viscous] fluids and ideal Hookean [elastic] solids), hence power law for non-Newtonian fluid is used to model the flow behavior of semiliquid filament from FDM extruder (Ramanath et al., 2008):

$$\dot{\gamma} = \varphi \tau^m \tag{16.2}$$

where $\dot{\gamma}$, shear rate; φ, viscosity; m, flow exponent; τ, shear stress.

As polymeric melt does not behave as Newtonian fluid, its viscosity depends on shear rate and is not constant. When plotting the dependence of viscosity on shear rate in a log graph, at low shear rate viscosity it remains constant, which can be termed as zero-shear viscosity. And with increase in shear rate, viscosity increases and is referred as pseudo-plastic or shear-thinning behavior. For Newtonian fluid the relation between shear rate and shear stress is linear with slope of 1 whereas any deviation from this slope represents non-Newtonian behavior.

As shown in Fig. 16.5(b) the entire part of filament is divided into five zones, and there will a pressure drop for zone (ΔP_1 to ΔP_5), which can be calculated with the help of Eq. (16.2) by assuming the process in the melt flow channel to be a steady state isothermal process. And the pressure drop for different zone will be:

$$\Delta P_1 = 2L_1 \left(\frac{V}{\varphi}\right)^{\frac{1}{m}} \left(\frac{m+3}{r_1^{m+1}}\right)^{\frac{1}{m}} \exp\left[\propto \left(\frac{1}{T - T_o} - \frac{1}{T_\propto - T_o}\right)\right] \tag{16.3}$$

$$\Delta P_2 = 2L_2 \left(\frac{V}{\varphi}\right)^{\frac{1}{m}} \left(\frac{m+3}{r_1^{m+1}}\right)^{\frac{1}{m}} \exp\left[\propto \left(\frac{1}{T - T_o} - \frac{1}{T_\propto - T_o}\right)\right] \tag{16.4}$$

$$\Delta P_3 = 2L_3 \left(\frac{V}{\varphi}\right)^{\frac{1}{m}} \left(\frac{m+3}{r_1^{m+1}}\right)^{\frac{1}{m}} \exp\left[\propto \left(\frac{1}{T - T_o} - \frac{1}{T_\propto - T_o}\right)\right] \tag{16.5}$$

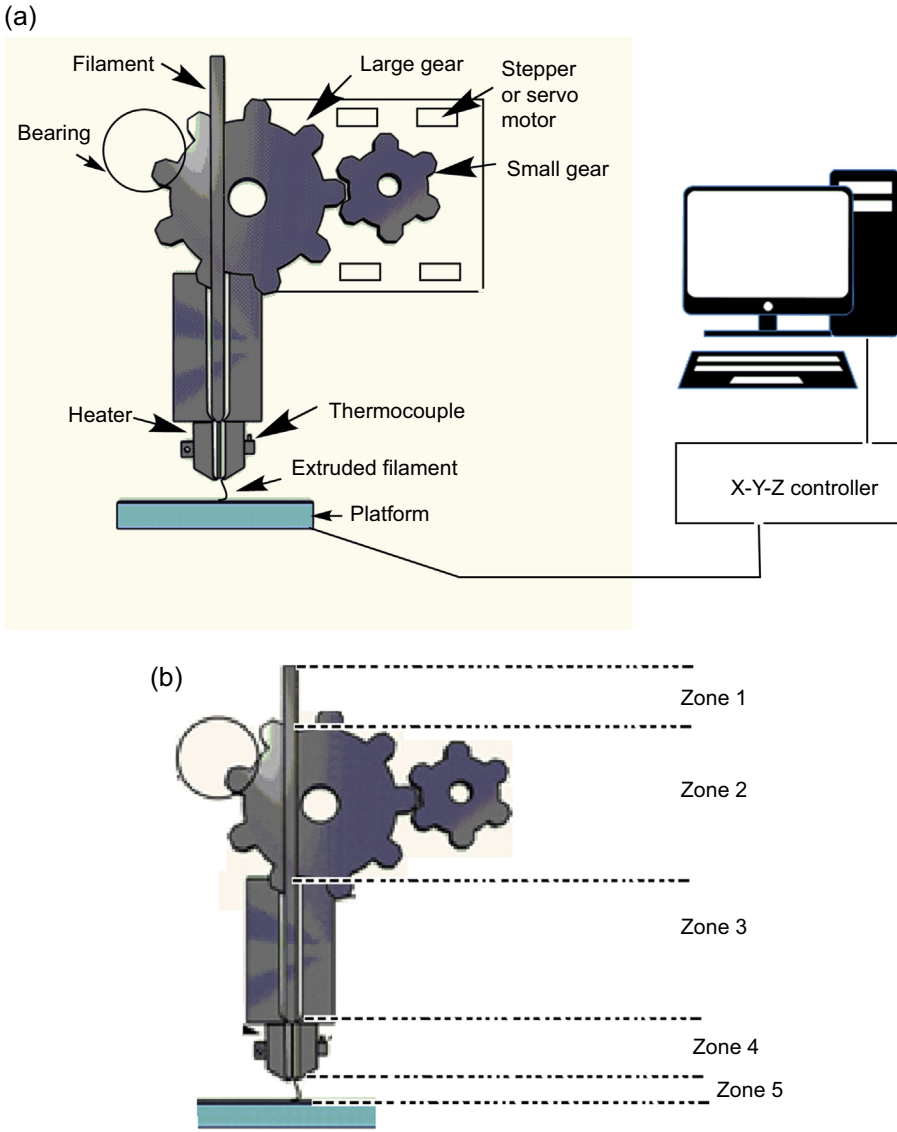

Figure 16.5 (a) Fabrication schematic of printing of three-dimensional structure by heating the thermoplastics in the form of filament using FDM technology (Gupta et al., 2018), (b) melt flow channel sectional view showing five zone areas.

$$\Delta P_4 = \frac{2m}{3\ tan\left(\frac{\propto}{2}\right)}\left(\frac{1}{r_2^{\frac{3}{m}}} - \frac{1}{r_1^{\frac{3}{m}}}\right)\left(\frac{V}{\varphi}\right)^{\frac{1}{m}}\left[r_1^2 2^{m+3}(m+3)\right]^{\frac{1}{m}} \exp\left[\propto\left(\frac{1}{T-T_o} - \frac{1}{T_\propto - T_o}\right)\right] \tag{16.6}$$

$$\Delta P_5 = 2L_5\left(\frac{V}{\varphi}\right)^{\frac{1}{m}}\left(\frac{r_1^2(m+3)}{r_2^{m+3}}\right)^{\frac{1}{m}}\exp\left[\propto\left(\frac{1}{T-T_o}-\frac{1}{T_\propto - T_o}\right)\right] \tag{16.7}$$

where $L_1 - L_3$ and L_5—length of filament in respective zones; $L_2 = \frac{\pi\left(R_2 + \frac{d_1}{2}\right)}{2}$, where R_2 is radius of channel at zone 2; r_1—radius of the cylindrical area of melt flow channel; r_2—exit radius; $\propto$—nozzle angle; φ—fluidity or viscosity; T—working temperature; M—flow exponent; $T_\propto$—temperature at which φ and m are calculated; V—filament velocity at entry; T_o—absolute temperature.

From above Eqs. (16.3)–(16.7), it can be seen that nozzle angle and diameter directly affects pressure drop occurring across the channel.

From this total pressure drop can be calculated, which will be:

$$\Delta P = \Delta P_1 + \Delta P_2 + \Delta P_3 + \Delta P_4 + \Delta P_5 \tag{16.8}$$

After knowing the total pressure ΔP, compression force (F) can be calculated as:

$$F = \Delta P \times A \tag{16.9}$$

where A—cross-sectional area of filament at entry.

Thus from Eq. (16.9), the force applied to push the filament can be calculated, this force influences the amount of extrusion of materials. Force calculation on filament through driver wheels helps to estimate the power of motor to push the filament. This force applied to filament is kept constant instead of pressure drop as FDM-based system lacks feedback system for pressure drop. As with change in pressure drop, the raster width of scaffold may vary, hence lowering the quality of the part printed.

16.2.1.2 Selective laser sintering

SLS 3D printing technique uses powdered material to print the desired geometry. A high-power laser beam having a controlled path determined by the computer scans the powder. On scanning the powder sinters due to heating. Due to molecular diffusion the neighboring powder fuses together. The processing of next layer again starts and the layer-by-layer printing out of powder starts. The remaining unbounded particles are then removed to get final product. The resolution of the printed part is determined by process parameters such as size of powder particle, laser power, and scan speed (Billiet et al., 2012).

The laser beam spot size (D), laser power (P), laser beam velocity (V), scan length line (L), and hatch spacing (HS) are the parameters that affect the intensity and mode of energy delivery to powder surface. The laser irradiation intensity is given as (Williams and Deckard, 1998):

$$P = \int_0^R I(r)\cdot 2\pi r dr \tag{16.10}$$

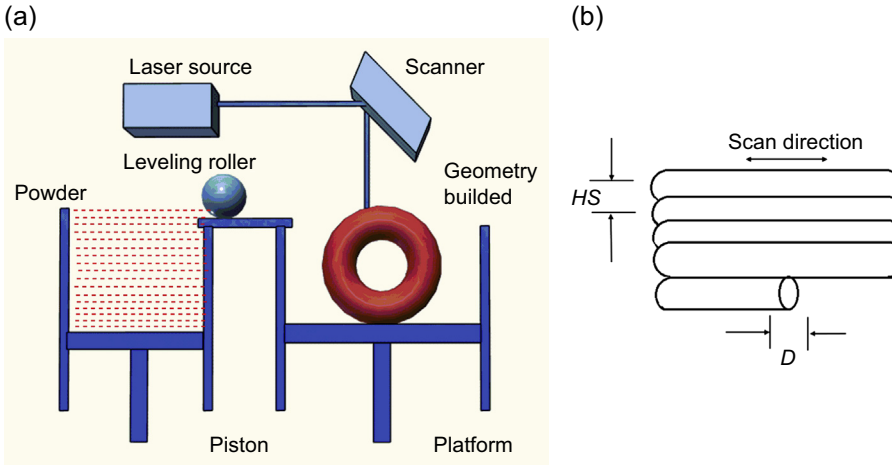

Figure 16.6 (a) Figure showing the use of laser beam and roller to form three-dimensional structures from the powdered form of polymer (Gupta et al., 2018). (b) laser scan pattern.

where $I(r)$—radial intensity distribution, r—radial distance from center of the spot.

The pulse duration is function of laser linear velocity and laser beam spot size. The period of exposure of laser irradiation is given as:

$$\tau = \frac{D}{V} \tag{16.11}$$

where τ—exposure period of laser irradiation, D—laser beam spot size, and V—laser beam velocity.

In SLS process a region is exposed to laser beam several times due to overlapping scanning pattern as shown in Fig. 16.6(b). The overlap, O, is related to the number of irradiation exposures by a point on the surface. And it is given as:

$$O = 1 - \frac{HS}{D} \tag{16.12}$$

where O—function of overlap, HS—hatch spacing, D—spot size.

On the powder surface due to overlapping scan lines, single point is exposed multiple times to laser irradiation. The cause of overlap is due to the less distance between HS and scan lines as compared to radius of laser beam i.e., $HS < R$. But as the energy at perimeter of laser beam is approximately zero, the total number of effective exposure is given as:

$$N_e = \frac{D}{HS} - 1 \tag{16.13}$$

Amount of energy that will be stored at surface will depend on time between exposures. But during this time between exposure, some amount of energy is lost in powder bed due to conduction and in surface due to radiation and convection. Thus a delay occurs between successive irradiation exposure, which is given as:

$$t_d = \frac{L}{V} \tag{16.14}$$

where t_d—delay period, L—scan line length, V—linear velocity of the laser beam.

During SLS process, for determining the energy stored at surface laser irradiation intensity (P), number of exposure (N_e), delay between exposure (t_d), and length of exposure (L) can be combined. As known, the mechanical property and geometry of the part produced decide response of the SLS-based printing process. And the geometry accuracy depends on the amount of energy delivered and time of energy to the surface of the part.

The energy carried on to powder bed is given by the general heat diffusion equation:

$$\rho C_p \frac{\partial T}{\partial t} = \nabla K \nabla T + A(x, y, z, t) \tag{16.15}$$

where $\nabla K \nabla T + A(x, y, z, t)$—energy storage, $\rho C_p \frac{\partial T}{\partial t}$—volumetric heat generation.

Boundary conditions are taken as:

$$-K\left(\frac{\partial T}{\partial z}\middle| z = 0\right) = \varepsilon_\theta \sigma \left(T_{z=0}^4 - T_{\text{sur}}^4\right) + h\left(T_{z=0} - T_{\text{env}}\right) \tag{16.16}$$

where ε_θ—effective emissivity, T_{env}—environmental temperature, T_{sur}—surrounding temperature.

The specific heat during melting phase is a function dependent on temperature within temperature range of 145−300°C such that,

$$C_p(T) = 932 + 2.28T \tag{16.17}$$

where T—temperature (K), $C_p(T)$—specific heat (J/kgK).

The effective thermal conductivity also depends on both density and conductivity as:

$$K = K_s(T) \cdot \frac{\rho}{\rho_s} \tag{16.18}$$

where $K_s(T)$—solid conductivity (W/mK) $= 0.0251 + 0.005T$, ρ—local powder density, ρ_s—solid density.

The rate of sintering that is assumed to follow general Arrhenius equation can be calculated from the rate of change of normalized height of a sample in powdered form.

$$K_s = A_s \cdot e^{\left(-\frac{E_s}{RT}\right)} \tag{16.19}$$

where A_s—pre-exponential factor, E_s—activation energy for sintering, R—gas constant, T—temperature (K).

This sintering rate helps to find rate changes in void fraction of bed-containing powder:

$$\frac{\partial \varepsilon}{\partial t} = -k_s \varepsilon \tag{16.20}$$

where ε—porosity of powder $= 1 - \frac{\rho}{\rho_s}$.

The two most important factors that influence the printing of geometry using SLS are the laser power and laser beam velocity and are related to sintering height, δ, as:

$$\delta\,(P) = A(P - P_{\text{thres}})^{\beta} \tag{16.21}$$

$$\delta\,(V) = C\left(\frac{1}{V} - \frac{1}{V_m}\right)^{D} \tag{16.22}$$

where V_m—maximum velocity after which sintering cannot occur, P_{thres}—minimum power to start sintering.

16.2.1.3 Stereolithography

SLA employs photopolymers as build material, which can be cured by UV laser. By tracing laser beam in a path defined by computer in the reservoir containing resins, it builds the object (Williams and Deckard, 1998). When the laser beam strikes the photopolymer it quickly solidifies, i.e., polymerizes into 2D structure due to curing reaction as shown in Fig. 16.7. For having a control over the final printed parts, it is necessary to consider the curing reactions occurring during polymerization (Liska et al., 2007).

On controlling the mirror position, entire layer can be cured at once, hence reducing production time (Gross et al., 2014). The thickness of the layer cured, i.e., depth where resin is cured to gel point (C_D) is given as:

$$C_D = D_p \; ln\left(\frac{E}{E_c}\right) \tag{16.23}$$

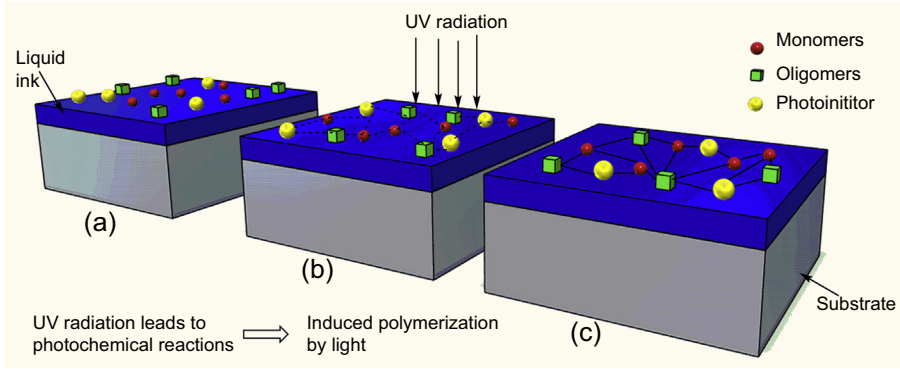

Figure 16.7 Figure explaining the curing reactions: (a) liquid ink applied to substrate; (b) the photoinitiators getting excited when exposed to UV radiation, hence passing energy to other components, stimulating bonding between molecules; (c) liquid resin get hardened and the final product is obtained.

where D_p—depth of light penetration (μm), E—light source intensity, E_c—resin's critical energy (mJ/cm^2) depends on concentrations of photoinitiator, dissolved oxygen.

The depth of polymerization due to the applied irradiation is adapted from Beer−Lambert equation, which states that there is exponential decay of intensity of light when passing through a medium in which it is absorbed. The absorbed light intensity I_a is obtained from a consideration of the Beer−Lambert law in the form (Andrzejewska, 2001):

$$I_a' = I_o - \ I_o e^{-\propto [A]D} \tag{16.24}$$

The plot of cured layer thickness versus applied irradiation dose (called as working plot) is plotted to find accurate SLA fabrication setting (Melchels et al., 2010).

The rate of photochemical initiation is given by:

$$R_i = 2\varphi I_a \tag{16.25}$$

where I_a—intensity of absorbed light (moles), φ—quantum field for initiation.

Under continuous illumination, monomers in the presence of free radicals start the radical chain polymerization whose equation for the polymerization rate can be given as (Odian, 2004):

$$R_p = \frac{k_p}{k_t^{0.5}}[M]\left(\frac{R_i}{2}\right)^{0.5} \tag{16.26}$$

where $[M]$—concentration of functional group, R_i—rate of initiation, k_p, k_t—propagation and termination coefficient, respectively.

The above equation shows that if the rate of initiation doubles, the rate of polymerization is increased by the factor $\sqrt{2}$:

$$R_p = \frac{k_p}{k_t^{0.5}}[M](\varphi I_a)^{0.5} \tag{16.27}$$

or

$$R_p = \frac{k_p}{k_t^{0.5}}[M]\left\{\varphi I_a\left(1 - e^{-\in [ln]b}\right)\right\}^{0.5} \tag{16.28}$$

where φ—quantum field for initiation, I_a—absorbed light intensity, I_o—incident light intensity, $\in$—extinction coefficient, $[ln]$—photoinitiator concentration, b—layer thickness.

After curing of layer, the movable stage at the reservoir lowers down by distance equal to the thickness of layer to print the subsequent layer, as shown in Fig. 16.8. During post-processing, the excess polymers are rinsed away, the parts printed from SLA has a high resolution. However, the application of SLA for scaffold manufacturing is limited due to the application of toxic resins and also the biomaterials are exposed to very high temperature, which limits its use.

Typical photopolymer materials used for SLA-based 3D printing are acrylic and epoxy resins, chemical structure of which is shown in Figs. 16.9 and 16.10.

Besides several advantages, there are some problems too that are associated with the 3D printing technology process. Seeding and penetration of cells is nonuniform throughout the rigid scaffold prepared. The scaffold generated is rigid, so it is not suitable for contractile tissues (heart) and also lacks the vascularization (Boland et al., 2003). Due to the abovementioned certain disadvantages of this traditional tissue engineering of seeding of cells into scaffolds, now there is emergence of a platform that does not use scaffolds, which are discussed in the following sections in detail.

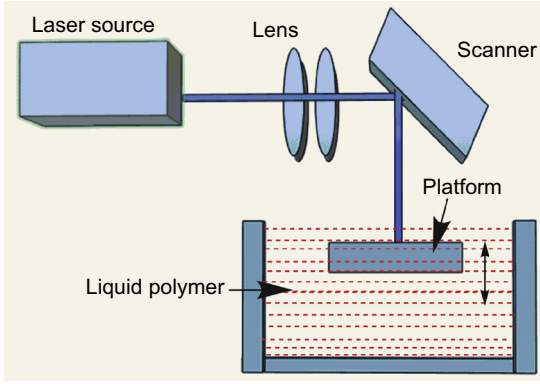

Figure 16.8 Figure showing the manufacturing of model using stereolithography technology (Gupta et al., 2018).

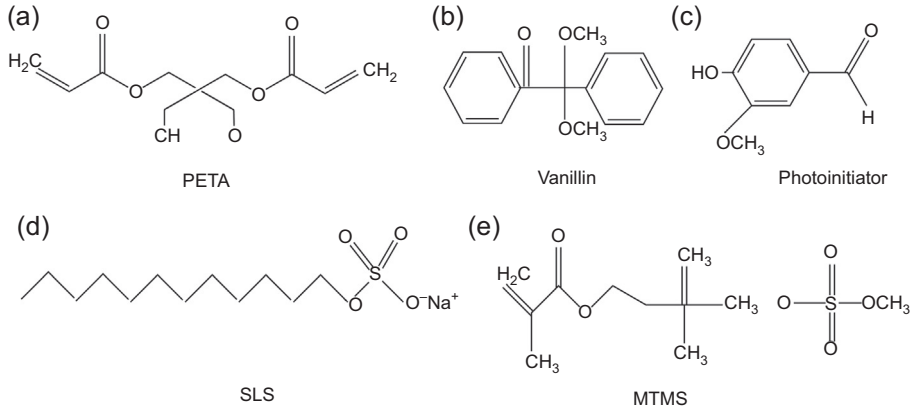

Figure 16.9 Chemical structure of stereolithography-based photopolymer acrylic resins. (a) PETA sub-unit, (b) Vanilin sub-unit, (c) Photo-initiator chain, (d) SLS sub-unit, and (e) MTMS sub-units.

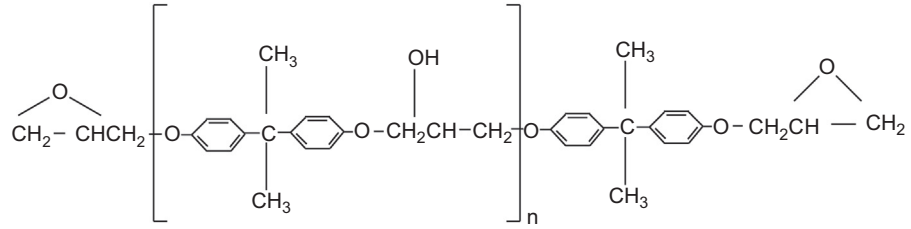

Figure 16.10 Chemical structure of stereolithography-based photopolymer epoxy resins.

16.2.2 Scaffold-free rapid prototyping

Over the last decades, a spectrum of researches based on tissue engineering technologies have evolved. The principal point of using scaffold-free technologies is to prepare a platform that does not requires cell seeding in a 3D support material, i.e., utilizing natural ability of cells to form tissues and respond to signals. In contrast to scaffold-based tissue engineering (TE), which consists of cells, signals, and scaffolds, scaffold-free TE consists of cells and signals. This possibility of development of relevant scaffold-free tissue is possible only with the ability of cells to self-organize on cell−cell interactions due to well-defined extracellular matrix (ECM) deposition. The scaffold-free tissue engineering approach involves techniques such as aggregate tissue engineering, self-organization technology such as cell sheet engineering, and self-assembly process. This technology does not involve the cell exposure to scaffolds and creates biomimetic microenvironment for communication between cells, resulting in increase in production of extracellular matrix. No consideration is required about the degradability of scaffold material after implantation, hence no release of toxic by-products. With scaffold-free RP technique, the dependence of tissue on cell proliferation on any support structure get eliminated and provide high cell density without

any support structures made up of biomaterials. This approach can be divided into two categories: self-organization and self-assembly. In self-organization approach some external forces are required as input to the system whereas self-assembly system employs the use of principle of free energy minimization. Self-assembly is a bottom-up tissue engineering technology that does not employ external forces. High-density cells are seeded in a nonadherent mold. Then cell adhesion receptors start binding due to minimization of free energy, which eventually results in the formation of ECM. Finally the tissue-specific matrix formed get matured to produce tissue (DuRaine et al., 2015).

16.2.2.1 Cell sheet technology

Cell sheet engineering, the self-organization category of scaffold-free approach, requires external energy to form a desired tissue structure. A temperature-responsive culture dish has hydrophilic surface at a temperature above 32°C and has hydrophobic surface at temperature below 32°C. Cultured cells are more likely to attach, proliferate on the hydrophobic surface. The cells when expanded for large duration, due to high confluence, a monolayer of cell sheet is formed. This sheet is then lifted out from the surface of the disc when sufficient ECM is developed. The cell sheet is further rolled or layered over molds. With further cell-to-cell contact, then cell-to-matrix contact, and then matrix-to-matrix contact leads to the formation of tissue. By stacking the monolayer cell sheet, multilayered tissue can be formed (Yamato and Okano, 2004). This scaffold-free tissue engineering technology has vast ability to promote tissue engineering−based research by constructing heart tissue (Masuda et al., 2008), myocardial tissue (Shimizu et al., 2003) (Fig. 16.11).

16.2.2.2 Aggregate tissue engineering

On application of rotational forces to cell in a suspension, cell aggregates can be formed in culture. As on application of external rotational forces aggregates are formed, it falls in the category of a self-organization technique. Process parameters

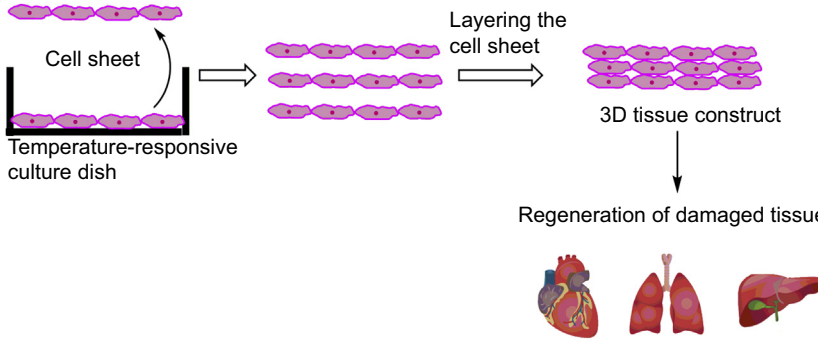

Figure 16.11 Representation of cell sheet−based scaffold-free rapid prototyping.

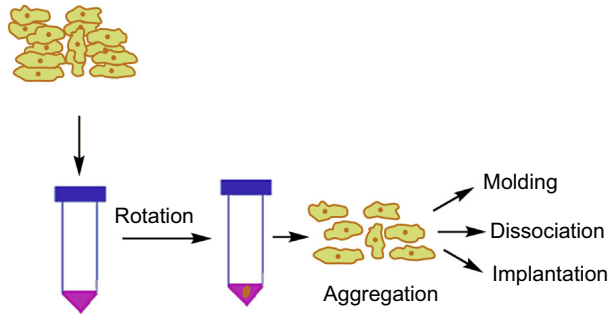

Figure 16.12 Diagrammatic representation of aggregate-based scaffold-free technology.

such as rotational speed and duration can be varied accordingly. The rotational motion leads to improvement in diffusion and also leads to exchange of nutrient/gas making it an appealing TE strategy (DuRaine et al., 2015) (Fig. 16.12).

16.2.3 Using bioink

3D bioprinting process of fabricating living construct uses an ink known as "bioink," which contains cells that are suspended in medium (Fig. 16.2). This is a recently introduced RP technique based on deposition of bioink into a biopaper. To obtain 3D tissue structure, bioink is deposited on biopaper at very low temperature (Billiet et al., 2012). The bioink is mostly derived from natural polymers such as gelatin, alginate, fibrin, or hyaluronic acid.

Biopaper is made up of biocompatible gel, e.g., collagen using a bioprinter Fig. 16.14. The gelation time of collagen is important for the smooth decomposition of spheroids. Also concentration of collagen must be properly tuned for proper fusion of spheroids. Biopaper can have different geometry depending on the type of target tissue to be printed.

Layer-by-layer printing of bioink leads to create a precise construct. After printing biopaper that acts as temporary support is removed (Masuda et al., 2008). Nowadays thermogels serve as biopaper, which are gels at temperature of 20°C, and at temperature above it they serve as biopaper into which tissue structures are printed. Dropping another layer of gel onto the already-printed surface could generate successive layers. One of the thermosensitive gels that can be used are poly [*N*-isopropylacrylamide-co-2-(*N*, *N*-dimethylamino)-ethyl acrylate] copolymer (Billiet et al., 2012). For printing scaffold, still there is need to develop bioink suitable for 3D organ and tissue printing.

16.2.4 Three-dimensional organ and tissue printing by rapid prototyping

For 3D organ and tissue printing, cell factors or cells termed as "bioink" is required to construct tissue structures. The advancement in the prevailing 3D printing in the field of tissue engineering leads to the creation of 3D living tissue/organ. The shortage of

donor organ can be overcome by a method called organ printing, the principles of RP technology. The computer-based deposition or printing of cells is done layer by layer. On completion of printing of desired structure, the gel can be eliminated by changing the temperature slightly. Nowadays bioink is prepared by encapsulating cells along with hydrogels. This 3D printing has the advantage of exact placement of cells. Various 3D tissue/organ printing techniques are available such as dispensing, droplet, and SLA developed to generate the microarchitecture with sufficient resolution. As per the prior description, medical image data are used to create biological design of organ/tissue. The different types of stem cells, biomaterials, and growth factors are selected. Then with the help of 3D printing system, the 3D tissue model is printed as per the code provided by computer containing all the printing strategies. Different types of techniques are available for the development of 3D tissue/organ. Each having advantages and disadvantages as per their need of biomaterials, printing speed, and resolution. This technique may be broadly classified into three main types as explained below (Boland et al., 2003).

16.2.4.1 Droplet technique

For the droplet-based printing technique, cell encapsulated hydrogels are developed. This formed hydrogel is then jetted in droplet form in predefined position on the substrate, allowing direct printing of cells in high resolution. Droplet techniques are mainly divided into three categories: pneumatic pressure-assisted, inkjet, and laser-assisted printing (Fig. 16.15). Inkjet bioprinting is mostly used in 3D tissue/organ printing. In inkjet-based technique, droplets can be produced using different forces such as thermal or piezoelectric forces and is then ejected from the nozzle head. In the thermal inkjet technique, a thin-film resistor is used as heating element. And on application of an electrical pulse at the head, through the resistor high current starts flowing, which makes the fluid in contact with it to vaporize, resulting in a formation of a vapor bubble over the resistor. This vapor bubble formed will then expand in the reservoir-containing fluid. And this leads to increase in pressure, which causes ejection of droplet from the nozzle. This technique maintains and uses a local heating temperature within the range of 200−300°C inside of the printing head for the production and ejection of the droplets by employing heater as shown in Fig. 16.13(a). Another technique called piezoelectric technique uses a piezo-crystal pulse actuator, which generates a voltage pulse that is either indirectly or directly in conjugation with fluid. Hence this volumetric change leads to creation of velocity/pressure change inside the

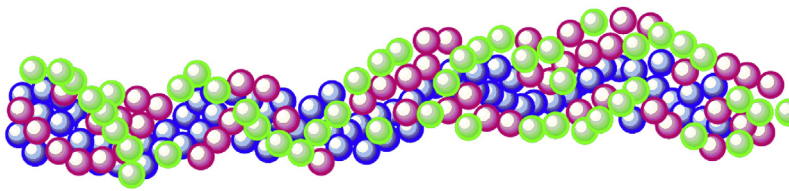

Figure 16.13 Figure showing bioink containing multicellular spheroids.

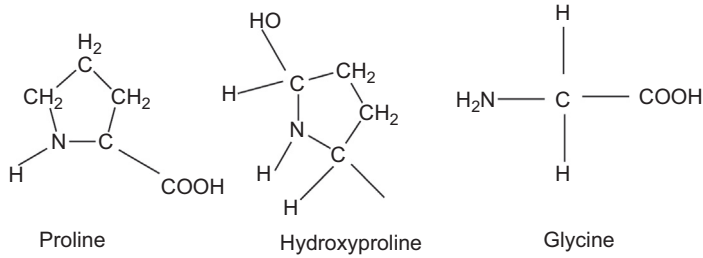

Figure 16.14 Chemical structure of collagen used as biopaper.

fluid, which is the cause of ejection of small droplets in continuous mode as in Fig. 16.13(b). In inkjet-based bioprinters, the biological materials containing living cells are used as ink. The pneumatic pressure-based printing technique consists of a set of electromechanical microvalves and droplets are produced by opening it under constant pneumatic pressure (Fig. 16.13(c)) (Murphy and Atala, 2014). Various types of liquid bioink can be used with this inkjet-based technique.

The desired property of the geometry required helps to find which droplet technique is to be used as each has its own printing speed, accuracy, and cost factor. Thermal-based droplet generation printers have higher printing speed as compared with piezoelectric inkjet printers but have the disadvantage of exposure of binder to thermal stress and even nonuniform droplet size. The binder is a material used to prevent spreading from nozzles. The binder concentration is also necessary for having desired precision in dimensions. For adjusting fluidic property of organic-based suspensions so as to be consistent with the printer head, viscosity must be 5−20 mPa s and surface tension must be 35−40 mJ/N (Shirazi et al., 2015). To have the above range, the ratio in Eq. (16.29) must have value between 1 and 10.

$$\frac{R_e}{\sqrt{W_e}} = \sqrt{\frac{\sigma \rho r}{\eta}} \tag{16.29}$$

where R_e—Reynolds number ($Vr\rho/\eta$), W_e—Weber number ($V^2 r\rho/\sigma$), ρ—ink density, η—viscosity, σ—surface tension, V—droplet velocity, r—droplet radius.

If ratio is too small, viscous forces become large, which requires high pressure for ejection; if it is too large, continuous column is ejected that can lead to the formation of satellite drops behind the main drop.

This droplet-based technique depends strongly on path of droplet, velocity, and initial size of droplet before spreading. Hence it is essential to have a control on these characteristics.

$$V_d = \pi r^2 \times \frac{V}{(2 \times f)} \tag{16.30}$$

where V_d—volume of droplet, r—radius of the nozzle, V—velocity of droplet, f—resonance frequency.

From the above equation, it can be inferred that with increase in V_d, velocity of droplet also increases, but the frequency of printer head movement decreases.

16.2.4.2 Laser-based bioprinting

Laser based bioprinting involves printing of donor layers over substrate layer by guided pulsed laser (Fig. 16.16). The donor layer comprises a layer transparent to laser radiation at top and a layer of bioink suspended at the bottom. The laser-assisted technique employs the use of principles of laser-induced forward transfer. The laser pulse

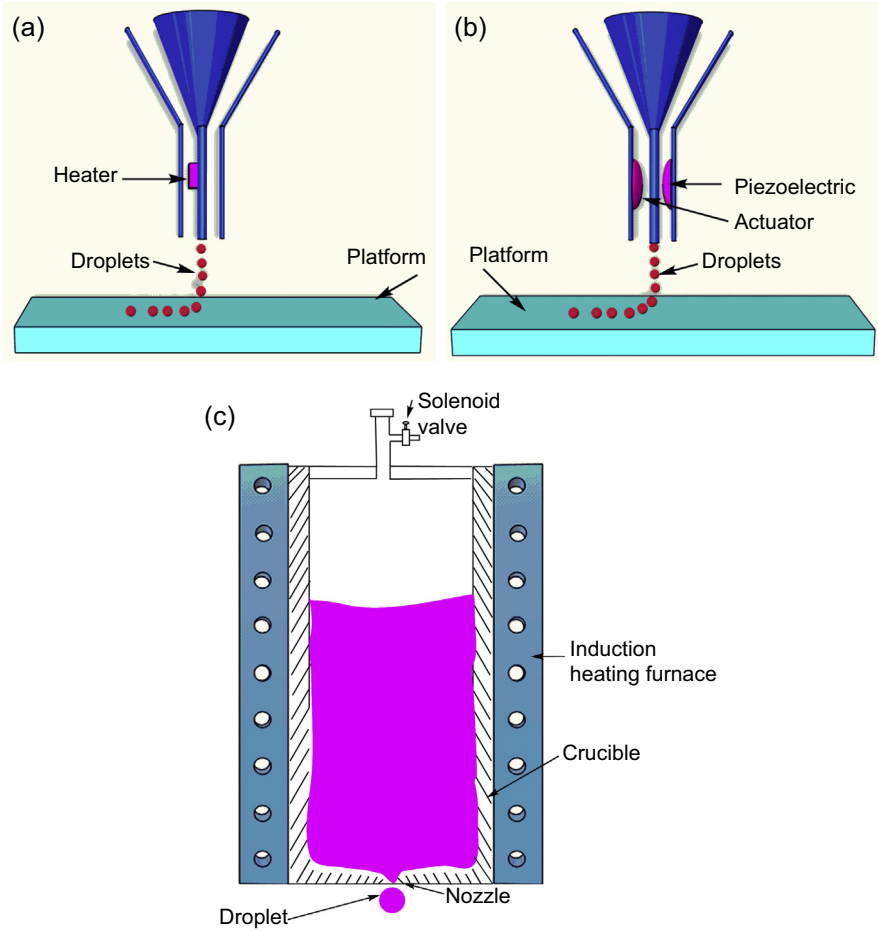

Figure 16.15 Diagrammatic representation of droplet-based 3D printing technique using (a) thermal, (b) piezoelectric actuator, and (c) pneumatic-based droplet generation.

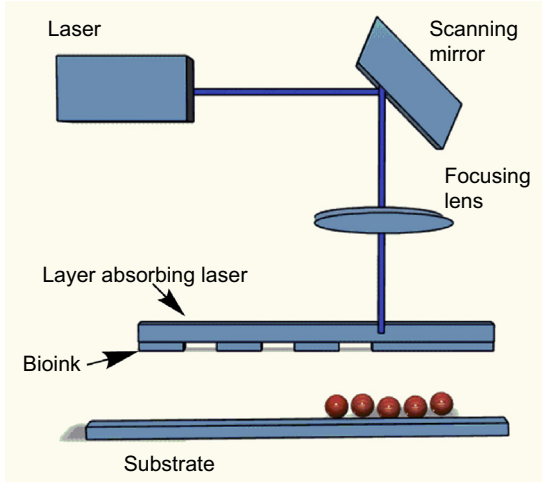

Figure 16.16 The laser-based droplet printing technique (Gupta et al., 2018).

focused stimulated a small area of the laser-absorbing layer, which leads to the creation of high-pressure bubble that further expands over free surface (Mandrycky et al., 2016).

It is possible to achieve very high resolution using droplet technique with low-viscosity biomaterial or bioink.

16.2.4.3 Dispensing technique

The dispensing-based technique prints the geometry by stacking the cross-sections of 2D layers, which are then consequently added to form the 3D object as shown in Fig. 16.1. The fluid-dispensing system consists of a platform, dispenser having heating controller, which moves accordingly in the x, y, and z axes. The cell-laden ink called bioink is dispensed from deposited system under computer control and results in formation of desired 3D geometry by precisely depositing the cells. More direct control on bioink is by use of piston-based extrusion, whereas screw-based extrusion system can result in large pressure drop across nozzle. To print microstructure using dispensing-based system, the types of dispenser commonly used are mechanical extruder (piston and screw) and pneumatic pressure dispenser. Out of which mechanical dispensing systems produce geometry with higher accuracy (Dababneh and Ozbolat, 2014). Pneumatic-based extrusion is basically a mechanical valve, which opens and closes on application of air pressure under regulation using a controller, but it is difficult to dispense small quantity of biomaterials because of residual air pressure. Mechanical extruder can have an accurate control on the volume of dispended biomaterial and also it allows extrusion of even highly viscous biomaterials. But it has a limitation that due to clearance between syringe and piston/screw, it suffers from leakage of biomaterials (Park et al., 2016) (Fig. 16.17).

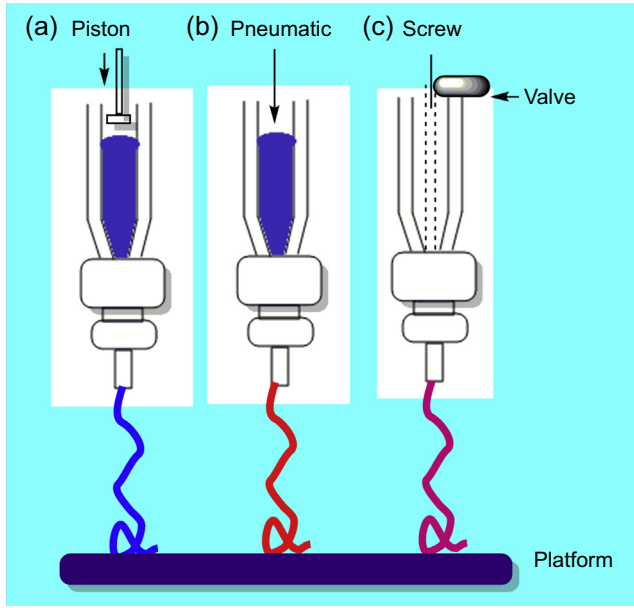

Figure 16.17 Figure showing printing technique using different types of dispensing systems: (a) piston based, (b) pneumatic based, and (c) screw based.

16.2.4.4 Stereolithography technique

One of the oldest 3D printing techniques is SLA, which is able to print geometry with very high resolution and accuracy. SLA employs photopolymers and through photopolymerization of liquid polymers as explained in Section 16.2.1.3 produces desired geometry as shown in Fig. 16.8. This photopolymerization can be induced either by single photon or two-photon absorptions, which are discussed further.

Single-photon–based SLA: This SLA-based technique is of two types depending on the type of mechanism to generate photon i.e., beam scanning and image projection. In beam-based scanning method, the focused laser beam scans 2D pattern and solidifies the liquid polymers in the path of laser beam. Whereas for the image-based projection method, a device such as digital micromirror generates a 2D pattern image. This image is then projected into the liquid photopolymer, and the 2D layer is created and solidified. This printing technology leads to reduction in printing time (Park et al., 2017).

Two-photon–based SLA: This process employs the use of two-photon absorption principle. The two photons are absorbed on same time and then it points toward the liquid. As it is generated at same time, it can act as single photon with double wavelength. This will lead to focusing of beam in a very small region (Park et al., 2016) (Fig. 16.18).

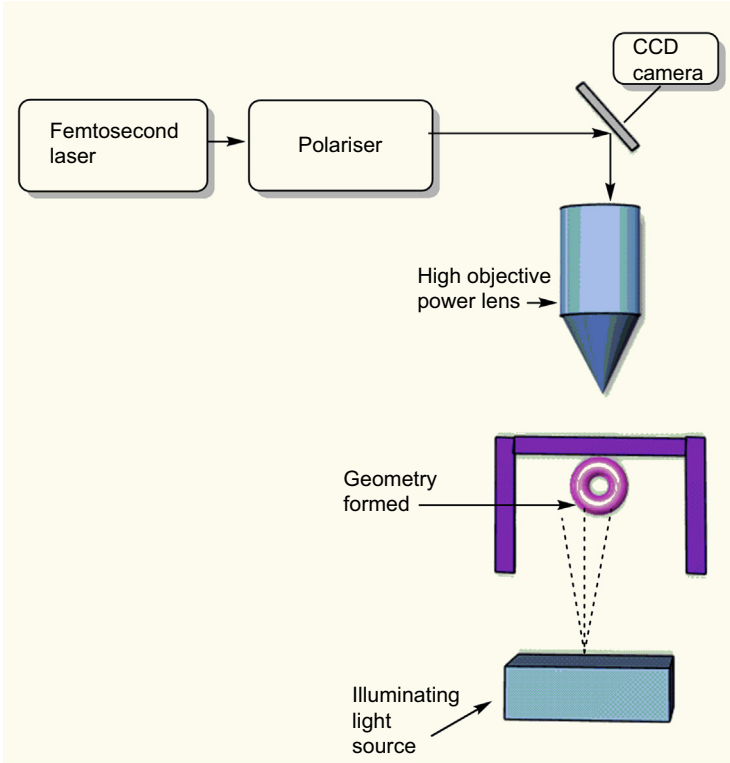

Figure 16.18 The use of two photons to allow focusing of beam in a very small region.

16.3 Constituents of polymeric gel–based printing

Many synthetic and natural materials can be prepared as hydrogel for making tissue-engineering–based scaffold.

16.3.1 Polymeric gel–forming materials

The polymeric gel for liquid base bioprinting is formed from natural or synthetic polymers. It can be classified as follows.

16.3.1.1 Synthetic materials

Various properties of biomaterials such as degradable linkages and structure molecular weight lead to the determination of dynamics of formation of gels, mechanical properties and degradation properties. Synthetic polymers possess an exciting property so as to be used in tissue engineering. Some of the synthetic materials used are PEO, PVA, and P(PF-co-EG). Out of which PEO, which is chemically similar to PEG

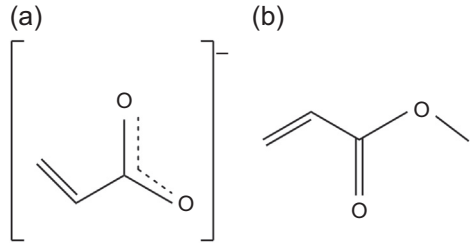

Figure 16.19 The chemical structure of (a) acrylates (b) methacrylates.

(poly (ethylene glycol)), is the most common polymer that can be used for various medical applications and even in tissue engineering. PEG and PEO are hydrophilic polymers that can be modified by cross-linking at each end with either acrylates or methacrylates whose chemical structure is shown in Fig. 16.19.

To make hydrogels out of this synthetic polymer, the modified PEO or PEG photocross-linked with acrylates or methacrylates is combined with photoinitiator (PI) and then cross-linked on exposure to UV. Also copolymers of PEO and PEG with poly (L-lactic acid) (PLLA) will lead to the formation of thermoreversible hydrogels. For the formation of PEO and PEG hydrogels that are degradable, a hydrolytically degradable poly (lactic acid) (PLA) and enzyme-specific cleavage sequences of oligopeptides are synthesized (Drury et al., 2004). PVA, a synthetic polymer can also be converted as hydrogel by having physical cross-linking with aqueous polymer solutions or can be chemically cross-linked by glutaraldehyde, adipoyl chloride, succinyl chloride, and sebacoyl chloride (whose structures are shown in Fig. 16.20) to form hydrogels (Drury and Mooney, 2003).

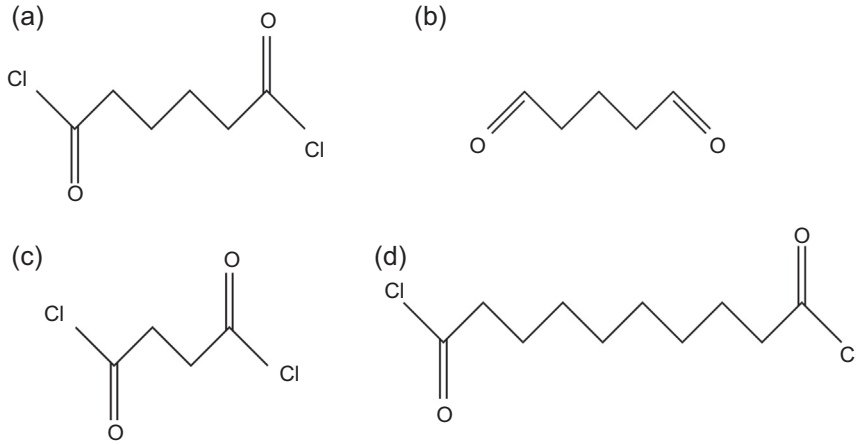

Figure 16.20 Chemical structure of (a) adipoyl chloride, (b) glutaraldehyde, (c) succinyl chloride, and (d) sebacoyl chloride.

16.3.1.2 Naturally derived materials

Naturally derived polymers are widely used in tissue engineering applications as they mimic the natural ECM. Some of them are collagens, hyaluronic acid (HA), alginate, and chitosan. Out of which HA, alginate, and chitosan are hydrophilic, linear polysaccharides. Due to all this reasons, it has a great chance to be used as hydrogel. Collagen has three polypeptide chains as shown in Fig. 16.14. These strands that are wrapped over one another can self-aggregate to form fibers. In addition, collagen can be used as hydrogel after chemically cross-linking it with glutaraldehyde, formaldehyde, carbodiimide, by physical cross-linking (may be freeze-drying, UV irradiation, and then mixing it with other polymers such as HA, PLA, PGA, PLGA, chitosan, or PEO). HA, a linear polysaccharide composed of a repeating disaccharide of (1−3) and (1−4)-linked b-D-glucuronic acid and *N*-acetyl-b-D-glucosamine units. Then for making HA hydrogels, it is covalently cross-linked with hydrazide derivatives by process of esterification and annealing. Next for using alginate (linear polysaccharide copolymer of (1−4)-linked b-D-mannuronic acid (M) and a-L-guluronic acid (G) monomers) as hydrogel, blocks of G monomers needs to interact with divalent cations (Ba^{2+}, Ca^{2+},or Sr^{2+}), which leads to formation of ionic bridges between the polymer chains (Drury et al., 2004; Drury and Mooney, 2003) (Fig. 16.21).

Chitosan (linear polysaccharide of (1−4)-linked D-glucosamine and *N*-acetyl-D-glucosamine) has a naturally occurring GAGs. Chitosan can be made as hydrogel by increasing the pH. It can also be converted into gel by chemically cross-linking with glutaraldehyde and physical cross-linking (UV irradiation, thermal variations).

16.3.2 Polymer matrix composites

Polymer materials are widely used in tissue engineering due to their low cost. They must have low melting point to allow the extrusion or be in liquid state to get solidified on exposure to UV. Instead of having many advantages, still there is a demand to increase its mechanical strength. For this, polymers are mixed with composite materials so as to enhance its mechanical properties with suitable flexibility. Various polymer-based composite materials are made as described below to meet the above-stated requirement (Shalin, 2012).

16.3.2.1 Particle-reinforced polymer composites

To form such composites, particles are mixed with polymers in an appropriate form (for SLS-based printing as powder, SLA as liquid, etc.). These particle reinforcement−based polymer composites have low cost; therefore, they are widely used (Kim et al., 2010). To enhance the property of polymer particles such as glass beads, copper or iron is used for enhancing tensile/storage modulus, aluminum and aluminum oxide(Al_2O_3) to improve wear resistance, and ceramic or tungsten particles for having good dielectric permittivity (Deligkaris et al., 2010).

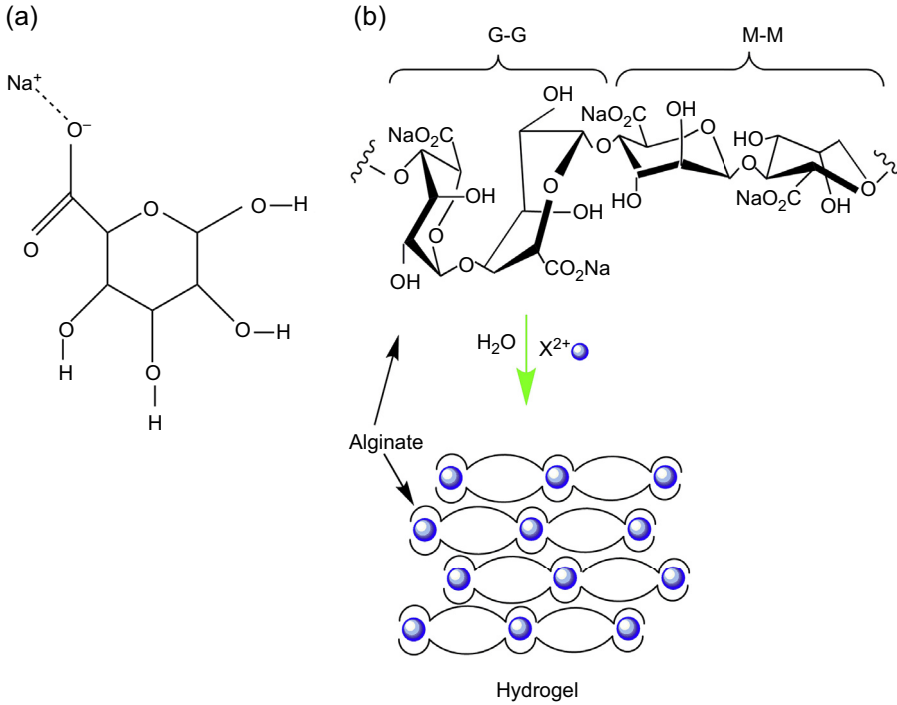

Figure 16.21 Figure showing (a) sequential distribution of M and G monomers that are then (b) interacted with divalent cations X^{2+} to form the ionic bridge between polymer chains resulting it to serve as hydrogel.

16.3.2.2 Fiber-reinforced polymer composites

Adding fiber too can significantly improve the properties of polymeric materials. Fiber-based printing is done mostly in FDM-based printing technique. For making fiber-reinforced polymer composites for FDM-based printing, firstly the fibers and pellets of polymer are mixed in a blender and then this mixture is passed to extruder so as to get fabricated in filament form, which is then used as build material in the FDM-based 3D printer. To increase mechanical properties, glass fibers and carbon fibers are commonly used as reinforcements. To determine the property of final printed parts, orientation of fibers is important (Ku et al., 2011). Also the fiber content must be low, as high fiber content may compromise with the porosity.

16.3.2.3 Nanocomposites

Nanomaterials have special properties of being lightweight and durable. Some of them possess unique electrical, thermal, and mechanical properties such as graphene, carbon

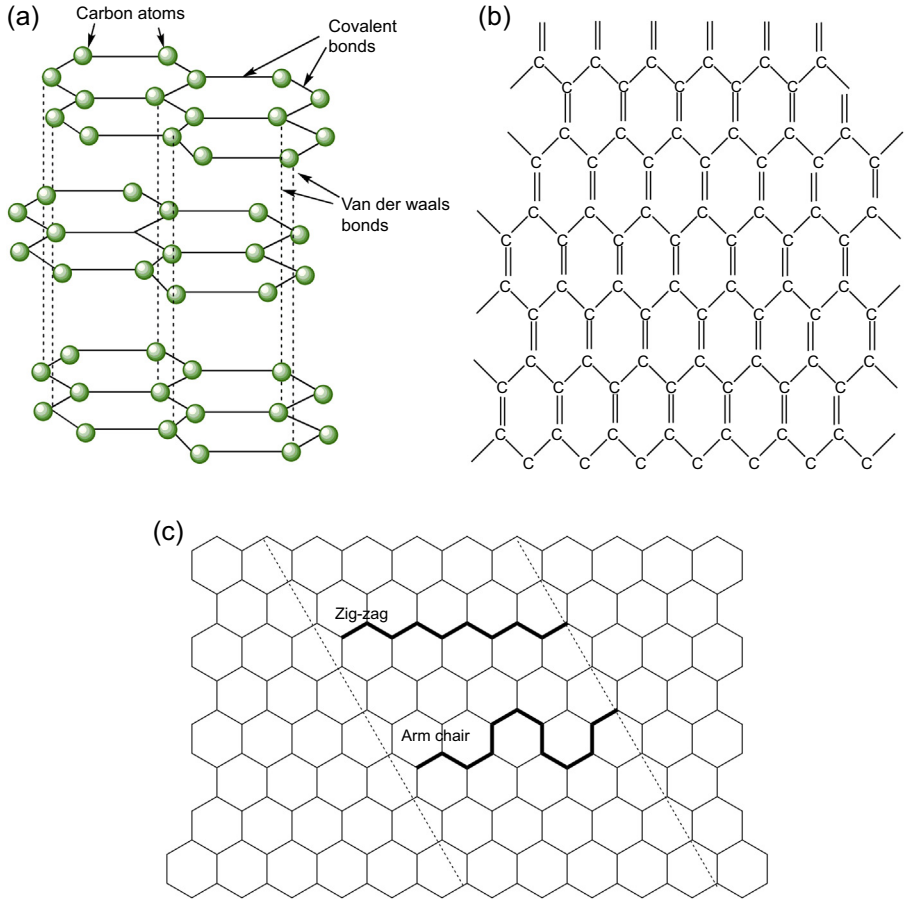

Figure 16.22 Chemical structure of different nanotubes: (a) graphene, (b) graphite, and (c) carbon nanotubes.

nanotube, graphite, metal, and ceramic nanoparticle. Hence the addition of such nanomaterials in a homogenous fashion into polymers could lead to the development of high-performance polymer composites (Kim et al., 2010; Yasmin et al., 2006) (Fig. 16.22).

16.3.3 Stimuli-response type hydrogels

Various stimuli are present in the body for example, ionic strength, pH, temperature, etc. This demands for the need of hydrogel that can be responded to such stimuli. Hence the following section explains in detail all such stimuli-sensitive hydrogels (Ahmed, 2015; Deligkaris et al., 2010).

16.3.3.1 pH-sensitive polymers

When polymer chains are bonded to acidic or basic functional groups for the preparation of hydrogels, the resultant hydrogel will then gain or lose the protons to respond to suitable pH with a change in ionic strength at aqueous media. This pH-responsive hydrogel possess desirable properties (both physical and chemical) at specific pH ranges. At high pH, acidic groups will release proton, whereas at low pH, basic groups with add protons. But in an aqueous solution, this adding or removal of ions to polymer may cause hydrogel swelling.

16.3.3.2 Thermoresponsive gels

With external temperature stimulus, these polymers undergo a phase transition. The external temperature stimulus dependence can be either positive or negative. The swelling of hydrogel will increase with positive temperature dependence and decrease for negative temperature dependence.

For negative temperature dependency, a temperature called lower critical solution temperature is considered, above which due to hydrophobic contents they collapse and below it due to hydrophilic content. With the increase in temperature, strength of hydrophobic interactions is strong whereas hydrogen bonding is weak. Due to breaking of hydrogen bond−based cross-links at high temperatures, shrinking in the hydrogel is caused.

16.3.3.3 Light- and chemical-responsive hydrogels

In this type of hydrogels, the activation process starts via light, hence making its use noninvasive and remote in areas of transport of molecular and cellular species. This hydrogel can be efficiently converted from gel to sol phase when irradiated with a light of suitable nm wavelength. And it can be recovered from sol to gel phase on irradiation with a light of different suitable nm wavelength controlled under mild condition. Examples for such hydrogels are deoxycholic acid−modified β-cyclodextrin derivative and an azobenzene-branched poly (acrylic acid) copolymer. The hydrogel was gelled at 350 nm and was able to recover on photo irradiation with light of 450 nm.

16.3.4 Polymer-based biomaterial for three-dimensional tissue printing

Due to biocompatibility, chemical and biological properties, polymers are used as biomaterials for the development of scaffolds or cell-laden constructs. And they can be classified into two categories of biopolymers: synthetic polymers (to develop 3D scaffolds that serve as constructs) and hydrogel (for printing living cells and growth factors), whose description is given below (Gupta et al., 2018).

16.3.4.1 Synthetic polymers

Synthetic polymers that are biodegradable can be widely used in biomedical fields due to its tailorable material properties. Its physical, chemical, and even mechanical properties can be easily altered and also are of low cost (Gunatillake et al., 2006). Some of the synthetic polymers that are at present extensively used for printing of tissue/organ are discussed here:

16.3.4.1.1 PCL

PCL is having a low melting temperature (59−64°C), hence are preferred in extrusion-based printing. It is also biocompatible and nontoxic and can acquire shape of the structure before degradation due to its hydrolysis-induced bulk erosion profile. Also, it is thermally stable and have appropriate rheological characteristic (Fig. 16.23).

16.3.4.1.2 PLGA

PLGA, which is a thermoplastic type of polymer is having properties such as biocompatibility and degradation (which can be controlled by appropriately adjusting the polymerization ratio between the PLA and PGA) (Fig. 16.24).

16.3.4.1.3 Poly(ethylene glycol)

PEG is another sacrificial material widely used due to its hydrophilic content, biocompatibility. For gel formation, PEG is modified by acrylation, and also modified chemically by cross-linking it with PI-induced polymerization under UV exposure (Fig. 16.25).

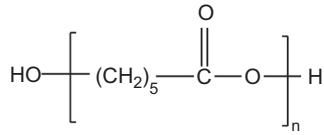

Figure 16.23 Chemical structure of PCL.

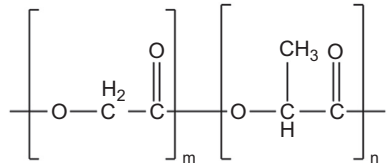

Figure 16.24 Chemical structure of PLGA.

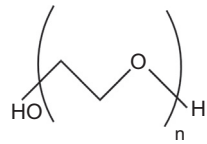

Figure 16.25 Chemical structure of poly(ethylene glycol).

16.3.4.1.4 Pluronic 127

Pluronic 127, a polymer with thermoreversible gelation. It is a liquid at temperature of 4−5°C and form as a gel at temperature of 16°C. This gel formation is reversible permanently (Fig. 16.26).

16.3.4.2 Natural polymers

Natural polymers as stated earlier are widely used in a hydrogel foam as printable materials that encapsulate and print living cells due to their similarity to the native tissue microenvironment. It can also provide tissue-specific biochemical and physical stimuli to guide cellular behaviors including migration, proliferation, differentiation, and maturation (Doppalapudi et al., 2015).

16.3.4.2.1 Alginate

Alginate is an anionic polysaccharide that can be derived from algae. Its structure and composition is already discussed in Section 16.3.1.2.

16.3.4.2.2 Collagen

Collagen consists of three strands of glycine, proline, and hydroxyproline residues. These materials mimics the ECMs resulting in a variety of collagen-mediated physiological interactions between cells and ECMs, and have the advantage of thermoresponsible gelation. Its chemical structure is already described in Section 16.3.1.2.

16.3.4.2.3 Gelatin

Gelatin is a polymer derived from denatured collagen Fig. 16. 27. It even promotes cell adhesion through integrin receptors, hence used as a gelling agent. Under aqueous condition, it coils its structure at temperatures above 40°C and forms an alpha helix structure below 30°C.

16.3.4.2.4 Fibrin

Fibrin is made up by fibrinogen and thrombin interaction, which is known as a blood coagulation mechanism. Due to its rapid gelation, it can be used as surgical glue. The chemical structure is shown in Fig. 16.29. It has mechanical stability, hence when printed remain soft and fragile, and hence difficult to maintain its shape (Fig. 16.28).

16.3.4.2.5 Hyaluronic acid

HA (linear polysaccharide) possess excellent properties of biodegradability, viscoelasticity, hydrophilicity, and biocompatibility for 3D tissue/organ printing applications.

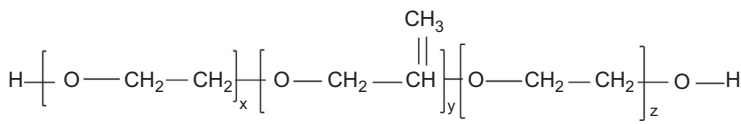

Figure 16.26 Chemical structure of Pluronic 127.

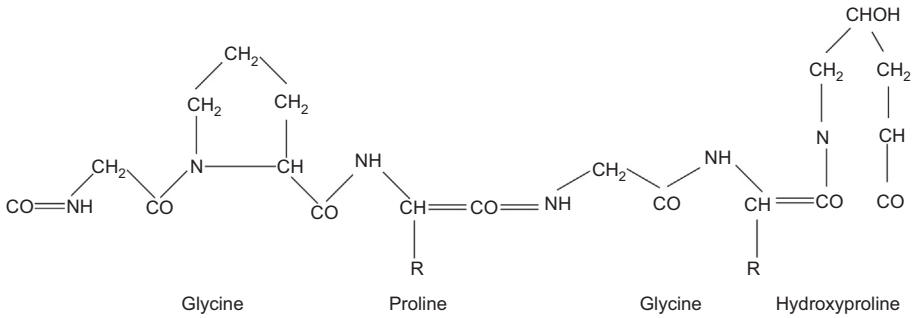

Figure 16.27 Chemical structure of gelatin.

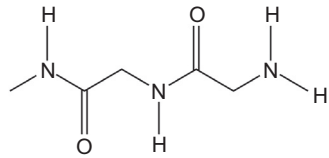

Figure 16.28 Chemical structure of fibrin.

It can be modified by chemically cross-linking methacrylate groups, which leads to gel formation due to process called free radical polymerization under UV exposure.

16.4 Principle of rapid prototyping

16.4.1 Direct rapid prototyping techniques

Direct RP has advantage of low cost and reduced time consumption. There are various direct RP techniques to develop implants: FDM and SLS. Or it can be broadly classified as particle bonding technique and melt-dissolution deposition technique depending on their assembly and are discussed in detail below.

16.4.1.1 Melt–dissolution deposition technique

In this technique, each layer is created through extrusion of appropriate biomaterial. After completion of printing of first layer, the consecutive layers get printed successively. The material extruded cools, solidifies, and glued to the previous layer. For having porosity, spacing between adjacent filaments is controlled along horizontal XY plane. A printing technique utilizing melt–dissolution deposition is FDM described previously (Yeong et al., 2004). With slight modification in this FDM-based printing technology, various new techniques have been developed, which are discussed in short below:

3D fiber-deposition technique
Pellet- or granule-based material is printed by giving pressure to the syringe.
PED
Consist of built-in heating unit in the extrusion system.

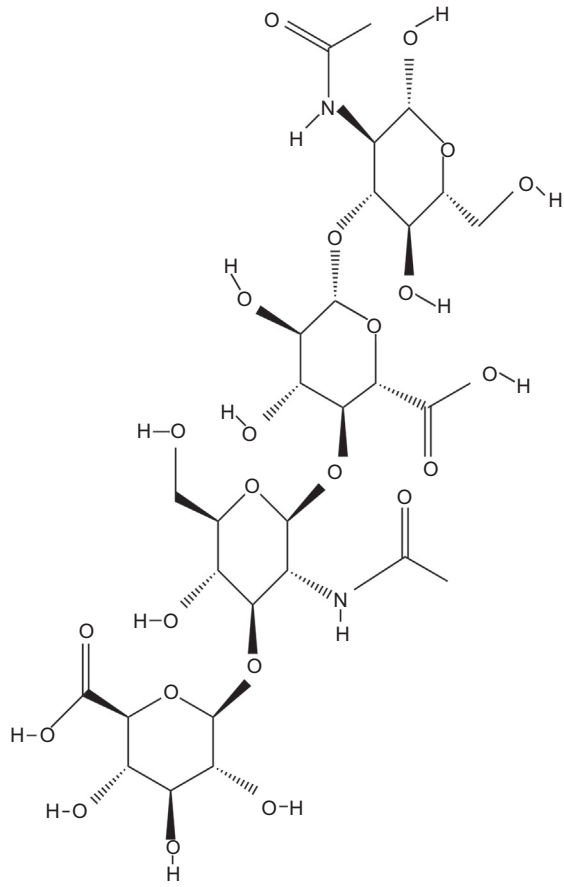

Figure 16.29 Chemical structure of hyaluronic acid.

PEM

Modification done by including more number of jetting nozzles to print multiple range of material simultaneously.

PAM

A microsyringe serves as extruder for jetting the dissolved polymer.

16.4.1.2 Particle-bonding techniques

In these techniques, materials are in particles form, which are then bonded as a layer of powder material. These 2D layers are then bonded to the previous one resulting in formation of complex geometry. After printing of complete layer, the object having a controlled macroporosity and microporosity is taken from the bed containing unbounded powder. The technology falling in this is SLS, which is explained in Section 16.2.1.2.

16.4.2 Indirect rapid prototyping techniques

The indirect RP techniques produce scaffolds with a defined pore structure. Using this method, a scaffold with controlled shape, pore geometry, and interconnectivity is obtained, which can be predetermined independently. Using this technique, a low-porosity scaffold can be generated (Hendrikx et al., 2016). This fabrication method may generate a sacrificial mold for fabrication of scaffolds, enabling user to control both the external and the internal geometry of the final product. This method has an additional advantage of using less raw scaffold material with increase in the range of materials used. Also as no heating is applied to the scaffold, the originality of the biomaterial remains conserved. Various biopolymers used for this method are collagen, PLLA, PCL, hydroxyapatite, gelatin, silk fibroin, and PLGA.

16.5 Hydrogel as polymeric gel

Polymer-based hydrogel has high water content making it suitable for soft tissues by possessing the desired biocompatibility, mechanical and diffusive properties. Mostly hydrogels consist of around 0.5 up to 20 wt% of dry polymer mass. That is they have water content of at least 20% (which is 99% by weight). It is a 3D network that is able to absorb water and release it when required according to the environment. Due to cross-linking of polymeric chains, they are insoluble in any solvent. Also, hydrogels contain tissue similar to phantoms in vitro condition; hence, they can be used to see the effect of external stimuli to soft tissues. Because of these advantages of being mimicking the ECM, rapid diffusion, low dry mass content, they are widely accepted in TE. For further increasing the biodegradation, chemical modifications can be done by cross-linking (Zhu and Marchant, 2011).

For the characterization and proper design of hydrogel-based scaffold degradation, swelling parameters are defined. Some of them are:

Mass swelling ratio

$$(Q_m) = (W_g - W_p)/W_p \tag{16.31}$$

Volume swelling ratio

$$(Q_v) = \frac{V_g}{V_p} = (Q_m + 1)\frac{\rho_2}{\rho_1} \tag{16.32}$$

Polymer volume fraction in the swollen state

$$v_{2,s} = \frac{V_g}{V_p} = \frac{1}{Q_v} \tag{16.33}$$

Number average molecular weight between cross-links

$$M_c = \frac{M_o}{2X} \tag{16.34}$$

Network mesh size

$$\xi = v_{2,s}^{\frac{-1}{3}} \left(Y_0^2\right)^{\frac{1}{2}} = Q_v^{\frac{1}{3}} \left(Y_0^2\right)^{\frac{1}{2}} \tag{16.35}$$

where W_g—equilibrium swollen gel weight, W_p—polymer weight, V_g—equilibrium swollen gel volume, V_p—polymer volume, ρ_2—density of polymer, ρ_1—density of solvent, M_o—polymer repeating unit molecular weight, $\left(Y_0^2\right)^{\frac{1}{2}}$—equilibrium state root-mean-square end-to-end distance of network chains between two adjacent cross-links, X—cross-linking degree.

Hydrogel can be grouped into different groups as shown in Fig. 16.30 below.

Inspite of all the advantages, it has one disadvantage that it cannot be shaped into desired geometry.

The synthetic hydrogels, such as PEG-based hydrogel are widely acceptable for in vivo use, which formation is already discussed in Section 16.3.4.1.3 and 16.3.1.1 (Fig. 16.31).

1. Hydrogels derived from natural biomaterials:
   Many types of hydrogels can be formed from natural materials. Some of them that are derived from natural polymers are already discussed in the previous sections such as collagen, fibrin, gelatin, alginate, hyaluronic acid. And the chemical structures of some of them are shown here:
   a. Hydrogels based on polysaccharides
      Cellulose is a natural polysaccharide material that cannot be dissolved in water. Hence it requires cross-linking for the fabrication of hydrogel network. Native cellulose nanofibers are normally generated from bacteria and plants. Those nanofibers prefer to disperse in an aqueous solution rather than dissolving (Fig. 16.32).
   b. Hydrogels based on polypeptides
      Hydrogel derived from polypeptides is gelatin, which is a product of collagen. Gelatin retains cell-binding motifs, which is important for cell encapsulation. This gelatin-based hydrogel is degradable and biocompatible as shown in Fig. 16.27.
2. Hydrogels based on synthetic materials
   Hydrogels can be even prepared by chemical polymerization of synthetic polymers. Various different types of monomers whose chemical structure is shown in figure below can be utilized for the preparation of synthetic hydrogels.

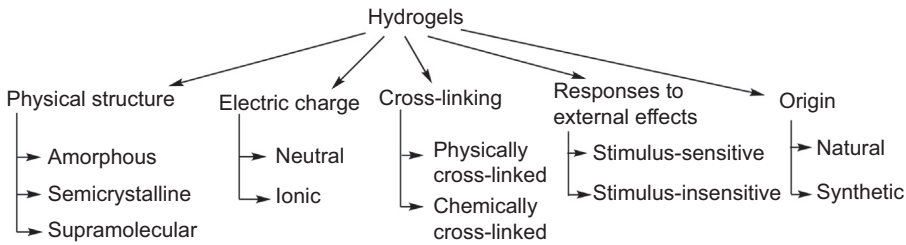

Figure 16.30 Classification of hydrogels.

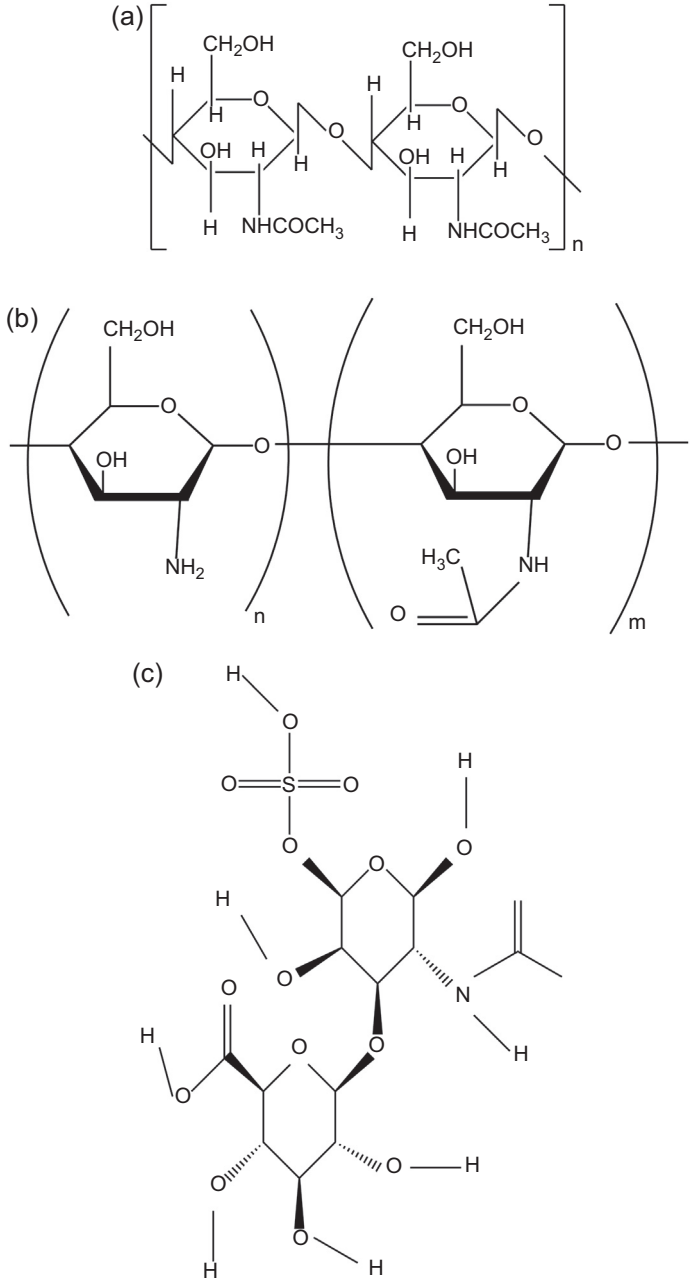

Figure 16.31 Chemical structure of (a) chitin, (b) chitosan, (c) chondroitin sulfate, and (d) starch.

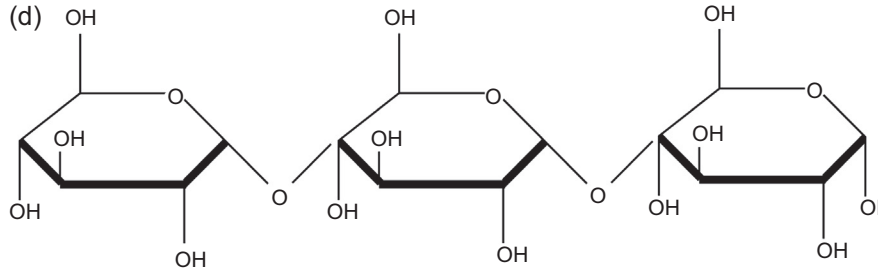

Figure 16.31 cont'd.

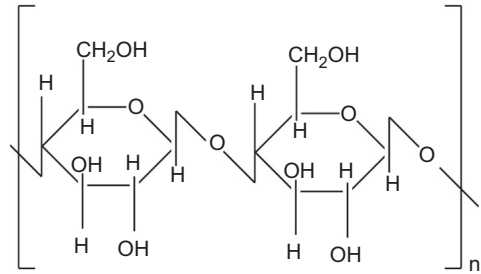

Figure 16.32 Chemical structure of cellulose.

The structure of this hydrogels along with desirable properties such as biocompatibility, swelling ability, mechanical strength, and porosity can be controlled by changing its preparation strategy and chemical composition (Hoffman, 2012).

16.5.1 Synthesis routes

Hydrogels are synthesized from copolymers or homopolymers by performing physical or chemical cross-linking to have gel prepared with desired chemical and mechanical characteristics. This cross-linking can be either through covalent or noncovalent interactions. Hydrogels that are covalently cross-linked gels are called chemical gels, whereas those that are noncovalently linked are called as physical gels. The covalent cross-linked hydrogels have good mechanical strength.

16.5.1.1 Physical cross-linking

These types of cross-linking are done when hydrogels synthesized are bonded with noncovalent interactions. The junction zone of polymer chains is interacting over certain length and not at each point. This type of cross-linking can be done in two

ways either by hydrophobic interaction in which hydrophobic content is coupled to hydrophilic content, resulting in the formation of polymer amphiphile. The temperature when increased, aggregation of hydrophobic blocks starts. The temperature at which these changes may occur depends on the length of hydrophobic's block and also chemical structure of the polymer.

16.5.1.2 Chemical cross-linking

These types of cross-linking are created when polymers are bonded with covalent bond. There are various ways to perform the chemical cross-linking such as by use of enzyme, high energy irradiation, radical polymerization, and chemical reaction of complementary groups.

16.5.2 Sample characterization

The scaffolds can be characterized using X-ray diffraction, H nuclear magnetic resonance, Fourier transform infrared spectroscopy (FTIR), swelling ratio, degradation ration, porosity measurement, differential scanning calorimetry, thermal gravimetric calorimetric analysis. To study the morphology, scanning electron microscopy (SEM), confocal fluorescent microscopy, and micro-CT can be done.

16.5.2.1 Fourier transform infrared spectroscopy

For FTIR analysis, samples are mixed with potassium bromide (KBr) and pellets are formed. The infrared radiation is passed through the sample, some of which is absorbed by sample and few are transmitted giving a molecular fingerprint of the observed sample. Each molecular structure has a different infrared spectrum. This helps to study the functional group and chemical bonding (Mansur et al., 2008) (Fig. 16.33).

16.5.2.2 UV–Vis spectrophotometer

Ultraviolet–visible spectroscopy uses visible and near IR and near UV ranges of light to measure the concentration (size and distributions) of components in the sample, as absorbance or reflection is directly proportional to the concentration (Tomaszewska et al., 2013).

16.5.2.3 Scanning electron microscope

For studying the morphology of hydrogel, SEM is done. For which the sample will be attached to stubs with carbon tape and placed in sputter-coating apparatus and a thin layer of gold is coated on the sample.

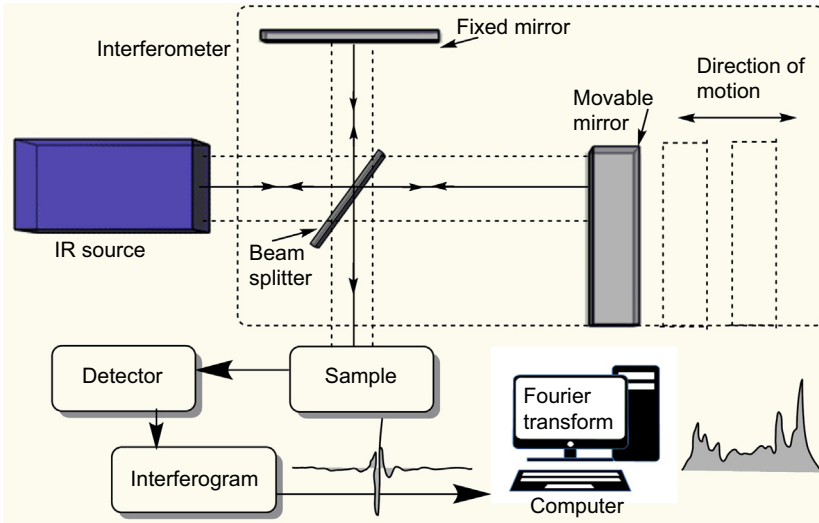

Figure 16.33 Operating principle of Fourier transform infrared spectroscopy Spectroscopy.

16.5.2.4 X-ray powder diffraction

This technique is used for identifying phase of crystalline material. On the hydrogel sample, X-rays are directed and after diffraction the rays are collected and counted.

16.5.2.5 Swelling ratio measurements

This is an important parameter for the characterization of scaffold. For hydrogel swelling capability testing, the specimens of scaffold are first weighed at dry state and the dry weight (W_d) is obtained. Then it is immersed in distilled water or PBS at a constant temperature of 37°C for fixed time interval and wet weight (W_w) will be assessed after the removal of extra water with the help of tissue paper. The mass swelling ratio (S) is calculated using following equation (Eq. 16.36)

$$S = \frac{(W_w - W_d) \times 100}{W_d} \tag{16.36}$$

where W_w—mass of the swollen wet hydrogel sample, W_d—mass of the dried sample.

16.5.2.6 In vitro degradation experiment

The in vitro degradability of the hydrogels to calculate the scaffold degradation that shows the material weight left was analyzed by means of gravimetric measurements.

$$S = \frac{(M_a - M_b) \times 100}{M_a} \tag{16.37}$$

where M_a—original weight of sample, M_b—residual weight of sample.

16.5.2.7 Porosity

Porosity plays a major role in tissue formation as porous products formed provides favorable conditions for growth of cells and transport of nutrients due to the availability of more surface area as compared to dense scaffold. Hence mostly an optimal pore size is considered versatile for tissue engineering.

$$\%P = 1 - \frac{\rho_a}{\rho_p} \times 100 \tag{16.38}$$

where ρ—density of the sample.

16.6 Applications

16.6.1 Tissue regeneration

In tissue engineering, scaffolds play an important role by mimicking as ECM to allow cell to differentiate and proliferate. As well known, the main challenge is the development of biodegradable, porous, and biocompatible scaffold, which leads to creation of reengineered tissue. This achievement is somewhat gained by combining hydrogels and RP. This has led to the creation of various reengineered tissues such as:

1. Cartilage and osteochondral regeneration
   Four cartilage-based, various hydrogels can be used such as gelatin, collagen (type I and II), and alginate hydrogel. The hydrogels are mixed with suitable growth factor such as fibroblast growth factor (FGH) and printed via RP.
2. Cardiovascular regeneration
   Various hydrogels such as natural, synthetic, and dECM-based polymers may be used to develop 3D patch-type structures. Also vasculature can be created in engineered tissues by either indirect printing of sacrificial materials or by directly printing the vascular cells.

16.6.2 Drug delivery

The structure of drug product may have an effect on drug release. The development of complex 3D structures using RP has led to a new opportunity for drug delivery. For example, the 3D printed drug product can have porous structure can bind powder

without any compression (Chai et al., 2017). Delivery of drug is a process of dispensing drug in which the porous structure of hydrogels serves as a matrix for drug loading and protect drugs from hostile environment at the same time to see its therapeutic effect. The porosity of the hydrogels is varied by controlling the density of the cross-link. An important parameter for drug carriers is the release rate, which mainly depends on the diffusion coefficient of the molecule through the gel network and is tuned according to the required. Due to the great potentials, hydrogels are used for drug delivery. Hydrogel scaffolds are utilized to stabilize several applications of space-filling agents and deliver bioactive molecules and encapsulate secretory cells. The scaffold used for local and specific delivery is highly desirable to make sure that the factors, which are beneficial to one tissue that may not be the same to other tissue. Diffusion and mechanical stimulations are used for the degradation of collagen gels by hydrogel. Osteogenesis promoting bone morphogenic proteins, angiogenesis promoting protein, basic FGH and thermal setting, degradable PEO−PLLA gels, thermally reversible PEG−PLLA gels, glutaraldehyde-cross-linked chitosan microspheres, and PEG bilayers have also been delivered from different hydrogel scaffolds. Cell encapsulation is a method by which a substance can be released according to the need of the body over long periods. Different types of primary cells, including pancreatic islets, hepatocytes, and adrenal cortical cells, are encapsulated using hydrogel to transfect the behavior of specific enzymes and growth factors. Depletion in oxygen diffusion process results in several limitations such as decreased viability of cell in the center of the capsule and also restricts its size. Hence to overcome the above limitation, an increase in oxygen diffusion of the microcapsule was done by immobilizing hemoglobin in the alginate matrix (Drury and Mooney, 2003).

16.6.2.1 Microfluidic flow−control devices

For the fabrication of microfluidic systems in an elastomeric material poly(dimethylsiloxane) (PDMS), a microfluidic channel is build layer by layer, which can mimic the heterogeneous multilayered tissue-like structure is made using a CAD program. Using a high-resolution printer, the channel design is converted into a transparency. This high-resolution transparency is used as masks in photolithography. Photoresist needs to be spin-coated onto silicon wafers to prepare the masters. Spin-coated at 5000 rpm for 20 s creates features of photoresist, and hence channels are developed (Eddington and Beebe, 2004). Then a 10:1 mixture of PDMS and curing agent stirred thoroughly, and then degassed under vacuum and is then casted on the master to produce a polymeric model incorporating a network of channels. Then curing is done for 1 h at 70°C, after which PDMS replica peeled from the master. The master can be developed using PDMS as shown in Fig. 16.35. For the removal of the PDMS, silanization of the master is done after molding. Creating masters using RP shows many advantages as compared with the conventional photolithography and micromachining such as transparencies

$$x Me_2SiCl_2 \xrightarrow[-HCl]{+H_2O} x\ "Me_2Si(OH)_2" \xrightarrow{-H_2O} y\ HO(Me_2SiO)_n + H\ z\ (Me_2SiO)_m \qquad (16.39)$$

Figure 16.34 Synthesis of poly(dimethylsiloxane).

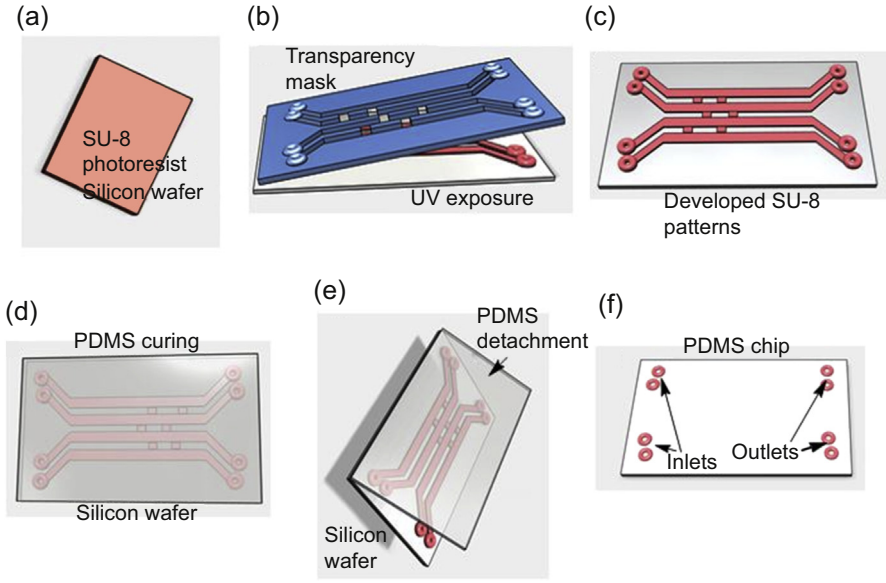

Figure 16.35 Schematic of fabrication of microfluidic chip. (a) SU-8 photoresist silicon wafer, (b) UV exposure phase through transparent mask, (c) Removal of mask, and channel patterned developed on SU-8 substrate, (d) PDMS Curing of masked silicon wafer, (e) detachment of PDMS sheet from silicon wafer, (f) engraved micro-channels on PDMS chip.

produced requires less production time and is less expensive and also photoresist development is easier to produce patterns. This leads to the development of 3D cell patterning by eliminating the limitations of 2D cell patterning technique. This microfluidic-based device can lead to mobilization of cells (Eddington and Beebe, 2004) (Fig. 16.34).

16.7 Future perspectives

Further progress in the printing resolution without increase in production time and cost with no sacrifice on the geometry of the product is the main future demand. Till now there is a need to work on the inner pore architecture and even outer geometry, as cell adhesion takes place on scaffold for the formation of tissue. However, reiteration and compactness of the product will still be a point for consideration. Still there is a need to have more research on development of biodegradable resins. The hydrogel can be used for the printing of soft tissues. One main limitation while employing hydrogel as

biomaterial is its low-mechanical strength and also its inability to give a proper shape of the geometry. Hence it is important to improve the mechanical integrity of the scaffold prepared. On printing complex 3D tissue or organ, with increase in complexity, time required for printing increases. This elongated printing time may have an unfavorable effect on viability of cells. The time consumption can be reduced by either incorporation of different printing technology or by modifying the raster width of the parts to be printed.

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Magnetic gels

17

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17.1 Introduction

As discussed in the preceding chapters of the book, exploitation of elastic and swelling properties of synthetic and natural polymers to form gels have attracted scientists and industrialists toward various biomedical devices and applications such as controlled drug delivery systems, smart muscle actuators, cell or biomolecular separation, and biosensors. Especially, elucidation of scientific principles of environment-responsive sol−gel transitions has been the basis of many exciting technological applications. These efforts are directed toward making the materials "smart" or "intelligent," which denotes that materials are able to detect alterations in environmental conditions, process the acquired information, and accordingly respond to the conditions. Typically, infinitesimal changes in certain environmental stimuli such as temperature, pH, ionic strength, induced electric field, or photoirradiation trigger a volumetric phase transition in several polymeric gels. In the initial developed smart gels, mostly the sol−gel transitions are affected as a result of volumetric phase transitions, which are kinetically constrained due to frictional forces between polymer and small molecules as well as limitations arising out of the collective migration of the long-chain molecules. This has been a principal bottleneck in development of gels with optimal swelling−deswelling properties. However, in the late 1990s, one class of gel materials has been developed, which can also exploit shape transitions under external stimuli (Barsi et al., 1996). Such adaptive materials are called as ferrogels or magnetics gels as the external environment is a magnetic field. When placed inside a nonuniform magnetic field, ferrogels undergo instantaneous shape distortions (elongation, coiling, torsion, bending, or rotation), which also disappear rapidly on removal of the field. They represent gels with the unique combinations of being mechanically soft and high elasticity with strong magnetic susceptibility. The nanoparticles cross-link the hydrogel or can be adsorbed onto polymer backbone, or endow gels to be able to respond to external magnetic fields (Muzzalupo et al., 2015). Ferrogels can also carry a larger amount of drug in comparison with magnetic nanoparticles dispersions. Other important advantages of developing magnetic stimuli-based smart materials are the fact that transitions can be controlled with remote access allowing interaction with deep tissues of the body, relative inertness of the body to magnetic energy compared with light or electric energy interventions, and the possibility to exercise both

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temporal and spatial control over the transitions. These advantages are making magnetoresponsive gels an important emerging class of smart materials. The major areas of their applications are as oil industry, as contrast agents in magnetic resonance imaging (MRI), cell or biomolecule (protein, nucleic acids) separation, drug delivery, hyperthermia treatments, and as artificial actuators.

Compositionally, magnetic gels comprise of a ferrofluid dispersed in a polymer gel network. Ferrofluids are considered as a stable suspension of solid ferromagnetic particles in a fluidic medium. The most interesting properties of ferrofluids arise when the particle size of ferrites is in the 3−50 nm range, in which such nanoparticles exhibit strong superparamagnetism—meaning material is ferromagnetic under applied magnetic field and paramagnetic in absence. The origin of this phenomenon requires the particles to be composed of a single magnetic domain. Thus, when nanoparticles are of sufficiently small size, minute thermal energies cause random flips in magnetization direction. The interval between subsequent flips is known as Néel relaxation time. When no external field is present, measurement time of magnetization exceeds the Néel relaxation time, and thus returning zero magnetization. In presence of external magnetic field, the particles show net magnetization as they possess high magnetic susceptibility giving rise to superparamagnetism and can be used for reversible magnetization without any residual magnetization. In another type of magnetic fluids, known as magnetorheological fluids, magnetic particles in size range of microns to submicron levels are often employed. In this class, after magnetic stimulation, viscosity of the medium increases to a high enough extent to impart solid-like character, whereas ferrofluids maintain fluidity after magnetic excitation. Therefore, in a magnetic gel, the responsiveness is generally governed by the properties of magnetic particles (Chatterjee et al., 2003) or ferrofluids. For example, in a spatially distributed magnetic field magnetic interactions are initiated in superparamagnetic particles, which move toward the strong field dragging with macromolecular chains and solvent. This causes fluid convection. In this way, noninvasive and precisely timed release of drugs can be triggered from the device. The alterations in molecular conformation in the gel caused by actions of cross-linking bridges lead to shape alteration and micromotion of gels (Szabó et al., 1998). The principle of motility and shape transformation of the ferrogels lies in specific magneto-elastic functions. The magnetic field controls and provides the driving force for the motion, and the ultimate shape is determined by the balance of magnetic and elastic forces. The other major component of magnetic gels is the polymer, which confers stability, elasticity, and swelling apart from providing a pivot for functionalization of gels with applications in immunological sensing and cell separations. Several times, polymer nanoparticle gel composites are administered intravenously followed by application of external magnetic fields of high gradient. The gel nanoparticles move to target site result of force balance on the particles by the blood flow and external field and as the applied field exceeds the linear flow rates of blood in arteries (10 cm/s) or capillaries (0.05 cm/s). At the target site, enzymatic activity or altered pH, temperature, osmolality, etc., may trigger the drug release and internalized in cells (Medeiros et al., 2011).

17.2 Compositions of magnetic gels

17.2.1 Magnetic materials

In selection of magnetic particles, metal oxides are mostly preferred over the pure metal for their stability. Magnetization curve behavior (Fig. 17.1) of this class of materials is determined by their composition, solid structure, and size and shape. Among all transition metals, iron oxides, have the highest magnetization properties and hence are most widely used. Magnetic iron oxides are particularly interesting because of their magnetic iron oxide nanoparticles (IONPs) are inexpensive synthesis, biocompatibility, physical and chemical stability, and environmental safety.

In nature, iron oxide exits in eight iron oxides forms though three among them hematite (α-Fe_2O_3), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3) are most popular because of the temperature-induced phase transition polymorphism (Medeiros et al., 2011). Hematite is the most stable of these oxides, accords high corrosion resistance, and is n-type semiconductor with a band gap of 2.3 eV. The other two iron oxides, magnetite and maghemite can be obtained from hematite. The crystal structure of weakly ferromagnetic or antiferromagnetic hematite is a rhombohedral. On the other hand, magnetite crystals are face-centered cubic spinel structure. Fe_3O_4 contains both divalent and trivalent cations. Stoichiometrically, Fe^{2+}/Fe^{3+} is 0.5 in magnetite, with the Fe^{2+} sometimes substituted by other divalent ions such as Mn, Co, and Zn. Fe_3O_4 has band gap of only 0.1 eV and can be both n- and p-type semiconductor.

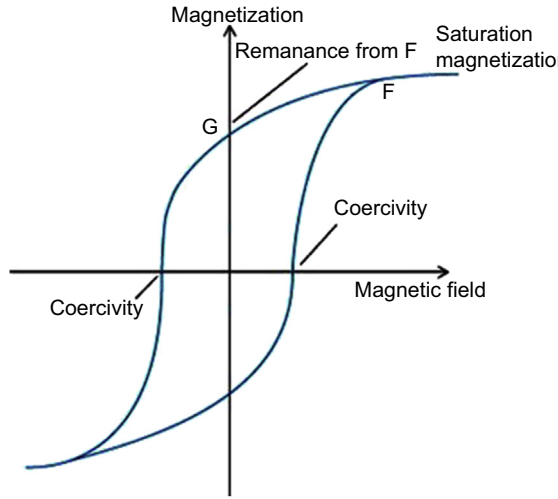

Figure 17.1 A typical magnetization behavior of ferromagnetic materials.
Reproduced with permission from Philippova, O., Barabanova, A., Molchanov, V., Khokhlov, A., 2011. Magnetic polymer beads: recent trends and developments in synthetic design and applications. European Polymer Journal 47, 542−559. https://doi.org/10.1016/j.eurpolymj.2010.11.006.

Maghemite is sometimes referred to as fully oxidized magnetite and has a band gap of 2 eV with a cubic structure. The three oxides exhibit different magnetic properties as weakly ferromagnetic or antiferromagnetic (hematite), ferromagnetic (magnetite), and ferromagnetic (maghemite) with curies temperatures of 956, 850, and 820−986K, respectively (Teja and Koh, 2009).

The strong magnetic moment in iron arises due to the presence of four unpaired electrons in its 3D shell. In the formation of crystal structures, magnetic states of different types can be originated. For example, when the magnetic moments of individual atoms align randomly with respect to each other, the paramagnetic state is observed with a zero magnetization for the crystal. The net magnetic moment of such crystals in external fields is small. On the other hand, each atom can have their magnetic moments aligned in a single direction, ferromagnetism is obtained. The third and fourth types, ferromagnetism and antiferromagnetism, are observed when the crystal has net magnetic moment from two sets of opposite atomic orientations, with different and equal strengths, respectively. It can be seen that domain size plays an important role in determining the magnetic properties and as particle sizes are reduced, materials with single domain structures are obtained. Though materials with strong magnetization are preferred in many applications, antiferromagnetic materials too have applications where recording of very small or minute magnetic changes is required. One such magnetic material is the Co_3O_4 (Raveau and Seikh, 2012). In general, cobalt can form many stoichiometric or nonstoichiometric oxides, exhibiting mixed valency of the cation, and/or oxygen vacancies. Cobalt oxides can exist in various spin states distinguishing it from other metal oxides and making the physics complicated. Co_3O_4 possesses a cubic crystal spinel structure (lattice constant *of* 0.8084 nm). 8 Co^{2+} (3*d*7) reside at the tetrahedral A-sites with a magnetic moment of 4.14 μB, whereas diamagnetic 16 Co^{3+} (3*d*6) ions occupy the octahedral B-sites. Co^{2+} show antiferromagnetic coupling near Néel temperature, between 30 and 40K. Like IONPs, significant size influence on magnetic particles is also shown by this material (Moro et al., 2013). Though cobalt and nickel are high magnetic materials, they have found lesser use in biomedical applications as magnetic gels because of their susceptibility to oxidation and biological toxicity effects (Akbarzadeh et al., 2012). Crystal structures of representative magnetic materials are represented in Fig. 17.2.

Though diamagnetic materials have weak response to external applied fields, they have also been explored for preparation of magnetoresponsive gels. This has been explored with the carbon fiber embedded in agarose system in which short deformations have been studied up to field strength of 8 T. Along with ferrogels, which show strong deformations but difficult to control sometimes, these materials can be applied for biomedical applications (Kimura et al., 2010).

17.2.2 Polymeric materials

Several polymers have been used for the fabrication of ferrogels. The most initial works have been reported with polyvinyl alcohol (PVA) and polydimethylsiloxane. PVA is a nonionic water-soluble polymer that can be conveniently cross-linked with different degrees of cross-linking by using various agents such as glutaraldehyde.

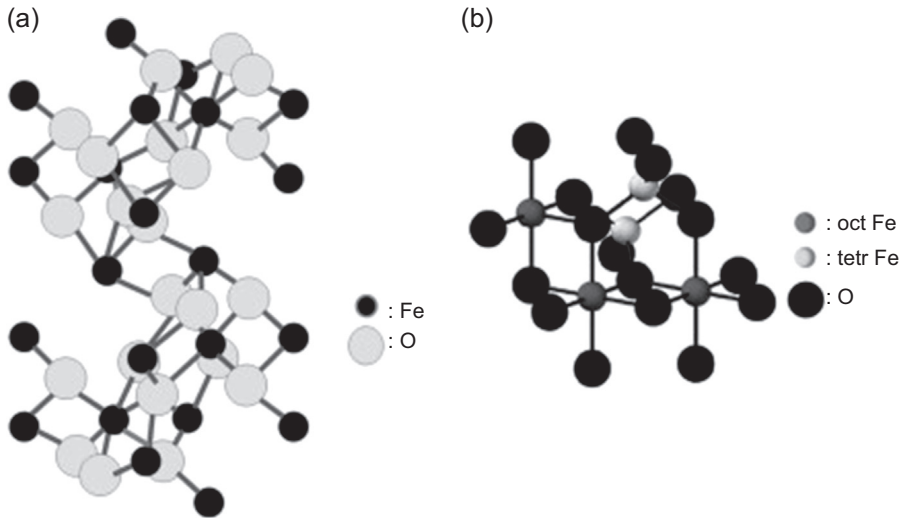

Figure 17.2 Representative crystal structures of hematite and magnetite.
Reproduced with permission from Teja, A.S., Koh, P.-Y., 2009. Synthesis, properties, and applications of magnetic iron oxide nanoparticles. Progress in Crystal Growth and Characterization of Materials 55, 22–45. https://doi.org/10.1016/j.pcrysgrow.2008.08.003.

PVA can also be gelled by physical processes such as repeated freezing–thawing cycles with such gels shown to have fibrillar structure with high aqueous swelling, elasticity, and high mechanical properties (Evans et al., 2012). Moreover, PVA has other desirable properties such as high hydrophilicity, biocompatibility and chemical resistance, which may be suited for biomedical applications. Polydimethylsiloxane, on the other hand is the polymer of choice to fabricate hydrophobic ferrogels, which are often required for application requiring chemical and osmotic inertness in water environments. For hydrophobic ferrogels, creating homogenous dispersion nanoparticles in the matrix requires substantial efforts (Evans et al., 2012). PDMS is available in two part solutions, which can be quickly mixed to cross-linked gel, and has received considerable interest as magnetorheological elastomers (Mordina et al., 2014). Poly(*N*-isopropylacrylamide) hydrogels, synthesized from free-radical polymerization of *N*-isopropylacrylamide (NIPA) is one of the most commonly investigated temperature-responsive gels. The sol–gel transitions occur due to changes in hydrophobicity–hydrophilicity of polymer domains (Zhang et al., 2001). The unique properties arise due to volume changes accompanying changes in external temperature with a lower critical solution temperature at which polymer chains collapse, usually around physiological temperature. Incorporation of magnetic particles within gels also raises the gel temperature due to hysteresis loss of magnetic material under alternating magnetic field. Thus, the in situ warming of the gel beads represents a process with higher efficiency compared to heating the whole surrounding environment (Xulu et al., 2000). Iron oxide/poly[NIPAM–AMPS] hydrogels were prepared to study their use in hyperthermia applications (Reddy et al., 2015) and

poly(*N*-tert-butylacrylamide-co-acrylamide) have also been reported (Guo et al., 2013). Among natural polymers, gelatin, obtained by partial hydrolysis of collagen, posses a sol−gel transition temperature in amenable temperature ranges have been shown to possess a favorable gel mesh for nanoparticles growths by small-angle neutron scattering (SANS) measurement (Helminger et al., 2014). Other polymers frequently investigated have been polystyrene (PS), polyacrylamide, PVA (Ramanujan and Lao, 2006), copolymers of acetoacetoxyethyl methacrylate, alginate, dextran, and *N*-vinylcaprolactam, copolymers of NIPA, sulfonated mesoporous styrene−divinylbenzene copolymer (Ramanujan and Lao, 2006), triblock polymer polyisopropene-block-poly(2-cinnamoylethyl methacrylate)-block-poly(*tert*-butyl acrylate), and glycidyl methacrylate, etc. (Caykara et al., 2009; Philippova et al., 2011).

Polyurethane magnetic composites are also reported as flexible sensors (Petcharoen and Sirivat, 2016). *kappa*-carrageenan is another polymer, which can form interpenetrating networks with PVA, and is particularly suitable for colon-specific drug delivery. Magnetic gel nanocomposites with iron oxide have been reported with this polymer (Mahdavinia and Etemadi, 2014). Hydrogels based on random copolymers of metal-chelating and hydrophobic 2-(acetoacetoxy)ethyl methacrylate with thermoresponsive, hydrophilic hexa(ethylene glycol) methyl ether methacrylate are also reported to exploit both thermo- and magnetic responsiveness (Papaphilippou et al., 2011).

Nanofibrillar cellulose nanostructures also serve as potential templates to form ductile or tough networks for obtaining functional materials. Using cellulose templates, magnetic aerogels can be conveniently synthesized. To achieve this, $CoFe_2O_4$ magnetic nanoparticles were synthesized using porous scaffolds of cellulose gel formed from LiOH/urea system and gels were freeze-dried. The aerogels contained nanosized pores with 50%−80% porosity, 300−320 m^2/g internal, and 0.25−0.39 g/cm^3 density. Thus cellulose nanofibrils can be used to form magnetic aerogels, which are considerably lighter in weight and flexible (Liu et al., 2012).

Organoferrogels with thermoreversible properties based on physical gelation in PS-block-poly(ethylene-co-butylene)-block-PS triblock copolymers, and ferrofluids are also synthesized (Krekhova and Lattermann, 2008; Krekhova et al., 2010). Ferrogels can be represented by a convenient notation such as FG/6.3/300/4.25 where sample name/%wt of polymer/ratio of monomer to the cross-linker/ concentration of magnetic nanoparticles (given by wt%).

17.2.3 Protein-based ferrogels

Biocompatibility of the polymer phase is an important determinant of magnetic gels in several biomedical applications. The polymers thus expected to allow favorable surface interactions with cells and produce nontoxic degradation products, if any. In this respect, most conventional natural or synthetic hydrogels have certain limitations. For example, natural magnetic hydrogels made from polysaccharides provide only limited tailoring of properties, whereas synthetic gels do not always provide suitable

tissue interface. Protein hydrogels offer a promising alternative to overcome the limitation of the natural or synthetic hydrogels. Likewise, Mody et al. recently reported protein-based magnetic microgels composed of IONPs. Interestingly, this method of magnetic gel preparation occurred concurrently, which is microgels formed only when iron was present and vice versa, i.e., nanoparticles were also formed only when protein was present. The method was based on addition of aqueous Fe^{2+} to a solution of hemoglobin KOH and KNO_3. The Fe to protein ratio determined the size and magnetic properties of gel nanoparticles. The combination of X-ray diffraction (XRD) and Fourier-transform infrared (FTIR) spectra showed Fe_3O_4 as phase of iron oxide. Hemoglobin ferrogels were ferro- or ferrimagnetic with the magnetism (per gram of sample) and coercivity (at 5K) in range of 90−100 emu/g and 600−800 Oe were obtained while considerable model dye payload (Mody et al., 2016).

17.2.4 Effect of colloidal size

Spectacular properties along with superparamagnetism, such as high saturation field, shifted loops after field cooling, high field irreversibility, extraanisotropy, are observed when size of magnetic particles are in the nanodomain. Surafce-, narrow-, and finite-size effects are the main contributors to the magnetic behavior of each nanoparticle. Frenkel and Doefman first predicted that uniform magnetization at any field a particle would be obtained if particle size of ferromagnetic material is below a critical size (usually 15 nm) such that it consists of a single magnetic domain. Concepts of magnetic domains influencing magnetic behavior can be visualized from orientation of atomic magnetic moments as shown in Fig. 17.3. Interestingly,

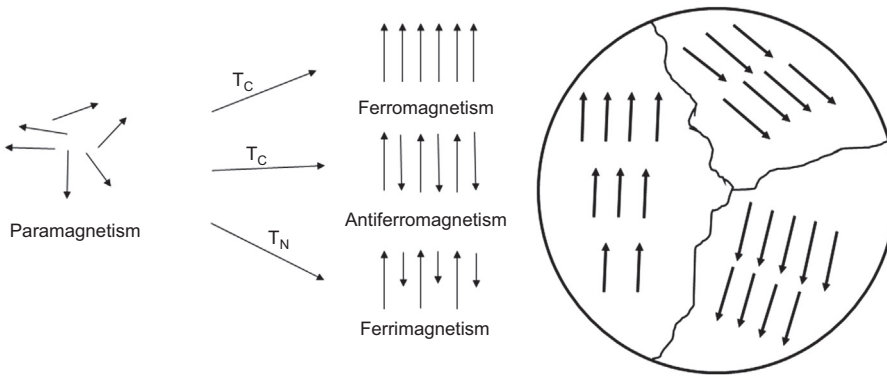

Figure 17.3 Orientation of atomic magnetic moments of each atom in types of magnetic materials and representation of domains in a bulk material. Size has an important role to play in determining domain sizes.
Reproduced with permission from Teja, A.S., Koh, P.-Y., 2009. Synthesis, properties, and applications of magnetic iron oxide nanoparticles. Progress in Crystal Growth and Characterization of Materials 55, 22−45. https://doi.org/10.1016/j.pcrysgrow.2008.08.003.

these materials exhibit identical magnetization to atomic paramagnets above a blocking temperature, but for large susceptibilities and magnetic moment (Akbarzadeh et al., 2012; Frenkel and Doefman, 1930). This apart, ferromagnetic materials in an applied field also exhibit hysteresis loop described by remanence and coercivity. Coercivity shows strong size-dependency as it first increases to a maximum with decrease in particle size and then falls toward zero, which is attained at critical size making ferromagnetic particle superparamagnetic. In superparamagnetic particles, thermal fluctuations cause spontaneous demagnetization of a saturated assembly resulting in the no hysteresis and zero coercivity. Theoretical models of the hysteresis in ferrogel magnetostriction of ferrogels have also been developed that explain deformation under the external field based on association of particles into linear chainlike aggregates and their rupture on removal of fields (Zubarev et al., 2016, 2017).

The magnetization M can be expressed as a vector sum magnetic moments of all atoms present per unit volume of any bulk ferromagnetic material. However, bulk materials consist of domains, which have their own magnetization vector. Hence, the bulk magnetization may be lower than the sum total of all atomic moments even if they are entirely aligned as the individual domains may not be aligned with each other. However, as the material is reduced to nanodimensions and approaches below critical size called dC, the number of domains decreases to a single domain. In presence of external applied magnetic field to a ferromagnetic material, magnetization increases with field strength and reaches a saturation value. However, when field is removed, magnetization is reversed by two mechanisms—Brownian motion (rotation of particle as a whole) or Neel mechanism (rotation of atomic moments inside particles collectively). However, all domains do not come back to their original alignment leading to magnetization hysteresis loop. This also gives rise to present of residual magnetization and can be removed by application of opposite coercive field. Interestingly, nanosized, single domain magnetic material does not display any hysteresis loop. Thus, IONPs ($<$20 nm) are said to display superparamagnetism at room temperature (Teja and Koh, 2009). In ferrofluids, both mechanisms take place but in polymeric ferrogels, the magnetic nanoparticles are entrapped in polymer chains, restricting the Brownian movement and the Neel relaxation is predominant. Depending on the nature of relationship between nanoparticles and polymer gel network, the relative importance of both phenomena is determined. Nanoparticles smaller than gel mesh have mobility inside the gel, whereas if particle size is bigger, then only rotation about their location takes place. Secondly, particles with strong affinity to the polymer molecules have limited rotational mobility. Comparative evaluation of both mechanisms in contributing to magnetic losses and heating under fields is thus required for specific applications (Safronov et al., 2016).

Temperature lowering to a blocking temperature can reverse superparamagnetic to ferromagnetic behavior, wherein such transition temperature depends on the volume of the magnetic particles implying slight changes in particle size can induce an

appreciable increase in blocking temperature (Chatterjee et al., 2003). Magnetic behavior of superparamagnetic materials is given by the Langevin function.

$$M = \Phi_m M_s L(\zeta) = \Phi_m M_s(\coth\zeta - 1/\zeta)$$

where, M is the magnetization of gel bead in an external magnetic field assuming that magnetization of each particle in gel is equal to saturation magnetization of pure ferromagnetic material, where Φ_m is the fractional volume of particles in the gel bead, and ζ is the parameter of Langevin function $L(\zeta)$ defined as $\zeta = mH/kT$, where m is the giant magnetic moment of nanosized magnetic particles, H is the external magnetic field, k is the Boltzmann constant, T is the temperature.

No hysteresis is observed in the field dependence of the magnetization and the magnetization of is directly proportional to the concentration and saturation magnetization of magnetic particles. Furthermore, superparamagnetic behavior at room temperature with no remnant is also observed experimentally in such systems. However, in real systems, magnetization values are sometimes lower than predicted values due to spin disorders from the higher surface area. In external fields, heating is also observed as thermal losses accompany fast remagnetization and forms the basis of hyperthermia applications (Philippova et al., 2011). M_s in nanoiron oxides has been found to be in the range 30−80 emu/g, which is below 100 emu/g of bulk particles (Wu et al., 2008).

For example, hematite is paramagnetic above 956K, its Curie temperature, whereas weakly ferromagnetic at room temperature and antiferromagnetic at 260K (phase transition at the Morin temperature, TM). Particle diameter, crystallinity, and degree of cation substitution can alter the magnetic behavior of hematite. A decrease in particle size causes decrease in the Morin temperature of hematite, which altogether disappears as the size approaches 8−20 nm. Magnetite, on the other hand, shows a Curie temperature of 850K and is ferrimagnetic at room temperature. As particle size is reduced to 6 nm, magnetite also shows superparamagnetic behavior. Though certain other parameters also influence such phase transitions. For example, in hematite, reduction in crystallinity, and cation substitution with Al, Ga, etc., can lower the Morin transitions, whereas in magnetite, changes in crystal morphologies or method of synthesis can change coercivity (high of octahedral, followed by cubic and spherical) in range of 2.4−20 kA/m corresponding to number of magnetic axes in each geometry (Teja and Koh, 2009). This apart, particles size optimization is also required for magnetic property point of view as below 50 nm particles, thermal motions affect manipulation of mechanical properties by applied field (Lopez-Lopez et al., 2017a). Magnetomechanical effects are more pronounced in composites with more than 100 nm size (Lopez-Lopez et al., 2017b).

Size also determines the biological internalization of magnetic beads. Gel beads with more than 200 nm diameter are sequestered by the spleen and eliminated by phagocytic cells, resulting in decreased blood circulation times. Furthermore, if

diameters are lesser than 10 nm fast renal elimination or extravasations may take place. Thus, 10−100 nm diameter has the highest blood circulation times and escape the reticuloendothelial clearance or penetrate small capillaries to achieve effective distribution in tissues of target (Philippova et al., 2011).

17.3 Physical properties of magnetic gels

17.3.1 Isotropic/anisotropic stress−strain behavior, swelling behavior under magnetic fields

One of the important physical properties of magnetic gels is their response to mechanical forces. The initial works on magnetic gels by groups of Zrinyi and Martinoty have been appropriately reviewed to understand the mechanical behavior of magnetic gels (Brand et al., 2011). Gels prepared under certain special conditions can respond to homogenous field strengths (uniaxial magnetic gels), whereas others respond only to magnetic field gradients (called isotropic magnetic gels). The static shear modulus G is given by the relation:

$$\sigma_n = G\left(\lambda - \lambda^{-2}\right)$$

σ_n = nominal stress = f/S; f is the tensile force, S cross-sectional area of undeformed sample; λ = compression ratio = l/l_0; l is sample length in force direction, l_0 is initial undeformed length (Brand et al., 2011).

The uniaxial gels show highly anisotropic behavior with a ~70% anisotropy in G and can also exhibit shear-induced softening of structure. These differences are illustrated with a PDMS gel containing either maghemite or carbonyl iron particles. The type of the magnetic particles, size, shape, and textural features determines the mechanical properties. Magnetic gels showing differential mechanical behavior in presence or absence of magnetic field have also been observed with silicon gel with 80% load of 3.8 μm carbonyl iron particles. Magnetic field (0.44 T) increases the stiffness and the load-bearing stress (up to 2 times) but decreases maximum strain of the material. The induced force shows a nonlinear decrease with increase in distance between magnet and the gel, whereas it shows a nonlinear increase with magnetic induction (Farshad and Le Roux, 2005). The particle−particle magnetic interactions result in columnar chainlike agglomerations increasing the elastic modulus of the beads under external magnetic field, a process referred to as temporary reinforcement. This effect is higher if the particle alignment, applied field, and the mechanical stresses are all parallel to each other (Philippova et al., 2011). For explaining the inhomogenous situation inside a ferrogels, density functional approaches can be used (Cremer et al., 2017).

Similarly, the magnetic gels can also show remarkable swelling anisotropy. Usually the gels show a lesser degree of swelling compared with swelling observed with gels with no magnetic particles as the particles induce additional cross-links in the structure (Philippova et al., 2011) as also suggested by higher modulus of elasticity modulus. Moreover, swelling also induces changes in remnant magnetization, and swelling/

shrinking events are associated with different degrees of magnetic properties, which may be related to number of nanoparticles contributing in swelling-induced effects. The effect of swelling can be incorporated into magnetization measurements as follows (Van Berkum et al., 2015):

$$H = \frac{M(t_0)}{2f}\left[\left(\frac{z + ft_0}{\sqrt{(z+t)^2 + R^2}}\right) - \left(\frac{z}{\sqrt{z^2 + R^2}}\right)\right]$$

where, $M(t_0)$ is the magnetization before swelling the gel of radius R, thickness t_0, and distance z to the center of the disk in perpendicular direction, $f = t/t_0$ is the swelling factor.

Because many magnetic gels are made by sol−gel transitions, it is imperative to characterize the gels for their dynamic rheological properties during the transitions. Generally, complex shear modulus, G,

$$G = G' + iG''$$

changes as the system undergoes sol to gel transition. In the sol phase, the low-frequency response of the system is analogous to liquids or,

$$G' = \omega^2 \eta \tau \text{ and } G'' = \omega\eta$$

where, ω is the angular frequency, η the viscosity, and τ is the relaxation time.

After gelation, the low-frequency response of the system is altered to represent solid-like behavior as

$$G' = G_P$$

$$G'' = \omega\eta$$

where, G_P is a constant plateau value (Brand et al., 2011). Incorporation of magnetic particles is also known to improve the thermal stability of certain hydrogels (Goiti et al., 2007). Moreover, anisotropy in electrical permittivity on external magnetic field stimulation is also seen in PDMS-based ferrogels (Kubisz et al., 2011). Viscoelastic changes accompanying the sol−gel transitions are also responsible for giving rise to nonlinear magnetic properties (Wisotzki et al., 2016).

17.3.2 Magnetostriction

Zryinyi found phase transition phenomena in the magnetostrictive deformation process. Gel networks prepared by freeze−thaw cycles of PVA (degree of polymerization of 1700 and 7600) and water or dimethylsulfoxide (DMSO) as the solvents illustrate the properties. A higher molecular weight polymer allows scaling down the weight fraction of polymer in the gel. Interestingly, both gels underwent

90% deformation in 0.1 s but aqueous gels deformed in the magnetic field direction, whereas DMSO gels showed the opposite behavior and contracted. Morphologically, aqueous gels remained homogenously dispersed, whereas DMSO gels showed agglomerates formations. The deformation is determined by ferrofluid content, polymer concentration, and gel elasticity. Between the hydrogel and DMSO gel, difference in strain direction is attributed to state of magnetic nanoparticles with hydrogels showing superparamagnetism and DMSO gels ferromagnetic properties (Hirai, 2014; Mitsumata, 2014). Several finite element modeling, including continuum and reduced models, has been proposed to understand the deformation mechanism of ferrogels (Attaran et al., 2017). Short-ranged order of particles spatial disposition changes the type of the deformation. Certain theoretical models have been developed to account for the effect of particle concentrations in magnetodeformation of gels and it has been proposed that short-range order of spatial distribution depends on the particle concentration with high loading of the particles showing to be able to only elongate (Hirai, 2014; Mitsumata, 2014). In addition, the deformation of hollow vesicle is much higher than a solid gel (Raikher and Stolbov, 2005), and shape effects in randomly packed composites on the magnetostriction properties are also modeled (Diguet et al., 2010). In addition, certain deformation behaviors can be explained analogous to analogous to a first- or second-order material phase transformations (Snyder et al., 2010). Magnetoresistive properties of magnetite−silver ferrogels in PDMS have been modeled (Mietta et al., 2016). Some models also incorporate free energy density to explain the magnetic effects (Liu et al., 2017).

17.3.3 X-ray and other characterization

In general, magnetic gels are characterized for particle size and distribution, functional group composition, crystallite size, etc., using dynamic light scattering, XRD, transmission electron microscopy (TEM), FTIR, scanning electron microscopy (SEM), thermogravimetric analyses, surface charge density, magnetic properties, among other techniques. A typical TEM and SEM image of magnetic nanoparticles is depicted in Fig. 17.4. For example, in the case of iron oxide ferrogels (magnetite or maghemite), most XRD peak is centered at 35.5 degrees (2θ) corresponding to (311) reflection plane and can be used to confirm the presence of iron oxide in the ferrogel matrix. The crystal size can be further evaluated using Scherrer equation (Reséndiz-Hernández et al., 2008). Magnetoimpedance studies of ferrogels have also been reported with values of $\Delta Z/Z_{max}$ about 80 ± 5 MHz to characterize stray fields of the magnetic nanoparticles and real part variation (Kurlyandskaya et al., 2017). Distribution of nanoparticles in a ferrogel can be probed by SANS, whereas measurement of magnetooptical birefringence properties allows estimating degrees of rotational freedom (Galicia et al., 2011; Hernández et al., 2009a).

17.4 Methods of preparation of magnetic gels

There are several methods for synthesis of magnetic nanoparticles and loading them into polymeric gel structures. For preparing magnetic gels, three principal methods

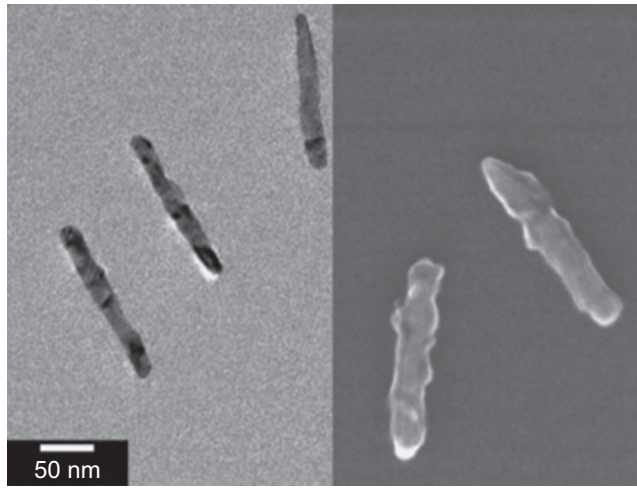

Figure 17.4 A bright-field transmission electron microscopy and scanning electron microscopy image of nickel−gelatin ferrogels
Reproduced with permission from Bender, P., Günther, A., Tschöpe, A., Birringer, R., 2011. Synthesis and characterization of uniaxial ferrogels with Ni nanorods as magnetic phase. Journal of Magnetism and Magnetic Materials 323, 2055−2063. https://doi.org/10.1016/j.jmmm.2011.03.016.

are direct blending method, grafting-onto method, and the in situ method. Proper selection of the method is made based on the properties of magnetic nanoparticles and polymer gel network, and the concentration and distribution homogeneity required for particular applications. The simplest method of soaking gel in a ferrofluid does not ensure retention of magnetic particle when placed in a different media (Lopez-Lopez et al., 2017a). In direct blending method, the method of preparation of nanoparticles themselves is also a crucial factor. The synthesis method determines the size, shape, particle size distribution, and surface chemistry of the particles, which all can affect the magnetic properties. The Fe_3O_4 generally produces irregular shapes when obtained by grinding of bulk materials, whereas regular, spherical are produced by plasma atomization, aerosol, or wet chemistry method. These spherical particles can be crystalline or amorphous depending on orderliness of aggregation of crystallites. Preparation method also determines presence and distribution of solid-state imperfections, defects or impurities. To obtain particles with highly controlled shapes and sizes, several methods have been described, which include coprecipitation, laser pyrolysis thermal decomposition, sonochemical, solvothermal, carbon arc, microemulsion, chemical vapor deposition, microwave assisted, and combustion synthesis (Akbarzadeh et al., 2012).

Gas-phase methods comprise reduction, thermal decomposition, disproportionation, oxidation, hydrolysis to precipitate solid nanomaterials from the gas phase. The chemical vapor deposition process is achieved by delivering a precursor-loaded carrier gas stream under conditions of vacuum at high temperature. Rapid expansion of dispersed gas stream at outlet of reaction chamber ensures growth or clustering

of the nanosized particles is restricted. Postsynthesis heat treatment allows further changes in composition, solid morphology, and particle size. Laser pyrolysis involves resonant energy transfer between laser photons and a gaseous species, reactant, or sensitizer. For example, sensitizer is excited by CO_2 laser photons and collides with the reactant to further transfer the absorbed energy. The reaction is carried out till a critical concentration of nuclei is achieved and leads to homogeneous nucleation. One-step laser pyrolysis to obtain uniform hematite and maghemite IONPs has been successfully reported. Gas-phase methods generally produce high purity particle though the yields low and setup is not amenable for large-scale production. On the other hand, liquid-phase reactions such as coprecipitation from aqueous solutions are more cost-effective with higher yields. Magnetite nanoparticles, spherical, 30−100 nm size is synthesized by reaction between ferrous and ferric hydroxides in aqueous media. In liquid-phase reactions, agglomeration of nanoparticles becomes a critical issue and often requires use of surfactant or coating of nanoparticles surfaces with proteins. Surfactants are also used to stabilize nanoparticles produced by thermal decomposition methods. However, for biomedical applications, presence of residual surfactants may pose problems in biocompatibility or magnetic properties of the materials. The water-in-oil microemulsion method is another method for preparing magnetic nanoparticles in which each emulsion phase provides a restricted microenvironment for growth of nanoparticles. In this method, nanoparticle size is determined by droplet size, which in turn is regulated by the water to surfactant ratio. However, microemulsion method is also difficult to scale up and contains residual surfactants. Pristine nanoparticles of iron oxide or its composite such as iron oxide−silica composites can also be prepared by sol−gel method involving hydrolysis or condensation of precursor solution metal alkoxides or alkoxide precursors. This method also poses problem of by-product contamination and requires substantial posttreatment. High-pressure hydrothermal methods exploit hydrolysis and dehydration of metal salts under exposure to water at high pressures and temperatures. The very low solubility of metal oxides in aqueous phase generates very high supersaturation, leading to formation of very fine crystals. Hydrothermal process is environmentally friendly and also does not require postsynthesis treatment except surface engineering and is amenable to industrial scale up (Teja and Koh, 2009).

On method to obtain crystal size of very narrow crystal size distribution is using biological principles or biomineralization process (Mirabello et al., 2016), which restricts the synthesis within smaller compartments. Spherical magnetoferritin with maghemite iron oxide particles with a 7.3 nm diameter has been reported. Similarly, Co_3O_4 nanoparticles synthesized through hydrothermal, reflux, template-assisted nanocasting, or thermal decomposition can produce nanoparticles with cube, needle, sheet, or wire type morphologies. However, they require surfactants as templates, multiple steps resulting in long reaction times, and are thus more suitable for lab-scale syntheses. Continuous flow hydrothermal reactors have been developed for large-scale synthesis of such nanomaterials (Moro et al., 2013).

Synthesis of magnetic nanoparticles can also be performed in presence of polymers gels, which offer the advantages of offering control over nucleation and

growth of magnetic particles by the constraints of the polymer network architecture. In this method, the polymer gels act as a reactor for in situ formation of IONPs. One example is provided by works of Breulmann et al. who prepared magnetite inside the pores of a polystyrene−polyacrylate gel (Breulmann et al., 1998). An iron oxide load of 3.5%−8% Fe_3O_4 (16 nm particle size) after one reaction cycle and maximum loading up to 20% iron was observed. In situ preparation can also be made before, during or post−cross-linking reaction. In yet other method, magnetic nanoparticle sol is made separately and mixed with the polymer solution after which cross-linking is initiated (Zrinyi et al., (n.d.); Zrınyi et al., 1997). Use of complexation reaction between PVA with Fe^{3+} and Fe^{2+} has also been applied to immobilize nano-sized magnetite particles. In such cases, complexation reaction achieves the cross-linking functionality and is achieved by titration of aqueous NaOH solution with PVA ion solution. Ferromagnetic gel beads of 2 mm diameter are readily synthesized (Hirai, 2014). Another demonstration of magnetic gel preparation included human fibrin as the polymer mixed with PEG-functionalized magnetite particles and incubated under CO_2 atmosphere. Gelation is performed with magnetic field applied vertically of 36 kA/m; a saturation magnetic property of 1.5−2.67 kA/m was obtained of ferrogels studied by vibrating-sample magnetometer and superconducting quantum interference device magnetometry. These ferrogels have been evaluated in vivo and found to be biocompatible (Lopez-Lopez et al., 2017a). In situ formation of ferrogels with polymerization reaction taking place within addition of magnetic nanoparticles has been demonstrated for free radical, atom transfer radical polymers also (Czaun et al., 2008; Shankar et al., 2017). PVP-stabilized Ni−nanorods−gelatine (∼150 nm)−based ferrogels have been synthesized. Nanorods were first prepared using porous aluminum oxide templates by current-pulsed electrodeposition of nickel. Isotropically and uniaxially aligned ferrogels of different elastic modulus can be obtained depending on distribution pattern of the nanorods (Bender et al., 2011). Radical polymerization to obtain gels with copolymers is also known (Korotych et al., 2013). In case of ferrogels synthesized by in situ coprecipitation method with polymer, polymer concentrations play a crucial role as it determines the diffusion in the matrix (Hernández et al., 2009b) and iron hydroxides may be formed in place of iron oxides as has been demonstrated for chitosan ferrogels. Oxidative coprecipitation has been shown for preparation of cobalt magnetic nanoparticles (Wan and Li, 2015). Low-temperature cross-linking can be exploited for fabrication of degradable PVA hydrogels (Bannerman et al., 2017). Magnetic gel scaffolds with silk fibroin/chitosan as polymer phase were made by freeze-casting method and a reverse coprecipitation method was used for synthesis of magnetite particles (Aliramaji et al., 2017). Performing the cross-linking reaction under external magnetic field for synthesizing the ferrogels can also impart superelasticity, nonlinear behavior in which stress−strain curve has a plateau regime, where small loads cause relatively higher reversible strains in the magnetic gels (Cremer et al., 2016). Ferrogels are also synthesized by ion exchange between the counterions in the DLVO layer of the gel beads and metal ions followed by suitable chemical treatments (Zhang et al., 2001).

17.5 Applications of magnetic gels

Magnetic gels find applications in magnetic storage media, biosensing, targeted drug delivery, contrast agents in magnetic resonance imaging, and destruction of the tumor tissue under the action of high-frequency magnetic fields ("intercellular hyperthermia") (Häring et al., 2015).

17.5.1 Monitoring diagnostics and display devices

For display device applications, high encapsulation is required for which modified methods have been proposed. Magnetic fluid is high enough and can be mixed with white powder such as TiO_2, the particles can be applied for magnetically controlled display as illustrated by (Hirai, 2014).

17.5.2 Stimuli-responsive drug delivery

Though porous magnetic gel scaffolds have been reported, which show large deformations under nominal field strength, one of the limitations is that small-sized devices (2 mm) suitable for implantation thickness does not exhibit significant deformations. Thus, to improve deformations at small sizes, biphasic ferrogels with iron oxide gradients have been developed. This was based on hypothesis that gel stiffness can be reduced and porosity increased by redistributing iron oxide from the deforming zone to site distal from field source would increase small ferrogel deformation of small-sized gels and consequently increase drug release. Such biphasic gels are poised to have applications in drug delivery, tissue engineering, and microfluidics (Cezar et al., 2014). Gels containing ferronanoparticles and formed from *N*,*N*_-dimethylacrylamide in presence of methacrylic acid are also able to modulate release of anticancer drug such as 5-Fluorouracil in response to by either permanent magnetic (0.25 T) or electromagnet (0.5 and 1.2 T) field stimulation. These gels are also pH sensitive as their swelling and drug release are different in tumor pH compared with normal physiological pH, and the combination of pH responsiveness with magnetic responsiveness increases drug release performance (Muzzalupo et al., 2015). Iron oxide/alginate−grafted heparin ferrogels prepared by ionic cross-linking ionic cross also show control over deformation controlled by applied magnetic. This ferrogel has been demonstrated for controlled release of TGF-β1, which can bind to heparin. The gels have increased potential to induce differentiation of ATDC5 cells toward chondrogenicity under magnetic field stimulation (Kim et al., 2016). PVA-Fe_2O_3 hydrogels can be optimized with respect to polymer and ferrofluid composition to obtain either a burst release or no release of drug molecules (Liu et al., 2008). Ferrogels based on Pluronic F 127 and SPIONS have been demonstrated to be attractive carrier system for reaching the round window membrane in inner ear, wherein they can target neuronal cells or organ of corti (Thaler et al., 2011). Remote-triggered ferrogels based on poly(*N*-isopropylacrylamide) and alginate (PNiPAAm) with specific power absorption in range 100−300 W/g are used to precisely deliver vitamin B12 in doses as low as $25(2) \times 10^{-9}$ g through AC magnetic fields has been shown (Bruvera et al., 2015). Stimuli-responsive release of gelatin−

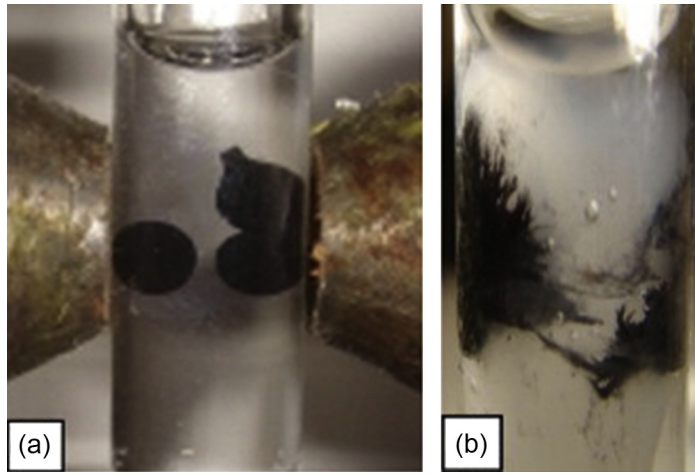

Figure 17.5 Principle of targeted drug delivery under guidance of external magnetic field demonstrated by accumulation of gel beads near source of external magnet and further disruption in a polyvinyl alcohol−borate polymer gel.
Reproduced with permission from Philippova, O., Barabanova, A., Molchanov, V., Khokhlov, A., 2011. Magnetic polymer beads: recent trends and developments in synthetic design and applications. European Polymer Journal 47, 542−559. https://doi.org/10.1016/j.eurpolymj.2010.11.006.

ferrite magnetic gels is also reported (Liu et al., 2006a). PVP hydrogels cross-linked by PVA ferrogels can be used for release of bleomycin under magnetic field (Guowei et al., 2007), whereas PVA-borate crosslinked gels can also be destroyed under influence of external field to effect drug release as shown in Fig. 17.5 (Philippova et al., 2011). Macroporous magnetic hydrogels with interconnected porosity in range ~700 μm and initial elasticity of ~2.5 kPa made from Pluronic-coated Fe_2O_3 nanoparticles, RGD-modified alginate is also used for delivery of cells and bioactive molecules. The macroporous ferrogels show about 70% decrease in height and volume compared with nanoporous gels under external magnetic field. Release of mitroxantrone, growth factor, DNA, and cells is demonstrated. Moreover, cells released from gels maintained appreciable viability (Nguyen and Alsberg, 2014; Gao et al., 2015; Zhao et al., 2011). Ferrogels can be fabricated by a layer-by-layer approach to desired geometries with core−shell architectures using microfluidic flow-focusing technique and used for drug encapsulation and controlled release (Chan et al., 2013; Gong et al., 2009; Campbell and Hoare, 2014). Ferrogels composed of iron oxide and PVA with different arrangement of magnetic nanoparticles were studied for biomolecule diffusions. It has been found that ferrogels with parallelly orientation of particles had the maximum biomolecule diffusion ability followed by isotropic gels, with the perpendicularly oriented gels exhibiting lowest drug diffusion rate (Liu et al., 2015). Switching time duration of the magnetic on−off control also influences drug release (Liu et al., 2006b). The magnetic gel consisting of sodium alginate and ferrimagnetic barium ferrite of particles has been demonstrated for

ketoprofen release (Mitsumata et al., 2008). Smart polymer ferrogels that undergo gelation in tumors under alternating external magnetic field and releases cytotoxic drugs doxorubicin for application as MRI-guided magnetic thermochemotherapy through combining with endoscopic technologies, simultaneously releasing the anticancer drug (Hayashi et al., 2016).

17.5.3 Sensors and actuators

Some of the first works on artificial muscle actuators with magnetoresponsive gels were performed with cylindrical PVA gels containing 0.72 g polymer and 0.96 g particles. Unidirectional change in elongation of magnetic gels excited by a nonhomogeneous field was reported and the contraction of gels was used to produce work by lifting loads. After tuning the field off, the gel is restretched. The theoretical equations for work functions were developed assuming homogeneous deformation and magnetization and magnetic field strength vary linearly, and mechanical and magnetic stress are additive as

$$\lambda_{M,H}^3 - \alpha\lambda_{M,H}^2 - \beta\left(H_h^2 - H_m^2\right)\lambda_{M,H} - 1 = 0$$

where, α is the ratio of nominal stress and the modulus, $\lambda_{M,H}$ is strain under applied external magnetic field where H_h and H_m are the field strength at the bottom and the top of a ferrogel cylinder, and stimulation coefficient is given by the relation

$$\beta = \mu_0\chi/2G$$

where, μ_0 is permeability of the vacuum, χ is susceptibility of gels, and G is modulus.

The mechanical work produced by the active strain can be expressed as follows:

$$W = M\ g\ \delta h$$

where, δh is the displacement of the load.

Approximately 5 mJ of work done was obtained (Zrinyi, 2000).

Magnetic gels are very attractive agents for hyperthermia-based cancer treatment. In this method, specific tissues can be exposed to mildly elevated (41−46°C) compared with physiological temperatures to cause death of targeted cells or increase their susceptibility to radiation. After accumulation of gels at the target site, external magnetic field is applied at certain amplitude and frequency. Magnetic particles undergo hysteresis loss when exposed to a magnetic field raising the temperature at the site while affecting volumetric alterations in a coadministered thermoresponsive polymer and causing cytotoxic drug release. The drug release from gels can be by diffusion-, swelling−diffusion, or degradation-mediated. Magnetic drug targeting potentially avoids the toxic side effects of chemotherapeutic agents. For example, almost two decades back, Gallo et al. used a Ferrofluid EMG II in combination with chitosan polymer microsphere gel to increase the blood−brain permeation of

oxantrazole and observed that the brain contained 100-fold higher accumulation of oxantrazole levels compared with solution administration (Hassan and Gallo, 1993).

MRI is a powerful noninvasive diagnostic modality in which image contrast is achieved due to specific tissue responses to applied sequence of radiofrequency pulses. The response is determined by the proton density of tissues and magnetic relaxation times. Paramagnetic metal ions decrease the T1 relaxation of tissue protons, increasing the intensity of signal, and resultant contrast. Fe_3O_4 is frequently used in combination with polymers such as dextran or silicone. Superparamagnetic iron oxide, on the other hand, causes reduction of T2 relaxation and decrease in signal intensity of normal tissues, enhancing the contrast with metastatic tissues. Magnetic gels, consisting of mostly superparamagnetic particles, can also be tagged with nonmagnetic cells and biomolecules, such as DNA, RNA, enzymes, and enzyme inhibitors (Albretsen et al., 1990; Karumanchi et al., 2002), and manipulated under externally applied magnetic field for in vitro bioseparation or sorting applications. This may be achieved by direct immobilization of ligands that are immobilized on magnetic gel beads incubated with analyte to allow complex formation followed by separation under the field. Alternately, secondary antibodies conjugated to magnetic beads may be used if antigens are not easily accessible or in case where antibodies have poor (Philippova et al., 2011). Fabrication of wireless laminated ferrogel sensor by embedded superparamagnetic nanoparticles has been demonstrated with pH sensor using a poly(methacrylic acid-*co*-acrylamide) pH with validated response up to 0.1 pH unit. The same can be useful for developing implantable sensors of glucose, antigens, etc. (Song et al., 2014). Integration with floroprobes can also be used for cancer theranostics (Alveroğlu et al., 2013). Novel applications of ferrogels in mechanosensing, magnetomechanoactuation for tissue engineering, and regenerative medicine are also anticipated (Santos et al., 2015). Magnetic iron oxide composites with PDMS have been shown to be useful for development of microfluidic channels, which may be an important direction of future research (Tsao and Lee, 2016).

17.6 Conclusions

Magnetic polymer gels are an attractive category of smart materials due to their magnetic susceptibility in presence of external applied magnetic fields. This enables their convenient segregation from other components in a mixture or accumulation at a particular zone of the mixture. Several effects of particle size and diameter are well elucidated. Conjugation with specific ligands further increases the sensitivity and specificity. It is thus expected that new applications of magnetic gels especially in microfluidic separations along with commercial products in drug delivery and artificial actuators will emerge in near future.

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Conducting polymer hydrogels

18

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18.1 Introduction

Gels are generally referred to a networked structure made of highly swollen and tangled polymers. These materials contain significant amount of suitable solvent in a networked structure. The maximum capacity of the liquid in the gel materials that could be held within network is dependent on the structure of the gel network and the polymer–liquid affinity (Siddhanta and Gangopadhyay, 2005). Therefore, a typical gel may contains cross-linked polymer network and holds large amount of liquid solvent within its network. The network is formed of following important components that are as below (Network; Rubinstein and Colby, 2003):

1. **Dangling/Loose/Free end**: It is a part of the original polymer chain that begins from a junction and remains as a free end. Its contribution toward the elasticity of the cross-linked network is negligible, and therefore, it can be considered as a defect in the cross-linked network structure.
2. **Junction**: It is a cross-link from which three or more strands spread out. The number of strands that are directly attached to a junction is called its functionality. Typically, in the random cross-linking (Fig. 18.1) the functionality is usually four. A cross-link may simply connect two chains, but this cross-link is not treated as a junction unless it is a part of an infinite network.
3. **Loop**: It is considered as a defect in the cross-linked gel structure. It is the part of polymer chain that begins and terminates at the same junction, without having any other intervening junctions. These loops come to existence due to intramolecular cross-linking reaction.
4. **Sol fraction**: The sol fraction contains material that could be extracted from gel, including the solvent present.
5. **Strand**: It is part of polymer network/chain that starts at a junction and ends at another without any intervening junctions.

When the liquid in the gel is water, the networked polymer structure is made of either chemically or physically cross-linked waters-soluble polymers, then the material is called as **hydrogel**. Therefore, the hydrogels are considered as 3D cross-linked network structures of hydrophilic polymer chains that possess excellent water-holding capacity because of their hydrophilic structure (Ahmed, 2015; Sun et al., 2016b). The hydrogel networks swell extensively in water and absorb large amount of water, therefore, offer great potential for its application for biomedical purposes (Yu et al., 2016; Billiet et al., 2012).

There are many different parameters that affect the structure of a hydrogel. For example, extent of charge, crystallinity, and association among hydrophobic moieties

Polymeric Gels. https://doi.org/10.1016/B978-0-08-102179-8.00018-1

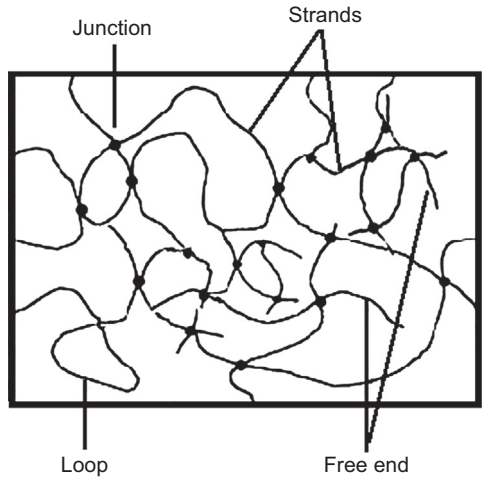

Figure 18.1 Components of network structure of gel.

on the chains are some important parameters. Due to their properties, hydrogels are utilized in contact lenses, foods, and water absorbents. Therefore, extensive research toward biocompatible hydrogels is in progress in the field of bioengineering and biomedical to be used in drug delivery, bioseparations, tissue engineering, etc. (Bahram et al.).

Conducting polymers (CPs) belong to group of artificial polymers and possess ability to conduct electrons. The backbones of these polymers are rigid, stiffened, and have presence of conjugate condition (Wijsboom et al., 2009). The main chain of a CP is made up of aromatic rings that consist of $\pi-\pi$ stacking in the chains, which contribute to the hydrophobic nature of the polymer backbone (Amrutha and Jayakannan, 2008). The stiffness in the CP, sometimes, is also due to undesired cross-linking. The organic moieties can be chemically modified; therefore, different functional group can be introduced to meet the specified requirements in biomedical applications. Consequently, CPs gained good popularity in the last decade, CPs can be used to prepare complex systems that can interact via electrical signals with tissues of brain, nerve, cardiac tissues, and muscle (Bendrea et al., 2011). The advancement in science and technology to process the CPs into diverse forms has favored the designing and fabrication of CPs for bioelectronics and therapeutic applications. Introduction of CPs into hydrogel as a conducting material is an attractive approach to obtain materials with very close resemblance to synthetic soft tissues. These hydrogels are so designed in a way that they possess the mechanical properties similar to that of the native extracellular matrix and also possess electrical conductivity needed for cell-to-cell communication.

Conducting polymer hydrogel (CPH) is the material that contains CP within a cross-linked network structure of insulating polymer in gel. CPHs become swollen in or with addition of water or electrolyte solution due to the supporting polymer as constituents. CPs, such as polyaniline (PANI), polypyrrole (PPY), and poly(3,4-ethylenedioxythiophene) (PEDOT), are electrical conductor whereas the cross-linked

polymers (the supporting and gel forming) constituents are water soluble. CPs are organic semiconducting polymers, exhibit electronic conductivity, and contribute ions in aqueous media (Stejskal et al., 2009; Malti et al., 2016). Besides, electronic conductivity: CPs also exhibit other important features, such as, high sensitivity toward pH change, redox (oxidized state and reduced state) transitions; can be observed from change in its color and electrical conductivity, catalytic and electrocatalytic activity, electromagnetic radiation absorption, adsorption of dyes/heavy/noble metals, or corrosion protection, etc. (Stejskal et al., 2015). Yet, they also have a noticeable disadvantage of their difficult processability. The CPHs may offer suitable application in various fields of interest with desirable properties. Therefore, these CPHs are treated as the most recent composite materials that possess CP of desired morphology. These CPHs exhibit following properties:

- Mixed electrical conductivity (electronic and ionic conductivity).
- Electrochemical reversibility between oxidized and reduced forms of CPs.
- Transition between conducting (salt form) and insulating (base form) in CPs.
- Good flexibility and mechanical integrity.
- Nontoxic and compatibility tissue or cell.
- Porosity and high specific surface area
- Macroscopic homogeneity and controlled morphology.

18.2 Preparation of conducting polymer hydrogels

There are various strategies to prepare CP; these are (1) chemical/electropolymerization of monomers of CP in a prefabricated hydrogel, and (2) mixing the precursor monomers followed by simultaneous or stepwise polymerization (Fig. 18.2). The final hydrogel network formed consists of CPs as physically entrapped within the hydrogel matrix. The most frequent approach is to prepare hydrogel of supporting polymer followed by the preparation of CP in the same hydrogel matrix. The insulating polymer forms the aqueous gel and the CP imparts conductivity to the gel (Stejskal, 2016).

Gilmore et al. (1994) for the first time reported CPH and described PPY-based hybrid composite, (PPY) was electropolymerized directly on a polyacrylamide hydrogel (Fig. 18.2). Water retention capacity was affected by incorporation of the hydrophobic PPY in the polyacrylamide hydrogel. Moreover, these hydrogels became conductive and electroactive. This report opened a new chapter in CP and tremendous increase in research publications describing the CPHs and their biomedical application was observed, due to availability of many hydrogels and number of CPs (Green et al., 2010; Molino and Wallace, 2015). List of important CPHs and their application is given in Table 18.1 (Mawad et al., 2016a).

18.2.1 Preparation of conducting polymer hydrogel (conducting polymers in hydrogel matrix)

In general, in this route, CPH is made of CP embedded in cross-linked water-soluble polymer matrix that swells in water or electrolytes solutions. CPs (PANI, PPY, and

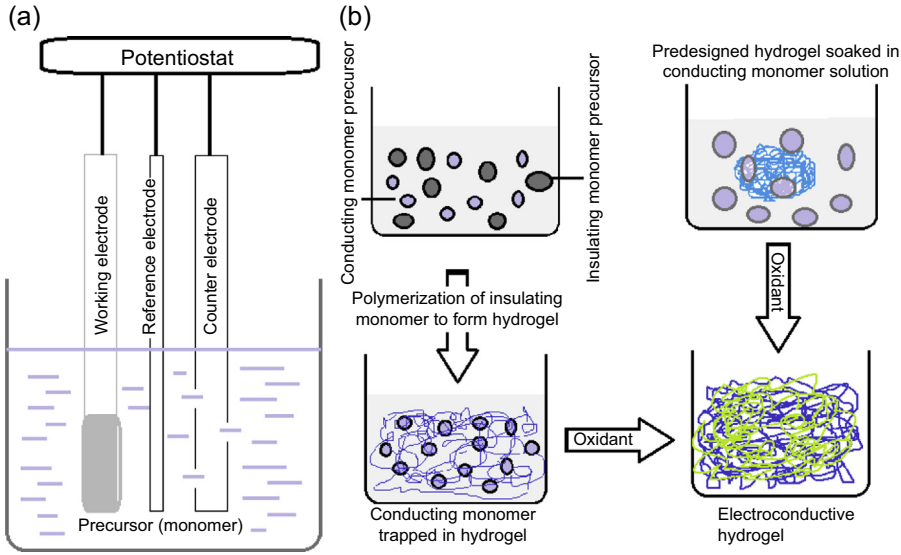

Figure 18.2 Preparation of conducting polymer hydrogen by (a) Electropolymerization and (b) Chemical Polymerization.

PEDOT) have been used in their preparation. The preparation of CPHs starts with the preparation of hydrogel made of water soluble and cross-linked polymer, and then polymerization of monomers such as aniline, pyrrole, etc. already prediffused into the prepared hydrogel matrix (Stejskal, 2016).

18.2.2 Preparation of conducting polymer hydrogels (conducting polymer in the presence of water-soluble polymer)

It is a one-step synthesis procedure to prepare CPHs. The monomer of CP is dissolved in a solution of water-soluble polymer and stabilizers and subsequently, oxidation/polymerization is carried out to get CPH. The presence of stabilizer and high concentration of monomer favor the hydrogel formation. In these hydrogels, hydrogen bonding and chain entanglements are the key factors that contribute to formation of cross-linked network structure in the hydrogel, where contributions of covalent or ionic bonds are negligible (Stejskal and Sapurina, 2005; Dispenza et al., 2006). For example, a hydrogel is formed when aniline is oxidized with ammonium peroxydisulfate in aqueous solution of poly(sodium 4-styrenesulfonate) (Słoniewska and Pałys, 2014). The same may also be prepared under modified conditions. In the oxidation of aniline in a reaction medium having low concentration of stabilizer, poly(sodium 4-styrenesulfonate) colloidal dispersion was the main product (Jia et al., 2012, 2015b). Meng et al. (2007) synthesized PANI hydrogel in presence of poly(ethylene glycol). A hydrogel mass was also obtained when aniline and *p*-phenylenediamine were copolymerized in presence of polyacrylamide (Stejskal, 2015; Smirnov et al., 2016).

Table 18.1 Some important conducting polymer hydrogels (CPHs) and their applications

CPs	Polymerization technique	Networked polymer/agent	Field of applications	References
PANI	Chemical	Heparin	Tissue Engineering	Ding et al. (2014)
	Chemical	Chitosan	Actuators	Marcasuzaa et al. (2010)
	Chemical	Poly(2-hydroxyethylmethacrylate-co-glycidylmethacrylate)	Biosensors	Bayramoglu et al. (2013)
	Chemical	Polyethyleneglycoldiacrylate (PEGDA)	Tissue Engineering	Guarino et al. (2013)
	Chemical	Supramolecular hydrogel	Biosensors	Ma and Zhang (2013)
	Chemical	Phytic acid (cross-linking agent)	Glucose Sensor	Zhai et al. (2013)
PPY	Chemical	Polyacrylic acid	Drug Delivery	Li et al. (2005)
	Electrochemical	Polvinylalcohol	Drug Delivery	Chansai et al. (2009)
	Chemical and Electrochemical	Agarose	Self-healing electrodes and patented films	Hur et al. (2014)
	Electrochemical	Sodium alginate	Supercapacitor	Huang et al. (2017)
	Chemical	Oligo(Polyethylene Glycol) fumerate (OPEGF)	Tissue Engineering	Runge et al. (2010)
	Electropolymerization	Poly(hydroxymethylmethacrylate) (PHEMA)	Implantable biosensors	Kotanen et al. (2013)
	Electropolymerization	PHEMA	Glucose responsive biotransducers	Kotanen et al. (2012)
PEDOT	Chemical	Alginate	Drug delivery	Paradee and Sirivat (2014)
	Electrochemical	Polyurethane	Tissue Engineering	Sasaki et al. (2014)
	Electrochemical	Fibrin	Tissue Engineering	Nagamine et al. (2011)
	Electrochemical	Aragose	Autograft	Abidian et al. (2012)
	Electrochemical	Poly(acrylic acid)/poly(vinylalcohol)	Optogenetic	Lu et al. (2012)

On the other hand, the stabilizer chains present in a colloidal dispersion can be cross-linked by a cross-linker or UV-irradiation. PANI dispersion was made in the presence of poly(2-acrylamido-2-methyl-1-propanesulfonic acid) and followed by cross-linking with poly(ethylene glycol) diglycidyl ether (Mano et al., 2007). A hydrogel was also obtained on irradiation of poly(*N*-vinylpyrrolidone)-stabilized colloid of PANI (Dispenza et al., 2006).

18.2.3 By penetration of conducting polymer in hydrogel

In this approach, CP in solution or colloidal form is allowed to penetrate into preformed hydrogel. This approach has its disadvantage of solubility of CP, besides, the incompatibility of two polymers in a solution is also a drawback of this approach and therefore, only limited reports are available. In addition to these issues, when a CP is allowed to penetrate into preformed polymer hydrogel, negligible or no penetration of CPs into the hydrogel is observed. Instead, only deswelling of hydrogel may be observed. This condition of CPs penetrating into hydrogel is therefore favorable, only if there is very strong interaction between the two polymers (CP and gel matrix) (Mawad et al., 2016b).

Recently, Martínez et al. (2015) successfully introduced PANI (emeraldine base) from its solution in *N*-methylpyrrolidone into a preformed hydrogel. These CPHs were homogeneous in nature and there were uniform distribution of blue color of emeraldine base into the hydrogel matrix. Later, the hydrogel was treated with acid to convert the PANI (emeraldine base) in the hydrogel to conducting PANI (emeraldine salt). Macroporous hydrogels could be formed utilizing this approach. Luo et al. (2015) reported penetration of poly(vinyl alcohol)-stabilized colloid of PPY into poly(*N*-isopropylacrylamide) hydrogel. Vishnoi and Kumar (2013) prepared composite hydrogel containing surfactant stabilized PPY colloid mixed with chitosan or gelatine solution. However, phase separation was also expected to take place with time. Molina et al. (2011) reported penetration of PANI colloid (stabilized in PVP) into poly(*N*-isopropylacrylamide)-based hydrogel.

18.2.4 Conducting polymer hydrogels preparation in presence of conducting polymer

This is, perhaps, the simplest route to prepare CPHs. Herein, particles of CP are dispersed in a solution of cross-linking materials. The cross-linking is completed with the aid of gelation or chemical or radiation. Though the process seems to be very promising yet it has drawback like other processes. Nonhomogeneous and insufficient dispersion of CP particles cause sedimentation during gelation or cross-linking. Zhang et al. (2009) dispersed polyamine nanofibers in agarose solution at 75°C and cooled it to obtained CPH on cooling it to ambient temperature. Baniasadi et al. (2015) used PANI-coated graphene to prepare CPH in chitosan solution. Liu et al. (2013) and Yuan et al. (2015) prepared PANI hydrogel in two different reaction mixtures, the former group suspended PANI powder in reaction mixture of

poly(acrylamide-co-sodium methacrylate) with tetramethylethylenediamine/peroxydisulfate, whereas the latter group suspended PANI powder in poly(acrylic acid) hydrogel. Petrov et al. (2016) used UV light to induce cross-linking in frozen suspension of PANI in hydroxyethylcellulose solution. Lee et al. (2016) and Castro et al. (2015) used similar method to incorporate PEDOT and PPY in polyacrylamide gel respectively.

18.2.5 Preparation by simultaneous polymerization/oxidation

It is a single-step procedure for preparation of CPHs. Herein in this method, the CPHs are prepared by performing two simultaneous reactions: polymerization and gelation/cross-linking. Sometimes, these reactions may or may not take place at the same time. During the process, some amount of heterogeneity may also be present and therefore should be avoided. To overcome this heterogeneity in the final hydrogel, Sharma et al. (2014b) used irradiation to induce polymerization and cross-linking to prepare PANI hydrogel.

18.3 Gel formation theory

The bulk structure of CPHs plays important role in determining their properties. Besides, few other parameters, such as, swollen state volume fraction, mesh size, and the molecular weight of the polymer chain between neighboring cross-link points are used for characterization of the network structure. The volume fraction of polymers in the swollen state is a measure of fluid that can be absorbed and retained in the matrix. The molecular weight between neighboring cross-link points, either covalent bond or physical interaction, describes the degree of cross-linking. These parameters are related to each other and can be calculated theoretically or determined by a variety of experimental techniques. The two most widely acceptable methods, the equilibrium swelling theory and the rubber elasticity theory, are discussed below (Ganji et al., 2010; Chai et al., 2017).

18.3.1 Equilibrium swelling theory

The mixing of polymers with liquid molecules in hydrogels, in ionic domains can be analyzed using Flory-Rehner equation (Flory and Rehner, 1943). The equilibrium of the hydrogel swollen in a fluid is a function of two opposite forces, swelling-favoring thermodynamic force and swelling-restricting force (stored in the stretched polymer chains) (Murakami and Maeda, 2005). These two forces balance each other as described in Eq. (18.1) for the physical situation in terms of the Gibbs free energy:

$$\Delta G_{total} = \Delta G_{elastic} + \Delta G_{mixing} \tag{18.1}$$

Where $\Delta G_{elastic}$ refers to elastic forces stored in stretched polymeric chains contained in the gel networks; ΔG_{mixing} is due to the mixing between fluid and the polymeric

chains. The mixing factor is a measure of the compatibility of the polymer and solvent molecules, expressed by (p-s) interaction (Flory, 1953).

Differentiation of Eq. (18.1) with respect to the number of solvent molecules, while keeping the temperature and pressure constant, gives Eq. (18.2):

$$\mu_1 - \mu_{1,0} = \Delta\mu_{\text{elastic}} + \Delta\mu_{\text{mixing}} \tag{18.2}$$

In the equilibrium state, the chemical potential outside the gel should be equal to the chemical potential inside the gel ($\mu_1 = \mu_{1,0}$). As a result, the chemical potential change because of free energy of mixing and elastic force (stored in the stretched polymer chains) cancels out each other. In aqueous system, there occur significant changes in chemical potential because of elastic forces that are responsible for the change of volume fraction density of the polymer in the cross-linking process (Peppas and Merrill, 1977). The situation becomes even more complex due to presence of ionic species in the hydrogel due to the formation of intricate system of ionic domain and polymer chains.

18.3.2 Rubber elasticity theory

From a mechanical perspective, hydrogels resemble elastomers that deform elastically due to applied stress. Treloar (1975) and Flory et al. (1949) described their structure with the aid of the elastic properties of the hydrogels. However, the original elasticity theory does not hold valid for hydrogels prepared in solvent. The theory of rubber elasticity by Peppas as in Eq. (18.3) (Peppas et al., 2000) is the only form applied to evaluate the hydrogel structure, when these are prepared in solvent.

$$\tau = \frac{\rho RT}{\overline{M}_c}\left(1 - \frac{2\overline{M}_c}{\overline{M}_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} \tag{18.3}$$

where τ, ρ, R, T, and M_c are applied stress to sample; density of the polymer; universal gas constant; experimental temperature in Kelvin; and molecular weight between cross-links, respectively. To utilize this elasticity theory for the analysis of structure of the hydrogel, experiments must be performed in the tensile mode (Lowman and Peppas, 1997).

18.4 Mechanism of gelation

There are numerous processes involved in a thermally induced sol−gel transition, for example, hydrophobic and hydrophilic interactions, coil to helix transition, micelle packing, etc. Therefore, to understand the gelation mechanism for certain polymers, information about the molecular level process plays major role (Klouda and Mikos, 2008). The most widely reported thermally induced gelation is based on the equilibrium between hydrophobic and hydrophilic interactions. For example, introducing a hydrophobic segment such as methyl, ethyl, or propyl to hydrophilic polymers is an

efficient way to tune the hydrophobicity of the polymer (Qiu and Park, 2001). There is a temperature value commonly known as lower critical solution temperature (LCST) below which the system will be miscible and above which phase separation will occur, forming gels. Interactions between polymer and polymer (p−p), polymer and water (p−w), and water and water (w−w) take place in aqueous polymer solutions. The LCST of the system depends on the equilibrium of these interactions. The most efficient way to determine LCST is by light scattering, with the collapse and aggregation of the polymer chains during gelation state inducing a dramatic increase of the light scattering (Gao et al., 2013). Thermodynamically, the thermally induced change in the solubility is controlled by the Gibbs free energy of mixing (Schild, 1992). A small change in temperature can cause negative change in the Gibbs free energy. As a result, the interaction between polymer and water (p−w) will be eliminated, and the water−water (w−w) and polymer−polymer (p−p) interaction will be favored. To compensate this negative Gibbs free energy change, there should be an increase in the entropy and enthalpy. In case, if the hydrophobic interaction increases between the polymer chains, at the sol−gel transition temperature, the polymer chains dehydrate quickly and collapse to a more hydrophobic structure (Ruel-Gariépy and Leroux, 2004). On the other hand, there will be formation of micelle structures of the amphiphilic block copolymers due to the hydrophobic interactions to equilibrate the decrease of the Gibbs free energy (Mortensen and Pedersen, 1993). Depending on the concentration, amphiphilic block copolymers can form micelles, which are aggregates of surfactant molecules dispersed in a liquid colloid and hydrogels by adding water and adjusting the temperature. These block copolymers build up a structure with a hydrophobic core and hydrophilic shell with typical micelle size between 20 and 100 nm. The mechanisms discussed are based on reversibility of physical linkage.

18.5 Properties of conducting polymer hydrogels

The common properties of CPHs are discussed in this section. It is important to mention that the structural inhomogeneity is most common in CPHs. Therefore, the properties of these materials depend on the shape and size of the hydrogel and so, comparison of their properties is very complex and inference toward these may be made only after critical examination. Some important properties are listed below (Stejskal, 2016).

18.5.1 Electrical conductivity

The electrical conductivity is an important property of CPHs. The comparative study of different hydrogels is difficult due to few reasons: (1) The electrical conductivity of hydrogels is also contributed by water (contains ionic species) in addition to the CP, (2) the hydrogels are heterogeneous and the electrical conductivity depends on the shape and the size of hydrogels, (3) other components present in hydrogel may also affect the electrical conductivity depending on their nature and proportion in the

hydrogel, and (4) the experimental set-up error during the experiments may also be present. Some general trends in different CPHs are discussed below:

The electrical conductivity in most of the CPHs is below 10 mS/cm. It is believed that major contribution to the electrical conductivity of these hydrogels is mainly from conductivity (ionic) of electrolyte solution in which the hydrogel swells and the contribution of CP is very small or negligible. It may be due to the small amount of CP in the hydrogel. The electrical conductivity of PANI: poly(acrylic acid)-graft-poly(ethylene glycol) hydrogels was reported to be ~11.50 mS/cm and the hydrogel without PANI possessed an electrical conductivity 9.28 mS/cm (Liu et al., 2015). The electrical conductivity of hydrogel based on PANI and phytic acid was approximately ~0.2 S/cm whereas an increase in the electrical conductivity to ~1.5 S/cm was found when certain definite amount of multiwall carbon nanotubes was incorporated into it (Chen et al., 2015). The PPY-based hydrogels have also possessed approximately same range of electrical conductivity to that of PANI hydrogels due to their overall small contribution. Qin et al. (2014) observed slight increase in the electrical conductivity of poly(2-hydroxyethylmethacrylate) hydrogel (9 mS/cm) on incorporation of PPY (13 mS/cm), while Wang et al. (2015a,b) reported higher electrical conductivity (~0.5 S/cm) of hydrogel containing PPY and reduced graphene oxide. A higher electrical conductivity (7.8 S/cm) was also observed in PPY prepared while sulfonated copper phthalocyanine was present during the reaction. The PPY and graphene oxide−based hydrogel had a conductivity of 18 mS/cm (Chen et al., 2014) or of the order of 0.1 mS/cm; in the latter case, the conductivity was lowered to significant amount when the sample was exposed to alkaline medium. The aerogel from PPY and polyacrylamide-based hydrogel had an electrical conductivity of ~0.08 S/cm (Shi et al., 2014b).

18.5.2 Mechanical properties

The mechanical properties of CPH depend on the chemical nature of its individual components. The mechanical properties of these hydrogels depend on the nature of covalent bonds, morphology, and cross-linking within the hydrogel. The hydrogels produced as a result of weak forces such as hydrogen bonding or van der Waals forces are found to have reduced mechanical strength. The mechanical properties of CPHs are also affected by amount of CP and the supporting polymeric component in the hydrogel, the swelling ratio as well.

Ideally, the CPs should be connected and form networked structure. But, in practice, CP often acts like particulate filler. It is present as microseparated areas of CP clusters widely dispersed in the matrix of supporting polymer (Meng et al., 2007). The mechanical properties of CPHs are also influenced by the presence of CPs in microcluster due to heterogeneous distribution. The mechanical properties, such as elasticity, have negligible or no role in elastic property of CPHs and the elasticity in provided mainly by the supporting polymeric components. Due to the heterogeneous distribution of CP in the CPH, the mechanical properties of CPH get affected due to the size and shape of cluster that enters the samples during the mechanical testing. The relative comparison of the mechanical properties of samples could be made possible, only if the details of their preparation route and testing methods are same.

18.5.3 Electrochemical characteristics

The electrochemical behavior of CPHs is mainly because of switching behavior of CPs into their individual redox (oxidized or reduced) forms. Cyclic voltammetry could be used to demonstrated the electrochemical behavior of PANI (Dou et al., 2016), PPY (Tang et al., 2015), and PEDOT (Dai et al., 2015) hydrogels.

18.6 Applications of conducting polymer hydrogels

The CPHs are utilized essentially for their applications in two main research streams: biosciences and energy conversion and storage. The research fields in biomedicine involve biosensors, drug-release devices, and neural prostheses, etc. (Guiseppi-Elie, 2010; O'Connor et al., 2015; Sun et al., 2016a). These may focus on growth simulation of stimulation of cell or tissues growth, artificial muscles, control and close observation of vital functions, etc. Some applications require the CPHs to be biologically compatible (Humpolicek et al., 2012) and CPHs-containing PANI were found to be suitable for such application at subcutaneous level (Li et al., 2015). The CPs are poor at biodegradability, environmentally very stable (Stejskal et al., 2012), and can be combined with biodegradable polymers, such as pectin (Zhao et al., 2016) to suit their application-wearable electronics.

Many energy conversion/storage devices are based on the electrical and electrochemical properties of CPs (Kim et al., 2015; Shi and Yu, 2016). Typically, the batteries convert the chemical energy into electrical energy; the capacitors store the electric energy, and supercapacitors combine both in various proportions. CPHs offer flexibility and stretchability as their valued properties for suitable application, as the redox (oxidized and reduced state) switching causes volume change, which is associated with elastic materials. Incorporation of electrolyte in CPHs offers ionic conductivity in CPHs in addition to electronic conductivity due to CPs. Various applications of CPHs have been proposed, which are discussed below.

18.6.1 Actuation

Mechanical characteristics are very important in such applications. Actuators are designed to response the change in volume, electric stimuli, etc. of the materials. It was tested on PANI-based CPH as a result of applied voltage (Siddhanta and Gangopadhyay, 2005), and PANI was reduced to its leucoemeraldine form at one end of the CPH and at the other end it oxidized to pernigraniline form (Stejskal et al., 1996). The structural gradient was demonstrated on PANI/alginate-based CPH (Srinivasan et al., 2015), and ethanol was released asymmetrically, which results in the surge of hydrogel on water surface. In another PANI-based hydrogel, crawling was observed in electric field, toward anode with approximate speed of 15 mm/s (Shi et al., 2013). The oscillatory bending was observed in PANI and chitosan-based CPH at an applied DC voltage, which could be attributed to the combined effect of protonation dynamic redox and phenomena (Kim et al., 2006).

18.6.2 Adsorbents

PANI (emeraldine salt; PANI-ES) acts as a polycation. The interaction of PANI-ES with anionic species is highly anticipated; however, this may or may not hold true in every case. PANI-based CPHs were tested for dye adsorption and release (Tang et al., 2008). In particular, PANI and phytic acid CPH was used to demonstrate the adsorption of methylene blue (Yan et al., 2015). It was explained that the dye molecules interacted with phosphate groups of phytic acid, instead with PANI. In PANI and polyacrylamide CPH, cationic safranine dye was removed due to electrochemical changes in the CPH as these gel undergo contraction on electrochemical oxidation, which results in expulsion (Lira and de Torresi, 2005). PANI-poly(acrylic acid)-gum ghatti CPH was analyzed for moisture retention capacity in soil cultivation (Sharma et al., 2014a). Carbonized PANI and poly(styrene-4-sulfonate)-based CPHs were used to adsorb creatinine or vitamin B12 (Jia et al., 2015a,b).

18.6.3 Biomedical application

Bajpai et al. (2009) studied biocompatibility of PANI and poly(vinyl alcohol)-based CPH for blood coagulation. No cytotoxic effect was observed when human skin fibroblasts were grown in PANI and bacterial cellulose−based CPHs (Shi et al., 2014a). PANI containing injectable hydrogel was tested for cell multiplication/growth and cytotoxicity, and in vivo inflammatory responses were found to fulfill the criteria for biomedical applications. PANI and gelatin CPH was studied for cell adhesion, myoblast cells, and mesenchyma stem cells. PANI and hydroxyethylcellulose CPH was found to affect the cell growth on application of electric potential. These CPHs were found to be nontoxic, bioactive material for tissue regeneration (Li et al., 2015).

Biodegradability of CPHs should be addressed with immediate consideration. Therefore, gelatin has become a favorite choice as supporting polymer while designing a biodegradable CPH (Tang et al., 2008). Gelatin gets easily hydrolyzed enzymatically or in acidic solutions (Blinova et al., 2009; Bhowmick et al., 2013). Hydrogels containing poly(acrylic acid) or guar gum (Kaith et al., 2015) or pectin (Zhao et al., 2016) were also found to be biodegradable. Though the supporting non-CPs used are usually biodegradable, the CPs are very stable (as discussed earlier) and do not biodegrade under normal conditions (Brozova et al., 2008).

18.6.4 Controlled release of drugs

Many drugs may interact with CPs, such as PANI, and get bonded by weak interactions. The combined PANI-drug system is released in a suitable condition, i.e., under specific physiological conditions. PANI/poly(acrylic acid−graft gum ghatti) CPHs and PANI/polyacrylamide CPH showed pH-dependent release of amoxicillin trihydrate (Sharma et al., 2016; Perez-Martınez et al., 2016).

18.6.5 Electrodes and electrolytes

PANI-based CPHs were utilized in supercapacitors as electrodes (Jayakumar et al., 2015). PANI and phytic acid−based CPHs containing nickel oxide (Zhang et al., 2015); tin (Zheng et al., 2016); or silicon nanoparticles (Oh et al., 2015) were used as electrodes in lithium ion (Li^+) battery. PANI/PPY and poly(acrylic acid)-based CPH electrolytes may also be utilized in dye-sensitized solar cell applications (Yuan et al., 2015).

18.7 Conclusion and future prospects

CPHs are emerging materials and their potential could be utilized in different fields, such as, biomedicine, energy conversion, and energy storage, etc. Presence of CPs in CPHs offers the electronic conductivity, redox activity, and response to external stimuli. The insulating or supporting polymer networks provides integrity, strength, and elasticity, etc. to the CPHs. The aqueous phase within the CPHs favors ionic conductivity. The combination of properties of hydrophobic/insulating polymer and CPs in the CPHs result in materials with unique properties. The control over the designing of polymer morphology in the nanometer range could be an advantage to produce new functional materials. As can be seen that the research is still in the laboratories, however, promising results of previous reports offer many possibilities for the future. By the use of carbon nanotubes, nanoscale CP could be used to develop superior quality biocompatible materials, advances in drug delivery, and so on.

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Polymeric gels for biosensing applications

19

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19.1 Introduction

The fast progress in medicinal therapies in past five decades has contributed to the higher demand for patient monitoring in healthcare systems. This growth led to the development of invasive and non-invasive monitoring of physiological parameters, such as blood pressure, heart rate, and body temperature and also levels of several essential metabolites such as glucose, proteins, lipids, hormones, neurotransmitters, antibodies and nucleic acids in the body. Early detection of these metabolites can save one from life-threatening events like heart failure, diabetes, neural disorders, obstructive liver disorders, kidney failure, cancer, bacterial and viral infections (Ridker, 2003; Rowe et al., 1999; Turner and Pickup, 1985; Schumacher et al., 2013; Kruse et al., 1987; Fragkou, 2010; Soper et al., 2006). This led to the emergence of a new category of sensing analytical devices need for quantification of these analytes in biological sources named as "Biosensors." Biosensors are analytical devices which require the perfect combination of transducer and biological recognition element in an appropriate sequence to detect the targeted analyte (Thévenot et al., 2001). Biological elements and transducers are explained later in the chapter in detail. The regular need for metabolites analysis leads to the miniaturisation of these devices, for example, blood glucose monitoring devices (Wang, 2008). The miniaturisation of biosensor poses the problem of the high enzyme, antibodies, or whole cell loading capacity to get better and rapid detection of the analyte. So bioelement loading capacity of support matrix should increase and signal should be propagated fast to the transducer for rapid and noise-free detection (Mehrvar and Abdi, 2004). Other than this, the microenvironment around the bioelement should favour its proper functioning. Polymeric gels especially hydrogel was identified as a unique answer to such problem (Mohamad et al., 2015). Hydrogels are hydrophilic polymeric molecule network and can absorb a large quantity of water and the water-soluble analyte. Thus, the hydrogel can provide a cell or tissue-like environment to the bioelement to function naturally (Tibbitt and Anseth, 2009). A bioelement can be either entrapped or adsorbed into a hydrogel which can be then deposited over the surface of the transducer element directly (Albareda-Sirvent et al., 2000; Cosnier, 1999). Later on, polymers with various functionalities such as conductivity for rapid electron transfer in case of the electrochemical biosensor, more transparency as in case of optical biosensors and stimuli-responsive gel to as an add-on to the whole biosensor detection setup introduced while fabricating the biosensor.

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19.2 Biosensors and its elements

According to International Union of Pure and Applied Chemistry (IUPAC) a biosensor or biological sensor is "A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" or in other terms convert a biological response to physically detectable response (Thévenot et al., 2001). Though, people used to check biological response towards toxic species from ancient times. Natural signals like massive reduction in fish population in water bodies, non-seasonal yellowing of leaves, crop dying in the fields were considered as the signs of danger for living beings caused by water or soil contaminants. In the nineteenth century, canaries and sometimes mice were used in coal mines to alert the miners for the presence of poisonous gas. Until recently, the behavioural or phenotypical disorders were considered to be a biological indicator of chemical hazards in the environmental impact assessment. Other than the detection of toxic species, biological responses have used for evaluation of food and drinks samples quality at industrial scale. What is typical for such approaches, the conclusion regarding the content and nature of the chemicals is substituted by the decision about their biological effect. Even though being very sensitive, biological indications based on taste, smell, and other senses, do not meet the standard requirements of the analytical detection systems. However, component from the biological origin (Bio-element) can be used to mimic such response recognition. That is why the biosensors invented and developed.

A biosensor can be considered to be a direct descendant of the coal mine canary, made up of bio-element and sensor/transducer element. The signals originating due to the interaction of analyte and bio-element/biological entity are utilised as recognition response in a typical biosensor assembly. The biological signals are then transformed via the sensor element (transducer) so that the output (current, potential, optical density) does not differ from that of conventional measurement devices. This signal can be amplified, processed and quantified against an internal and external standard. A combination of the high sensitivity and biochemical "sense" exerted by the biological part with accuracy and certainty of the physical counterpart was the main idea of the biosensor development. A general scheme of the biosensor assembly is presented in Fig. 19.1.

19.2.1 Biosensor classification

The most frequently used classification of biosensors is based on the type of biological recognition element or bio-element being used during the designing process. Though, biosensors are also classified by mode of signal transducer employed. Different kind of bioelement and transducers utilised in the fabrication of a biosensor has been highlighted in Fig. 19.2.

The biosensor recognition element or bio element may further be classified into biocatalytic recognition element (composed of mono or multi-enzyme, whole cell or tissue) and biocomplexing or bioaffinity recognition element (e.g., antibody-antigen, Receptor/antagonist/agonist). Biocatalytic recognition element based biosensor or

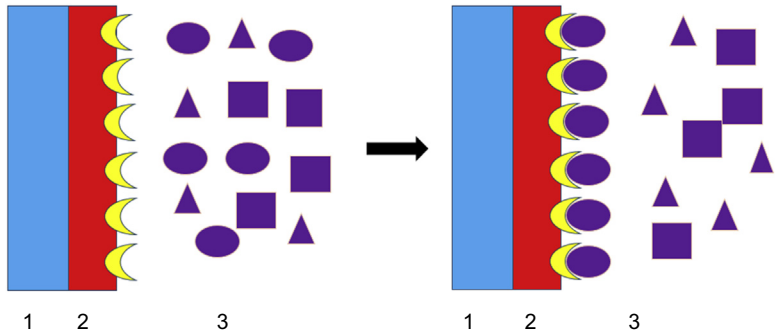

Figure 19.1 Scheme of a biosensor assembly, 1-transducer or sensor element, 2-bio element, 3-analyte.

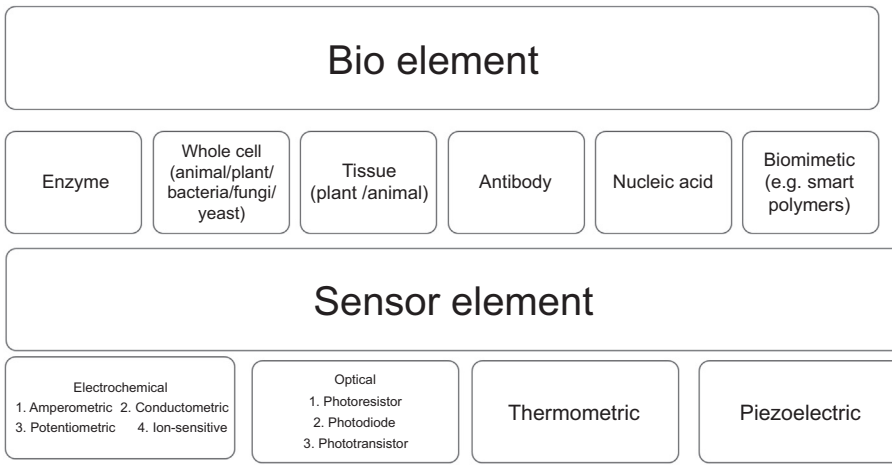

Figure 19.2 Biosensor classification chart according to bioelement and sensor element.

biocatalytic biosensor is the best known and well-studied one. In the biocatalytic biosensor, a reaction is catalysed with the help of an immobile biomolecule. One or more analyte (substrate and co-substrate) are converted to products. The analyte consumption is then monitored either by measuring the consumption of co-substrate or formation of the reaction product. Other than an enzyme, whole cell (microorganisms, such as bacteria, fungi, eukaryotic cells or yeast), cell organelles, cellular particles (mitochondria, cell walls) and tissues (plant or animal tissue slice) are also used as biocatalytic recognition element in biosensors. Among various available biocatalytic recognition element enzymes were the first element to be used in biosensors and remains the most widely used bioelement (Clark and Lyons, 1962).

In the case of biocomplexing or bioaffinity recognition element, the biosensor operation is based on the interaction of the analyte with macromolecules or organised molecular assemblies. The macromolecules are either isolated from the biological entity

or engineered. There is no further net consumption of the analyte by the immobilised bio-complexing agent once the analyte and macromolecule interaction has reached an equilibrium. The integrated detector monitors these equilibrium responses, and in some cases, this bio-complexing reaction itself is monitored by a complementary biocatalytic reaction. The most common examples of such biosensors are immunochemical reactions (antibody-antigen interaction) based biosensors. In immunochemical reactions basically, an antigen (Ag) binds to a specific antibody (Ab), and this complex formation is then quantified using enzyme-coupled antibodies or antigens. Formation of Ab-Ag complexes has to be detected under the condition where nonspecific interactions are minimised.

Other than this, receptor/antagonist/agonist can also be utilised as bio-complexing elements. Ion channels, membrane receptors or binding proteins are used as molecular recognition systems. A developing field in electrochemical biosensors is the use of chips and electrochemical methods to detect binding of oligonucleotides (gene probes). There are two approaches currently in use. The first approach involves intercalation of the analyte into the oligonucleotide duplex, during the formation of a double-stranded DNA on the probe surface, a molecule that is electro-active. The second approach directly detects guanine which itself is electroactive (Thévenot et al., 2001).

On the basis of signal transduction or transducer/sensor element used biosensor can be broadly classified as electrochemical biosensors, optical biosensors, a piezoelectrical and thermal biosensor.

19.2.2 Electrochemical biosensors

Electrochemical biosensors are simple devices based on the measurements of electric current, ionic or conductance changes carried out by bioelectrodes. The electrochemical biosensor may be further classified as Amperometric, Potentiometric and Conductometric depending on the type of electrochemical parameters measured.

19.2.2.1 Amperometric biosensor

An Amperometric biosensor as the name suggests based on the measurement of current resulting from the electrochemical oxidation or reduction of an electroactive species. It is operated by maintaining a constant potential at the working electrode concerning reference electrode if currents are low (10^{-9} to 10^{-6} A). The resulting current is directly correlated to the bulk concentration of electroactive species or its production or consumption rate within the adjacent biocatalytic layer. The oxygen probe introduced by Leland C. Clark Jr. in 1956 (Clark and Lyons, 1962) was the first and simplest form of the Amperometric biosensor. The probe indicates the amount of dissolved oxygen using electrochemical reduction of oxygen and associated electrolytic current as a response signal. Amperometric transduction is also suited for affinity sensors provided that an electrochemically active compound be attached to the recognition product and act as an electrochemical label. Some of the nucleobases included in the nucleic acid structure are electrochemically active, and their electrochemical reactions are used to monitor the recognition by hybridization.

19.2.2.2 Potentiometric biosensors

In potentiometric Biosensors, the electromotive force (e.m.f.) generated in the galvanic cell consisting of two electrodes immersed in the electrolyte solution is measured by a high impedance voltmeter (Fabry and Siebert, 1997). The measurement is performed without any external polarisation of the circuit at about zero currents. By the theory, the e.m.f. value is mainly determined by the difference in the potential of the electrodes. One of them, the potential of which is assumed to be constant, is called a reference electrode and the other a working electrode. Most commonly used potentiometric devices are pH electrode, gas electrode and ion selective electrode (Winquist et al., 2000). For potentiometric measurements, the relationship between the concentration of the analyte and the potential is given by the Nernst equation (Compton and Banks, 2011) which is as follows:

$$E_{cell} = E_o - \frac{RT}{zF} \ln Q$$

Where E_{cell} is the electromotive force, E_o is the standard electrode potential, R is the universal gas constant, T is the Kelvin temperature, z is the charge no of electrode reaction, F is the Faraday constant and Q is the ratio of the ion concentration at the anode to ion concentration at the cathode.

19.2.2.3 Conductometric biosensors

Conductometric biosensors are based on the measurement of the ability of an analyte or a medium to conduct current between electrodes or reference electrodes. Many enzyme reactions result in the changes in the ionic strength of the sample, which can be measured by the conductometric device. Because the sensitivity of the measurement is hindered by the parallel conductance of the sample solution, usually a differential measurement is performed between a sensor with enzyme and an identical one without enzyme. The technique does not differ significantly from the conventional conductometers used. A typical conductivity meter applies an alternating current at an optimal frequency to two active electrodes and measures the potential. Both the current and the potential are used to calculate the conductance. Different analyte such as glucose, creatinine acetaminophen, urea and phosphate have been reported to be determined by using conductometric biosensors (Razavi and Janfaza, 2015).

19.2.3 Optical biosensors

Optical biosensors are based on the light emission or light absorption by the sample. Optical biosensors have been developed parallel to the electrochemical biosensors. Though, initially, they were found in the form of test strips or indicating tubes with visual detection of the colour change (Lodeiro et al., 2010). These biosensors are based on the optical measurements such as absorption, reflection, refractive index, fluorescence, chemiluminescence and phosphorescence (Velasco-Garcia, 2009). Optical biosensors allow a safe non-electrical remote sensing of materials. Another advantage

is that these biosensors usually do not require reference sensors, as the comparative signal can be generated using the same source of light as the sampling sensor. The absorbance-based optical biosensor is the most simple and easy to operate devices and based on the process of absorption. Absorption is a process in which light energy is absorbed by an atom or a molecule, promoting the molecule from the ground energy state to a higher energy excited state. The resulting energy is dissipated non-radiatively (i.e., thermally) to the medium when the excited state relaxes to the ground state.

The absorbance changes are related to the concentration [C] via the Beer−Lambert relationship:

$$A = \log\left(\frac{I_o}{I}\right) = \varepsilon \cdot [C] \cdot l$$

Where A is the optical absorbance, I_o and I are the intensities of transmitted light in the absence and presence of absorbing species. In practice, an optical biosensor consists of a light source, the optical transmission medium (fibre, waveguide, etc.), immobilised biological recognition element (enzymes, antibodies or microbes), optical detection system. The light source can be a Light emitting diode (LED) or lasers, and optical detection can be done by using photoresistor (Light depended resistor), photodiode and phototransistor. A photoresistor is extremely sensitive to light irradiation and is more sensitive than photodiodes and phototransistors. Though, the linear response may vary according to the application. The most common application of this type of biosensor is to determine the glucose level in blood (Newman and Turner, 2005; Moreno-Bondi et al., 1990), in which immobilised glucose oxidase oxidised the glucose to produce hydrogen peroxide which further oxidised the weakly coloured chromogen (e.g., *O*-toluidine) to a highly coloured dye (Bauer, 1983).

19.2.4 Piezo-electrical biosensors

Piezoelectric biosensors are based on the principle of acoustics (sound vibrations). Hence they are also called as acoustic biosensors. Piezoelectric crystals form the basis of these biosensors. The crystals with positive and negative charges vibrate with characteristic frequencies. Adsorption of specific molecules on the crystal surface alters the resonance frequencies which can be measured by electronic devices. Enzymes with gaseous substrates or inhibitors can also be attached to these crystals. Piezo-electric crystals (e.g., quartz) vibrate under the influence of an electric field. The frequency of this oscillation (f) depends on their thickness and cut, each crystal having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorb from the surface of the crystal, obeying the relationships:

$$\Delta f = \frac{Kf\Delta m}{A}$$

Where Δf is the change in the resonate frequency (Hz), Δm is the change in mass of absorbing material (g), K is a constant for the particular crystal dependent on such

factors as its density and cut, and A is the absorbing surface area (cm^2). A piezoelectric biosensor for organophosphorus insecticide has been developed incorporating acetylcholine esterase (Bachmann et al., 2000). Likewise, a biosensor for formaldehyde has been developed by incorporating formaldehyde dehydrogenase (Dennison et al., 1996). A biosensor for cocaine in the gas phase has been created by attaching cocaine antibodies to the surface of the piezoelectric crystal (O'Sullivan and Guilbault, 1999).

19.2.5 Thermal biosensors

Thermal Biosensors is based on the reactions associated with the absorption and production of heat, which in turn changes the temperature of the reaction medium. Many enzymes catalysed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. This represents the most applicable type of biosensor. The temperature changes are usually determined using thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment. Devices such as thermistors or thermopile are usually incorporated to measure the heat change. These biosensors are mostly used in bioprocess monitoring (Ramanathan et al., 1999) and simultaneous determination of multiple analyte cholesterols, glucose, penicillin G. and other metabolites (Paul, 2008; Subramanian et al., 2002; Bataillard et al., 1993).

19.3 Polymers as immobilisation matrix in biosensors

For proper functioning of a biosensor, its biological element should recognise and generate a readable signal. As discussed earlier the biological element can be an enzyme, whole cells, antibodies, molecular assemblies or tissues. These bioelements should be immobilised onto an appropriated matrix surface which in turn is in connection with the sensor element that can register and read the signals (Albareda-Sirvent et al., 2000). The immobilisation matrix should provide not only a support and stable medium like an extracellular matrix for the bioelement but also a medium which efficiently transfers the generated signals to sensor element (Cosnier, 1999). Polymeric gels especially hydrogels are an active matrix suitable for bioelement immobilisation (Fig. 19.3) (Teles and Fonseca, 2008). As previously discussed hydrogels are hydrophilic, water imbibing polymeric network, that can provide a cell-like environment to bioelement especially enzymes to function. There are different types of immobilisation strategies available (Garcia-Galan et al., 2011). The simplest one is adsorption of bioelement on immobilization matrix, which has been explored in the development of various glucose biosensors those are commercially successful (Fig. 19.3) (Fragkou, 2010; Wang, 2008; Wilson and Turner, 1992; Tang et al., 2004; Liu et al., 2005).

Covalent attachment of bioelement especially enzyme to a solid support produce a highly stable system. However, sometimes it reduces specific activity of an enzyme (Iyer and Ananthanarayan, 2008; Sheldon and Van Pelt, 2013; Brena and Batista-viera, 2006). The sensitivity of such system is limited as the monolayer of the enzyme is

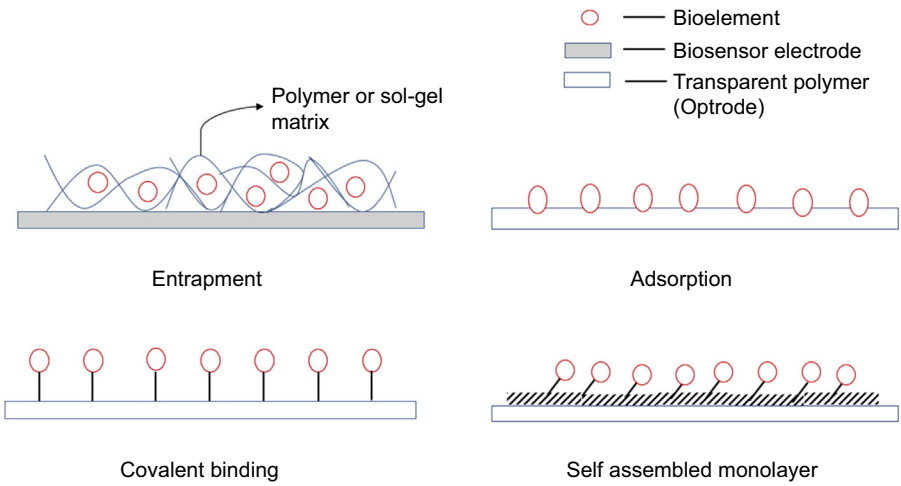

Figure 19.3 Bioelement immobilization over metal/optical electrode for biosensor development.

deposited on solid support (Urban and Weiss, 2010). This limitation can be resolved by entrapping the bioelement specially enzyme inside a biocompatible three-dimensional inert structure of hydrogels (Sheldon and Van Pelt, 2013; Brena and Batista-viera, 2006). Entrapment procedure enhances the loading of enzyme and also support continuous measurement of the signal. The hydrogel environment can also be tweaked according to the specific need of enzyme and thus can give long-term stability to the enzyme (Sheldon and Van Pelt, 2013; Brena and Batista-viera, 2006). Hydrogels are also useful as they can link the enzyme/other bioelement to the solid surface. As a result of hydrophilic nature and porous structure of hydrogels, the native conformational state of the bioelement is maintained (Iyer and Ananthanarayan, 2008; Wang et al., 2005; Shiroya et al., 1995). Hydrogel can be a suitable matrix for bioelement immobilisation due to following reasons

- They increase the bioelement loading due to increased surface area of the solid matrix
- Provide suitable environment for the long-term stability of the bioelement
- Provide an environment for ligand diffusion and interaction with bioelement for recognition and detection process
- Can propagate the signal or electrochemical change in the environment to the sensor/electrode surface due to its hydrophilic nature
- Being inert in nature does not create any interference during course of detection
- They can be readily synthesised and manipulated.

Hydrogel as three-dimensional inert matrix only allows the substrate/ligand to diffuse to or from the matrix while diffusion or leaching of the bioelement across the matrix is restricted/negligible. Even though hydrogel lacks thermal and chemical stability, sol-gel based technology for bioelement immobilisation on the solid surface can be and is utilised nowadays for biosensor development (Gill and Ballesteros, 2000; Jin and Brennan, 2002). Sol-gels are mechanically rigid, chemically inert and

thermally stable structures (Gupta and Chaudhury, 2007). Mostly used sol-gel are oxides of silica and are transparent hence quite frequently used in the preparation of optical biosensor (Jerónimo et al., 2007). The bioelement can be attached to a transparent film, optical fibre ends, or any other glass/polymeric transparent surface with the help of sol-gel. Sol-gel provides dual benefit when used in an optical biosensor; it works as an immobilising matrix as well as a light propagating medium (Jin and Brennan, 2002; Jerónimo et al., 2004; MacCraith et al., 1995). Sol-gel based optical biosensor for urea, heavy metal, and other analyte determination has been reported in the literature (Doong and Tsai, 2001; Jerónimo et al., 2007; Tsai et al., 2003; Braun et al., 2007).

Based on hydrophilicity, compatibility with bioelement, pore size and mechanical property of the hydrogel or sol-gel the immobilisation procedure or protocol can be designed and standardize to achieve an efficient sol-gel/hydrogel based biosensor.

19.4 Polymers as conducting material in biosensors

In biosensor development immobilisation of biological recognition element/bioelement is a critical aspect of concern. Transfer of signal generated by the interaction of bioelement with the analyte to the transducer is also equally important. This process is more applicable for an electrochemical biosensor as they detect a change in conductivity, resistivity, potential and current (Guiseppi-Elie, 2010). Thus an excellent current propagating medium is essential for electrochemical biosensors response. Electrically conductive polymers or conducting polymers can be an ideal suitor for such cause (Guiseppi-Elie, 2010). Conducting polymers or CP as they are referred, are a class of functional polymers that have alternating single and double bonds along the polymeric chains. The electrochemical properties of these conjugated polymer chains can be altered via doping/de-doping process, hence making them a desirable transducing material in various biosensors (Seeber et al., 2014). The conducting polymers belong to polyenes and polyaromatic compounds such as polyaniline (PANI), polyacetylene, polypyrrole, poly (p-phenylene), poly furan (PF), poly(p-phenylenevinylene) (PPV) and other polythiophene derivatives etc. (Fig. 19.4) Conducting polymers can be synthesised either by chemical or electrochemical oxidation (Guiseppi-Elie, 2010; Balint et al., 2014). The electrochemical oxidation procedure is often preferred over chemical method while depositing polymeric films over anode surface (Urban and Weiss, 2010). The conducting polymers have remarkable switching capabilities from conducting oxidised (doped) to insulating reduced (de-doped) state (Xia et al., 2010). As mentioned earlier, conducting polymers can efficiently transfer electric charge produced by the biochemical interaction between bioelement and analyte to the sensor element. Moreover, conducting polymers can also be used to entrap bioelement on a defined electrode surface, this ability of conductive polymers has been widely exploited in the fabrication of amperometric biosensors (Fig. 19.5) (Gerard et al., 2002; Ahuja et al., 2007). Other than this, conductive polymers have been used in the production of various immunosensors (Gerard et al., 2002; Bangar et al., 2009), DNA sensors (Chang et al., 2007), environmental and food safety monitoring biosensors too (Ahuja et al., 2007).

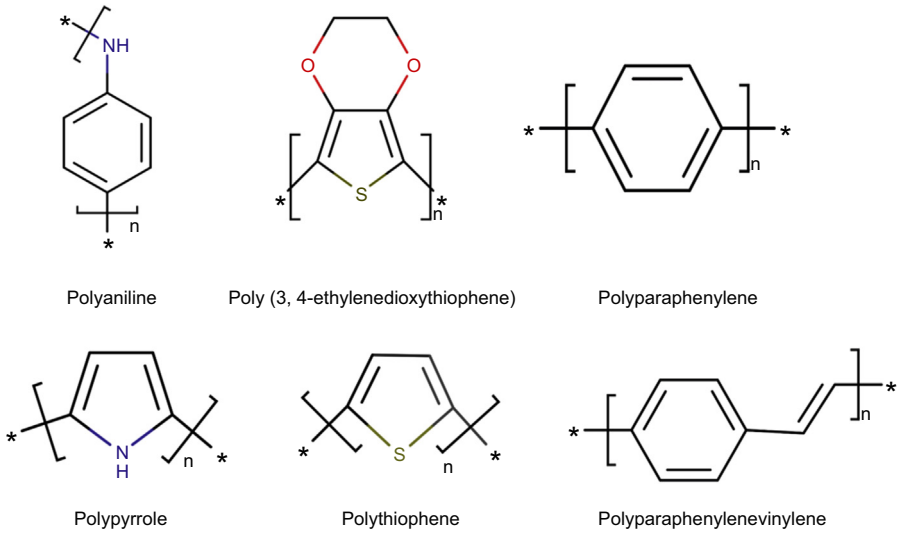

Figure 19.4 Structures of some common conducting polymers.

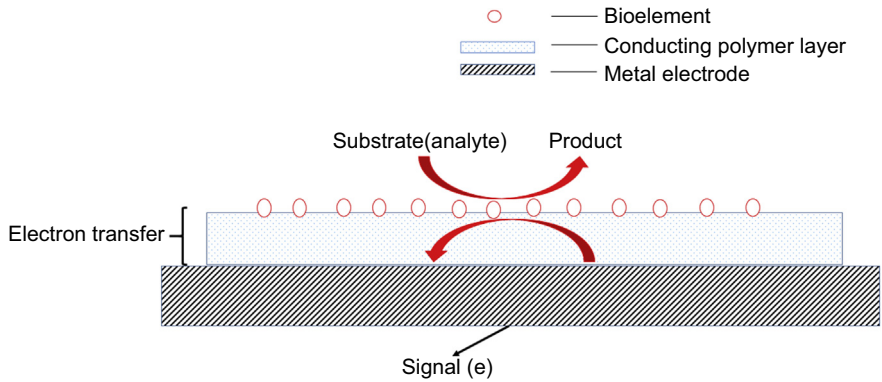

Figure 19.5 Electron/current transduction via a conductive polymer over a metal electrode.

The sensitivity of conducting polymer depends on the surface area and doping/dedoping processes used during the synthesis of conducting polymer. However, surface area plays a significant role in the sensitivity and response time of biosensor. Nowadays, conducting polymer nanostructures are being used in the biosensor or nanobiosensor fabrication (Xia et al., 2010). Nanostructure provides high pore size and larger surface area (as in case of nanotubes and nanofibers). Thus, the generated signals or charged species produce due to the interaction between analyte and bioelement would efficiently transfer to electrode/sensor, making biosensor more sensitive and responsive as shown in Fig. 19.5 (Xia et al., 2010). Various comparative studies report a comparison between bulk film and nanofiber deposition over the electrode and its effect on the sensitivity and response time of biosensor (Lahiff et al., 2010;

Xian Guo and Ming Li, 2010; Deepshikha and Basu, 2011). The synthesis of nanostructures of conducting polymers is an issue thus currently limiting their application in biosensor development (Xia et al., 2010). Electrospinning (Huang et al., 2003), hard physical template-guided synthesis and soft chemical template synthesis (Long et al., 2011) are various methods of conducting polymer nanostructures synthesis. However, these methods are only suitable for small or lab scale synthesis of nanostructures, large-scale synthesis methods are still not being developed (Xia et al., 2010).

Conducting polymers exhibit excellent signal propagation property and are also very effective entrapment matrix for continuous detection of an analyte. Hence studies and development of conducting polymers either as bulk material or in nanostructured form are desirable in the field of biosensor fabrication.

19.5 Application of transparent polymers in biosensors

The optical biosensors are based on methods such as UV−Vis absorption, bio/chemiluminescence, fluorescence/phosphorescence, reflectance, scattering and refractive index, caused by the interaction of the biocatalyst with the target analyte. Transparent polymers with the high refractive index can enhance the efficiency of optical biosensors. Transparent, recyclable polymers, commonly called thermoplastics have a high refractive index. Further, the refractive index of such polymers can be further hence by incorporation of nanoparticles in it. Some examples of the transparent polymer include Acrylic (polymethylmethacrylate), Butyrate (cellulose acetate butyrate), Lexan (polycarbonate), and PETG (glycol-modified polyethylene terephthalate).

A high-refractive-index polymer (HRIP) is a polymer that has a refractive index higher than 1.50. Such materials are required for anti-reflective coating and photonic devices such as light emitting diodes (LEDs) and image sensors. A micro-lens array is a critical component of optoelectronics, optical communications, CMOS image sensors and displays. Polymer-based micro-lenses are easier to make and are more flexible than conventional glass-based lenses. The resulting devices would be using less power, smaller in size and cheaper to produce (Liu and Ueda, 2009). As of 2004, the highest refractive index of a polymer was 1.76. Substituents with high molar fractions or high-n nanoparticles in a polymer matrix have been introduced to increase the refractive index (Seto et al., 2010). Other examples of such material include: PMMA is a transparent material with an extensive range of applications, e.g., in injection moulding as well as extrusion. PMMA features good weather resistance combined with high scratch resistance and transparency. PMMA is also available as high-impact and light diffusive grade. High Heat Polyethylene terephthalate glycol-modified (PETG) is a transparent material containing a bio monomer which is suitable for a vast range of applications. It features high chemical resistance combined with high heat resistance. Polyethersulfone (PES) offers high heat deflection, excellent chemical resistance, and excellent electrical properties and is inherently flame retardant. Transparent polymers have found its more application in the construction of medical devices.

19.6 Stimuli-responsive polymers in biosensing application

Stimuli-responsive polymers as the name suggests can convert chemical and/or physical stimuli into observable physiochemical changes in the polymer/polymer containing the responsive compound. Stimuli-responsive polymers can mimic functional properties of biomolecule like polynucleotides and proteins (Song et al., 2002; Alexander and Shakesheff, 2006). In the above sections, the discussion has been focused on the polymeric gels those support bioelement or sensor element in the recognition or signal propagation for fast and reliable detection of an analyte. Stimuli-responsive polymers can be directly used for the detection of an analyte. Stimulus active or responsive polymers can vary their shapes or produce mechanical power in response to external stimuli such as temperature, electric, light, magnetic field and hydrodynamics (Fig. 19.6) (Stuart et al., 2010). The unique properties of such polymers show structural dependence and make them very useful for different applications such as sensing, drug delivery, the application as artificial muscles, tissue engineering, and self-healing material (Urban and Weiss, 2010; Alexander and Shakesheff, 2006; Stuart et al., 2010). The property of responsive polymer can be modified/changed at the time of polymer synthesis by altering the chain architecture. Structural modification in the chain such as linear, cyclic, graft, star and so on has been successfully done in the past decade using techniques like group transfer polymerisation, ring opening polymerisation, click reaction and various controlled radical

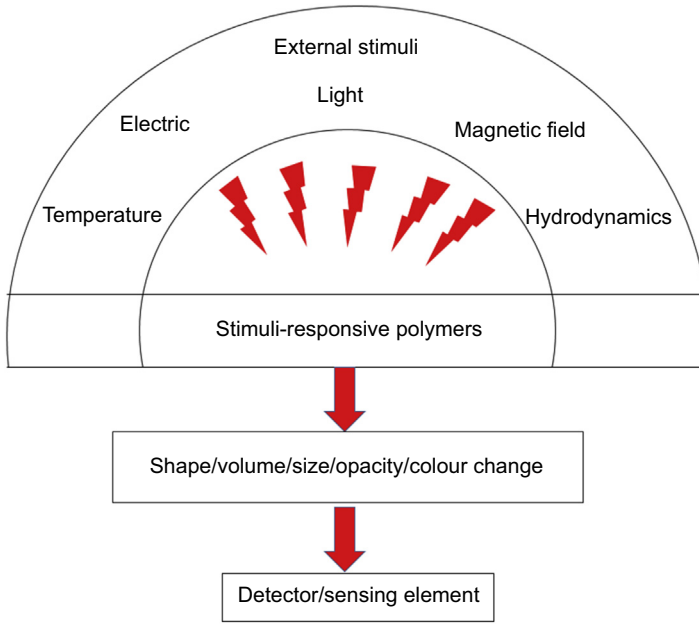

Figure 19.6 Schematic representation of working of stimuli-responsive polymers in biosensor.

polymerisation techniques(Stuart et al., 2010; Gauthier et al., 2009; Dechy-Cabaret et al., 2004). These new chain topologies gave rise to the new novel self-assembling nanostructure with novel morphologies and functions both in solution and in bulk state.

The stimuli-responsive polymers based detection system can be categorised into two major types. First is molecular recognition based small molecular sensors, that is the small polymer molecule act and behave like bioelement and try to recognise an analyte and produce a signal which can be detected by sensor element (Hu and Liu, 2010; Meng and Hu, 2010). Second is chemical/biochemical reaction based sensors, i.e., the change in the environment due to the chemical/biochemical reaction between bioelement and analyte would be picked up by stimuli-responsive polymer (Hu and Liu, 2010; Meng and Hu, 2010). The stimulation leads to change in the shape of the polymer or any other change in the mechanical property of the polymer creating a signal that can be monitored or registered (Urban and Weiss, 2010; Hu and Liu, 2010). The second type of setup is more utilised in biosensor fabrication, as per favorite definition of a biosensor it consists of a biological element and a sensor element. Moreover, molecular recognition based small molecular sensors have low stability, and weakly functional (Hu and Liu, 2010; Mendes, 2008). One example for biochemical reaction based sensor would be, the coupling of pH-sensitive hydrogel-like poly(methacrylic graft-ethylene glycol) (P(MMA-g-EG)) with proton generating an enzymatic reaction. During the reaction process, the enzyme would act on the substrate or analyte and generate proton, which lowers the pH of the reaction mixture. This pH change alters the microenvironment of the pH-responsive polymer leading to either a size reduction or increment of the polymer (i.e., volume change of polymer). This volume change can be easily detected by piezoelectric sensors (Urban and Weiss, 2010). Similarly, thermo-responsive polymers like poly (*N*-isopropylacrylamide (pNIPAm)) also change their size according to temperature change so an endothermic or exothermic biochemical reaction can be linked to the polymer as a measure the analyte concentration (Meng and Hu, 2010). Other than this, light sensitive or photonic responsive polymeric gels or hydrogel-like poly(*N*-isopropylacrylamide (NIPAm)) and its derivatives based 1D or 2D arrays have been utilised in label-free signal transduction in biosensors(Hu and Liu, 2010; Meng and Hu, 2010). The photonic sensitive hydrogel changes their shape, size, which leads to change in diffraction, opacity and also change the colour of the reaction medium. That can be easily detected using an optical transducer (Meng and Hu, 2010). So stimuli-responsive polymeric gels should play an important role in detection and sensing/biosensing application. However, stimuli-responsive polymeric gels based sensing/biosensing systems are still in its infancy stage as compared to the use of conventional polymeric gels.

19.7 Summary

It is evident that polymeric gels have a significant role to play in the development of the biosensors. Especially in the last few decades, polymeric gels especially hydrogels have gained popularity in the field of biosensor development and fabrication. Intrinsic properties of hydrogels and other polymeric gels predetermine their use in biosensing

application. It started from enzyme immobilisation via sol-gel or hydrogels and went to conducting polymers, transparent polymers and stimuli-responsive polymers role and application in designing of electrochemical, optical, thermal and piezoelectric biosensors. With advancement and development in the field of material science and polymer chemistry, multifunctional polymers are coming into the picture that will decide the future or next generation biosensor technological development.

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Polymeric gels for cartilage tissue engineering

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20.1 Introduction

Articular cartilage present in diarthrodial knee joints is avascular, aneural, alymphatic, multiphasic connective tissue with limited regenerative ability. It helps in distribution of loads and reduces friction between joints during movements (Mistry et al., 2017; Temenoff and Mikos, 2000). It mainly consists of solid phase and fluid phase. Chondrocytes, collagens, and proteoglycans are main constituents of solid phase, whereas water and dissolved salts are major portion of fluid phase (Cohen et al., 1998). These two phases work in coherence to develop compression resistance and distribution of loads (Buckwalter and Mankin, 1998). Cartilage is present in different forms such as hyaline and fibrous in knee joints. The types of cartilage are defined on the basis of molecular composition of extracellular matrix (ECM) and appearances (Kinner et al., 2005). Articular cartilage is hyaline in nature with 2−4 mm thickness. It is mainly present in knee joints, the surfaces of which are covered with hyaline cartilage. Synovial membranes cover the joints and retain the synovial fluid to provide sufficient lubrication and nutrients to cartilage (Sophia Fox et al., 2009). The hyaline cartilage contains only type II collagen, whereas only type I collagen and low content of proteoglycan aggrecan are present in fibrocartilage-consisting meniscal regions. The articular cartilage shows glassy appearance with no evidence of fibers under microscopic examination (Poole et al., 2001). As illustrated in Fig. 20.1, the articular cartilage is subdivided into superficial zone, middle zone, deep zone, and calcified zone. Each zone also contains pericellular region, territorial region, and interterritorial region (Sophia Fox et al., 2009). Normally, articular cartilage contains low amounts of specialized cells known as chondrocytes that are responsible for synthesis of ECM, consisting of type II collagen, proteoglycan, and water, which provides specific physiochemical properties such as load dissipation and frictionless movements. Structural components of articular cartilage are discussed in detail in further sections.

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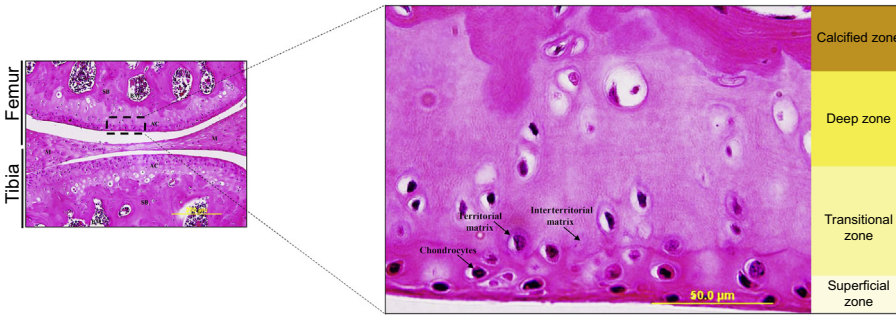

Figure 20.1 Histologic structures of knee joint articular cartilage showing chondrocytes and different zones and regions. *AC*, articular cartilage; *M*, meniscus; *SB*, subchondral bone.

20.2 Chondrocytes

Chondrocytes are specialized cells that are differentiated from mesenchymal stem cells (MSCs). In the final stage of development, the chondrocytes at central zone secrete proteins that assist in calcification of the matrix, whereas chondrocytes present at peripheral zones synthesize and release collagen and other ECM molecules to develop hyaline cartilage. The mature chondrocytes remain entrapped within ECM, appear glassy and rounded, and possess limited regeneration potential (Kinner et al., 2005). The chondrocytes only represent 1%−2% of total volume of articular cartilage and play key role in synthesizing the degraded ECM to maintain its functional nature (Stockwell, 1978). The cellular density of chondrocytes is highest in early ages (0−2 years) and decreases with the advancing age of individual (Mitrovic et al., 1983). The shape, size, and number of chondrocytes vary according to zones and regions of articular cartilage (Stockwell, 1978). In superficial zones, they are flat and small. The density of chondrocytes in deeper zones is less as compared to superficial zone. Chondrocytes secrete ECM and are immobilized within this specific environment, which affects the turnover of ECM. This entrapment inhibits direct cell-to-cell interaction. However, the cells respond to stimuli such as growth factors, mechanical loads, piezoelectric forces, and hydrostatic pressure (Buckwalter et al., 1988). It has also been reported that chondrocytes contains a cell-autonomous circadian rhythm, the disruption of which was observed in osteoarthritic individuals (Doody and Bottini, 2016).

20.3 Extracellular matrix

ECM consists of collagen, water, proteoglycan, noncollagenous proteins, glycoproteins, metabolites, and other dissolved salts (Temenoff and Mikos, 2000). Water and dissolved matrix molecules represents 65%−80% of the total weight, whereas remaining is represented by solid phase molecules such as collagen, proteoglycan,

etc. Some other biomolecules such as lipids, phospholipids, glycoproteins, and noncollagenous proteins are also present in low quantity (Sophia Fox et al., 2009; Shoulders and Raines, 2009).

20.3.1 Collagen

Collagen is major composition of ECM and imparts stability to the matrix. Collagen networks provide mechanical support and structural integrity to bear the stress and load. The efforts have been (theoretical, mathematical, and computational) made to develop engineered collagen network to replicate the function of collagen (Wilson et al., 2006); however, these efforts were not able to mimic the function of natural collagen of articular cartilage (Responte et al., 2007). Collagen constitutes about 60% of dry weight of ECM (Cohen et al., 1998) and 10%−20% of wet weight (Bhosale and Richardson, 2008). Collagen type II (Fig. 20.2) is the most abundant collagen in articular cartilage and consists about 90%−95% of collagen in the matrix. Further, the collagen types VI, IX, X, and XI are also present in small amount (Eyre, 2002). The other type of minor collagen helps to stabilize fibril network of collagen type II. Importantly, the collagen types II, IX, and XI form a fibrils mesh, which entraps macromolecules and support tensile strength of the matrix (Eyre, 2002). Glycine (Gly), proline (X), and hydroxyproline (Y) are the most abundant amino acids in sequence of $(Gly-X-Y)_n$ and are present in α-chains of right-handed triple helix

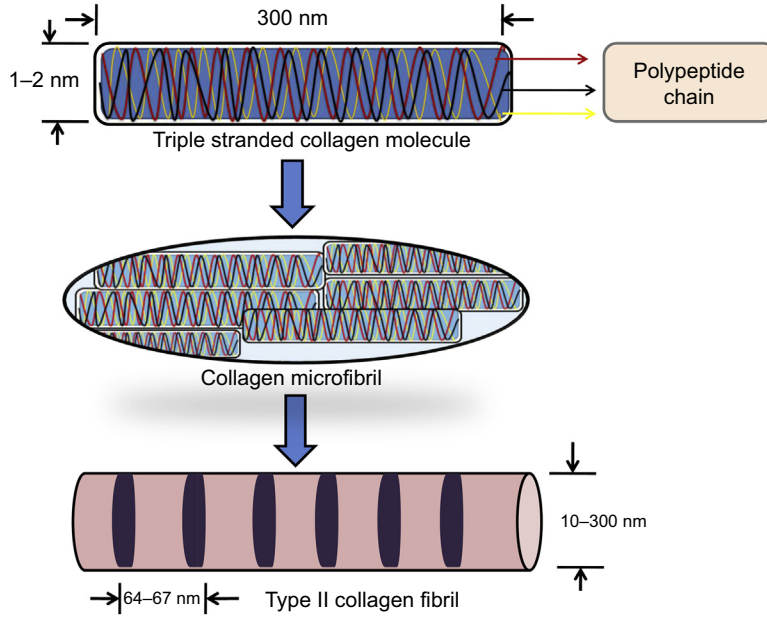

Figure 20.2 Schematic representation of type II collagen fibril structure.

collagen molecules (Yingst et al., 2009; Shoulders and Raines, 2009). Collagen type II and type XI are structurally similar; however, collagen type II is homotrimer, whereas collagen type XI is heterotrimer (Thomas et al., 1994). Collagen orientation and depth charge densities affect the mechanical behaviors of cartilage (Korhonen et al., 2008).

20.3.2 Proteoglycans

Proteoglycans are the major constituent after collagen, which represent 10%−15% of wet weight. Five percent of proteoglycans is protein, whereas remaining 95% proteoglycan is constituted by polysaccharides (Cohen et al., 1998). The proteinaceous core of proteoglycan is covalently bonded with one or more types of glycosaminoglycans (GAG) linear chain (Fig. 20.3). These various kinds of GAGs include hyaluronic acid (HA), chondroitin sulfate, keratin sulfate, dermatan sulfate, and heparin sulfate (Poole et al., 2001). GAG chains emerge out from protein core and stand separated due to charge repulsion of negatively charged sulfate or carboxylate group. The presence of this negative charge on the GAG helps it to bind with water and other positively charged macromolecules. The ECM also contains various kinds of proteoglycans such as aggrecan, decorin, biglycan, and fibromodulin. Out of these, aggrecan, which is the most abundant proteoglycan by weight, contains more than 100 keratin and chondroitin sulfate. Other proteoglycans, the decorin, biglycan, and fibromodulin are smaller as compared to aggrecan and also contribute to chondrocytic function and organization of ECM (Sophia Fox et al., 2009). Further, the link proteins attach the HA to the monomers of keratin sulfate and chondroitin sulfate chains. The interfibrillar space of matrix is filled by aggrecans (Eyre, 2002), which binds to water molecules to provide osmotic properties and are responsible for load distribution and resistance to shear/tear forces (Sophia Fox et al., 2009).

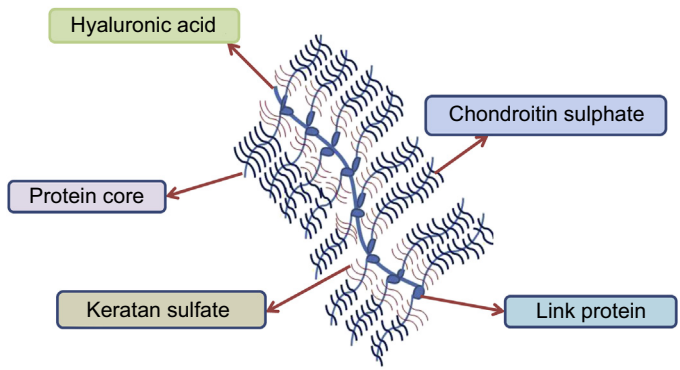

Figure 20.3 Structural representation of proteoglycan aggrecan present in extracellular matrix of cartilage.

20.3.3 Glycoproteins and noncollagenous proteins

The protein core of glycoproteins stabilizes the ECM of articular cartilage and assists not only in intercellular interaction but also between cells and ECM (Eyre, 2002). Other noncollagenous proteins such as fibronectin, anchorin CII, tenascin, and cartilage oligomeric protein play a significant role in maintaining the structural integrity of ECM (Sophia Fox et al., 2009). Besides, the growth factors affect the matrix synthesis and increase the formation of collagen type X, which regulates the synthesis of proteolytic enzymes thereby assisting in clearance of ECM, vascularization, and calcification in cartilage (Poole et al., 2002).

20.3.4 Pericellular matrix

Pericellular matrix (PCM) differs from ECM and it surrounds the chondrocytes with the narrow layer (Poole et al., 1987). The chondrocytes encircled with PCM is termed as chondron (Vanden Berg-Foels et al., 2012). It has also been reported that the PCM transduces biomechanical and biochemical signals to chondrocytes (Macri et al., 2007) and also interacts with ECM to participate in chondrocyte metabolism of joints (Halloran et al., 2012). The major components of PCM include types VI and IX collagen, perlecan, hyaluronan, aggrecan monomers, biglycan, and other small aggregates. These macromolecules determine organizational and functional role of PCM. Notably, the PCM is characterized by the presence of type VI collagen around the chondrocytes (Youn et al., 2006). It is also to be noted that the transport properties and structure of PCM matrix may affect the response of chondrocytes to growth and other factors (Steward et al., 2013).

20.3.5 Tissue fluid

Tissue fluid is an essential part of hyaline cartilage, comprising up to 80% of the wet weight of the tissue. In addition to water, the fluid contains gases, metabolites, and large amount of cations to balance the negatively charged GAGs in the ECM. It is the exchange of this fluid with the synovial fluid that provides nutrients and oxygen to the avascular cartilage. Besides, this fluid through interaction with ECM components provides the tissue the ability to resist compression and return to normal shape after deformation (Kinner et al., 2005).

20.3.6 Zones and regions of articular cartilage

20.3.6.1 Zonal organization

Based on the ultrastructure and function, the articular cartilage is divided into four zones: superficial, transitional, deep, and calcified zone. The superficial zone is covered by synovial fluid, which reduces friction between bones. This zone contains layer of flattened chondrocytes, type II and type IX collagen fibers. The high amount of collagen, fibronectin, and water in ECM, not only strengthens tensile property of matrix but also enhances resistance property of zone against shear and compressive

forces on articular surface (Sophia Fox et al., 2009). This zone is approximately 10%−20% in thickness of articular cartilage (Buckwalter and Mankin, 1998). Transitional zones come next to the superficial zone, and it covers almost 40%−60% of total cartilage volume. This zone contains more spherical chondrocytes and high content of proteoglycan, the aggrecan in ECM with random distribution of collagen fibrils arranged perpendicular to the articular surface (Buckwalter and Mankin, 1998). Chondrocytes are present in low numbers and arranged parallel to the collagen fibrils, whereas collagen fibrils are perpendicular to the articular surface (Temenoff and Mikos, 2000). Deep zone lies between calcified and transitional zone and consists about 30% of volume of articular cartilage. The high amount of proteoglycan and low amount of water provides highest resistance to compressive forces (Decker et al., 2015), whereas partly calcified layer of deep zone provides resistance to vascular invasion and works as interface between uncalcified region of cartilage and subchondral bone to provide structural integrity (Sophia Fox et al., 2009). The tide mark distinguishes the deep zone from the calcified cartilage (Buckwalter et al., 1988). Calcified cartilage attaches articular cartilage to subchondral bone, and this region generates considerable shear stress (Buckwalter, 1983).

20.3.6.2 Regional organization

Chondrocytes are surrounded by matrix that is further organized into different regions. These regions are characterized based on their distance from articular surface, chondrocytes composition, and structure of collagenous fibrillar matrix (Sophia Fox et al., 2009). In addition to zonal variations in structure and composition, the matrix consists of several distinct regions based on proximity to the chondrocytes, composition, and collagen fibril diameter and organization. These regions in ECM can be divided into pericellular, territorial, and interterritorial (Buckwalter et al., 2005). The PCM is situated adjacent to the cell membrane, and it completely surrounds the chondrocyte. It contains mainly proteoglycans, glycoproteins, and other noncollagenous proteins. This matrix region may play a functional role to initiate signal transduction within cartilage (Eggli et al., 1985). The territorial matrix surrounds the PCM, which is composed mostly of fine collagen fibrils, forming a basket-like network around the cells (Guilak et al., 2006). This region is thicker than the PCM, and it has been proposed that the territorial matrix may protect the cartilage cells against mechanical stresses and may impart resiliency to articular cartilage structure to withstand substantial loads (Buckwalter and Mankin, 1998). The interterritorial region is the largest of the three regions of ECM and contributes most to the biomechanical properties of articular cartilage (And and Guo, 2002). This region is characterized by the randomly oriented bundles of large collagen fibrils, arranged parallel to the surface of the superficial zone, obliquely in the middle zone, and perpendicular to the joint surface in the deep zone. Proteoglycans are abundant in the interterritorial zone (Kuettner et al., 1991).

20.4 Tissue engineering in treatment of articular cartilage injury

Different therapeutic approaches including surgical procedures such as abrasive chondroplasty, microfracture and spongialization, and transplantation of tissue or chondrocytes/stem cells have been employed to regenerate articular cartilage in knee joints (Hunziker et al., 2015). Tissue engineering approaches might be developed as an alternative to conventional treatments, which applies concept and techniques of multidisciplinary fields including biological sciences, biotechnology, chemical engineering, material science, and mechanical engineering to regenerate or grow tissues from cells in vitro (Luyten et al., 2001). As demonstrated in Fig. 20.4, these techniques employ the cell source, scaffold, and growth factors to stimulate cellular environment to regenerate tissues (Griffith and Naughton, 2002). However, applying tissue engineering techniques to regenerate articular cartilage is complex due to complexity of zonal structure, which is either calcified or uncalcified (Poole et al., 2001). To engineer articular cartilage, the chondrocytes are inoculated in biodegradable scaffold (defined shape and volume) with essential growth factor under inducible environmental condition (Lynn et al., 2004). Engineered articular cartilage needs to assimilate with existing cartilage and subchondral bone without causing any major immunological or inflammatory response (Kim et al., 2006). Further, the biomechanical properties of engineered cartilage should be incoherent with adjacent native cartilage tissue for its survival and function in microenvironment of knee joint (Malaviya and Nerem, 2002). Therefore, the extensive preclinical studies are needed before considering it

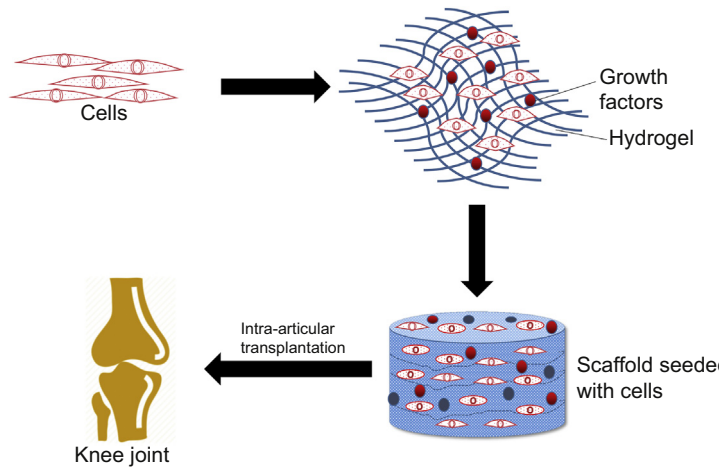

Figure 20.4 Elements of tissue engineering. A mixture of living cells, hydrogel scaffold, and growth factors to form a tissue-engineered construct to promote the repair and regeneration of cartilage tissues.

for therapeutic purpose (Lynn et al., 2004). In this section, we will discuss about the use of hydrogels and other biopolymer as scaffold, in addition to environmental/growth factors promoting the development of cartilage from cell sources.

20.4.1 Cells in articular cartilage repair

The choice of cell source for regenerating articular cartilage tissue is a significant factor and affects its growth and characteristics. For this, the chondrocytes are most evident choice, as these cells can be isolated from various sources such as articular cartilage, nasal septum, costal cartilage, or auricular cartilage and can develop into tissues with similar peculiarity of native cartilage (Isogai et al., 2006). Moreover, the chondrocytes could be preprogrammed to produce ECM (Kessler and Grande, 2008). High cell density for seeding in scaffold, prolong release of growth factors, and chemical and mechanical stimuli are essential components for developing highly effective cartilage (Madeira, 2015). Isolated chondrocytes from normal articular cartilage are expended by passaging, where passage number should be kept optimum to maintain chondrogenic property of cells (Huang et al., 2016). As, chondrocyte dedifferentiate, a shift from round to fibroblastic cellular morphology, an increase in cell size, and decrease in cartilage matrix are also observed (Schulze-Tanzil, 2009; Demoor et al., 2014). Besides, the sustained 3D chondrocyte cultures promote redifferentiation and cells stop proliferating. Low number of passaging ($P < 4$) does not significantly affect the neocartilage formation (Darling and Athanasiou, 2005; Benya and Shaffer, 1982; Mandl et al., 2004; Schulze-Tanzil et al., 2002), whereas, the high passage number ($P > 4$) leads to loss of redifferentiation potential (Huey and Athanasiou, 2013; Dell'Accio et al., 2001; Giovannini et al., 2010); thus, the passage number is a critical factor and it need to be defined for regenerating cartilage. Further, the cellular aging negatively affects the chondrogenic potential, high cell density, synthesis of appropriate aggrecan, and response to stimuli (Adkisson et al., 2001). These hurdles compel to identify other cell source.

Stem cells especially MSCs are germane option due to its potentiality for chondrogenesis and cartilage regeneration. MSCs could be isolated from various sources such as bone marrow (BM), adipose tissue, synovium, periosteum, skeletal muscle, skin, amniotic fluid, or umbilical cord blood matrix (Zhao et al., 2010). MSCs from synovium, periosteum, and BM were able to maintain expansion level and chondrogenic potential even after 10 passages. Further embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have also been studied for their capacity to regenerate damaged articular cartilage. To promote differentiation of MSCs into chondrocytes, specific culture medium and physiochemical environment must be provided (Baghaban Eslaminejad and Malakooty Poor, 2014). Along with concentration of bovine serum in medium, other factors such as transforming growth factor-β1, insulin-like growth factor-1, fibroblast growth factor-2, and dexamethasone, also influence the induction of chondrogenesis. The 3D scaffold system provides enough mechanical stimuli to advance cartilage regeneration and maintain cartilage phenotypic characteristics (Ronziere et al., 2010; Pires de Carvalho et al., 2014; Di Nicola et al., 2002). However, it is extremely cumbersome to differentiate MSCs into

chondrocytes in traditional 2D culture system due to high oxygen tension and absence of proper mechanical stimulation (Baghaban Eslaminejad and Malakooty Poor, 2014). Overall, the application of MSCs for cartilage replacement seems promising; however, the ossification of cartilage tissue and development of thin cartilage are some limiting factors (Koga et al., 2009; Madeira et al., 2015). The high density of MSC is required per lesion volume or surface area for effective chondrogenesis (Undale et al., 2009). Among MSCs, though ESCs are also promising option for cartilage regeneration, the ethical concerns make it difficult to exploit (Takahashi and Yamanaka, 2006). So, the iPSCs are developed to overcome the complexity of ethical issues and chance of cross-reactivity (Park et al., 2008).

20.4.2 Scaffold design and biomaterials

Scaffold for articular cartilage engineering should be biodegradable, biocompatible, smooth to process, nontoxic to native tissues, and sufficiently porous for exchange of factors, nutrients, and cell migration. Additionally, it should also be able to withstand load during tissue regeneration in knee joints. The degradation of scaffold must be in coherence with load distribution and neocartilage formation. Scaffolds should catalyze the assimilation of engineered cartilage to host tissue and encourage colonization of native cells (Coutts et al., 2001). Notably, the FDA-approved materials or polymer need to be used for scaffold purposes (Frenkel and Di Cesare, 2004; Hollister, 2005; Camarero-Espinosa et al., 2016). Both natural (e.g., collagen, silk) and synthetic polymers [poly(α-esters), polyurethanes, poly(propylene fumarates), polyphosphazenes, and poly (ethylene glycols)] are used as bioscaffold for regeneration of cartilage (Camarero-Espinosa et al., 2016). The scaffolds such as hyaluronan, agarose, alginate, and chitosan foster 3D structure to promote cell proliferation through delivering essential factors and stimuli to trigger regeneration of tissues with defined shape and function.

In conventional approach of engineering tissue, a porous scaffold is designed, fabricated, and seeded with cells. Thereafter, the seeded scaffold is placed into dynamic condition to bioreactor to mimic the physiological environment to develop mature tissue-bearing similar mechanical load as native cartilage. Finally, the tissue-scaffold construct is surgically transplanted and enough time is provided to acclimatize the construct in host (Hutmacher, 2000). Further, the choice of scaffold is a critical factor as it controls shape, function, and development of tissues from seeded cell. Additionally, the scaffold type and its structure varies according to the tissue requirements and cell source (Hutmacher, 2000). Chondrocytes are able to synthesize ECM and maintained their cellular activity when grown in compatible 3D structural scaffold, and any deviation from required structure leads to dedifferentiation. Nonlinearity, nonhomogenousity, and viscoelasticity are major characteristics of polymers that enhance the possibility of successful articular cartilage regeneration (von der Mark et al., 1977). The solid gel phase consisting of honey comb, porous body, mesh sponge, and unwoven fabric (Lu et al., 2001) provides higher mechanical supports during regeneration. However, the exploitation of solid phase as scaffold is confined due to its pore size limitation (Yamaoka et al., 2006).

20.4.3 Hydrogels

The other promising scaffolds are injectable hydrogels. These are polymers of synthetic or natural units (Drury and Mooney, 2003). These hydrogels swell in aqueous environment without rupturing their structural integrity, penetrate deep into tissues with high adaptability, and recover entire tissue damage (Sivashanmugam et al., 2015). Hydrogels mainly function as 3D support (Kim et al., 2011) and permeable matrix for chemical signals and soluble factors (Tibbitt and Anseth, 2009) and show less mechanical strength compared to solid gel (Yamaoka et al., 2006). Hydrogels polymerizes either chemically or physically to attain 3D structure, which efficiently entrap cells and provide suitable environment for cell growth and transport of nutrients and other factors in and out from mesh. To regenerate tissues in vivo, a long surgical cut is required to insert scaffold at targeted site; however, the hydrogels overcome this surgical complexity. Hydrogels have closet similarity to natural tissue as compared to other biomaterials due to its high content of water (Zhu and Marchant, 2011). Hydrophilic groups present in the polymers determine the water-absorbing capacity of hydrogel, whereas the insolubility of hydrogel is the result of cross-linking of polymeric networks (Ahmed, 2015). The properties of cartilage are similar to hydrogel, as it mainly contains long polymers of GAGs and collagen, which swell in excess of water (Broom and Oloyede, 1998). The characteristics of hydrogels could be controlled and modified by changing the properties such as polymer composition, network structure, degradability, and cross-linking density. The application of hydrogels has been explored in the field of drug delivery, diagnostics, biomedical implants, and tissue engineering (Peppas et al., 2006). However, this chapter would only discuss the role of various hydrogels in engineering articular cartilage.

20.4.3.1 Designing parameters of hydrogels for engineering articular cartilage

The physical and biological parameters are critical design criteria for the selecting hydrogels as scaffold materials for regenerating or engineering new tissues (Lee et al., 2001). The functions of hydrogels is tuned by controlling the variables, such as polymer types, cross-linking density, degradation pattern, mechanical properties, loading dose, source of cells and seeding density, release of growth factors, biocompatibility, and integration of hydrogels with native tissues (Spiller et al., 2011). Notably, the physical properties of the scaffold are determining factors for successful regeneration of tissues. The intrinsic physical characteristics includes gel characteristics, diffusion rate, mechanical properties, and the degradation rate of hydrogel could be controlled by cross-linking density, type of cross-linkers, and environmental conditions (Drury and Mooney, 2003; Spiller et al., 2011). Further, the swelling and water retention capacity of hydrogel is determined by the cross-linking density. The low cross-linking density results in large pore size in hydrogels, which increases the diffusion rate of nutrient and other factors (Bryant and Anseth, 2002). Therefore, the cross-linking density of hydrogels such as polyethylene glycol (PEG) hydrogel may be stimulated by increasing the concentration of PEG or by using branched

PEG instead of linear PEG (Bryant and Anseth, 2002; Söntjens et al., 2006; Bryant et al., 2004). It has also been reported that chondrocytes seeded within lower cross-linking density PEG hydrogel synthesized sufficient ECM with desired characteristics. Besides, it is established that pore size should be large than proteoglycan aggregate and collagen fibril to ensure effective diffusion of nutrients and other factors (Bryant and Anseth, 2002, 2003).

The cellular and structural arrangement of hydrogels and chondrocytes within the scaffold is also determined by the procedure followed for cell seeding and delivery of scaffold. Variation in processing factors such as pH, temperature, and solvent may exert negative effect on the cells (Anseth et al., 1996). To overcome this problem, scaffold is prepared and the cells are seeded in last stage; however, injectable hydrogel have also been successfully used to develop tissues in vivo (Drury and Mooney, 2003).

The mechanical properties of hydrogels are controlled by the choice and concentration of polymer, behavior of cross-linker, temperature and pH, swelling, and biodegradation rate (Anseth et al., 1996). Though swelling decreases the mechanical strength of hydrogels, it could be easily manipulated by selecting the branched or linear cross-linker and changing the concentration (Anseth et al., 1996; Lee et al., 2000). The biodegradation of hydrogels during tissue regeneration subverts the 3D support of scaffold, yet the ECM synthesized during regeneration strengthens their mechanical strength (Bryant and Anseth, 2002; Bryant et al., 2004).

Hydrogels are degraded via hydrolysis, enzymatic degradation, and dissolution. Generally, the rate of tissue generation is directly corelated to the degradation rate of scaffold (Drury and Mooney, 2003). Hydrolysis promotes the degradation of synthetic hydrogels at constant rate, and the ester linkage is hydrolyzed (Suggs and Mikos, 1999; Metters et al., 2000; Saito et al., 2001). The rate of hydrolysis of synthetic hydrogels depends on the type and compositions of hydrogel; however, the environmental factors do not contribute in degradation of hydrogels (Saito et al., 2001). Other hydrogels such as collagen, HA and chitosan are enzymatically degraded, whereas cross-linked alginate is hydrolyzed. Further, the enzymatic degradation depends on available cleavage sites on hydrogels and amount of enzymes exposed to the scaffold (West and Hubbell, 1999; Mann et al., 2001). The degradation rate is also determined by the ionic environment of the scaffold (LeRoux et al., 1999). Diffusion of nutrients, growth factors, and waste is adjusted by manipulating the degradation rate of hydrogels (Bryant et al., 1999). Because the amount and rate of degradation significantly affect the production of ECM, a balanced designing of effective scaffold for engineering functional articular cartilage is highly needed (Spiller et al., 2011). Besides, the photodegradation might play significant role in degradation of scaffold and regulation of cell behavior in tissue development within designed hydrogels (Kloxin et al., 2009).

Transfer of gasses, nutrients, growth factors, and wastes in and out or vice versa from scaffold are critical parameters for engineering functional tissues. The mass transfer phenomena in scaffold are mainly controlled by the diffusion rate. The pore size plays important role in diffusion and it is regulated by polymer concentration and size along with type and concentration of cross-linker (Smidsrod and Skjak-Braek, 1990). Diffusion rate is also affected by compound's molecular weight and size too. For low molecular weight compounds such as glucose, oxygen, and growth factors,

the pore size of 1 nm is sufficient for the diffusion (Tanaka et al., 1984). However, for larger molecules such as proteoglycan aggregate, collagen fiber, myoglobin, albumin, and fibrinogen, this pore size is insufficient for free diffusion. Mechanical signals also regulate the cell metabolism and induce chondrocytes differentiation. Because static loading hinders cartilage synthesis whereas predetermined cyclic loading enhances ECM synthesis and suitable development of cartilage, an optimum level of mechanical loading need to be established to enhance the productivity. Besides mechanical signals, the nature of hydrogels, level of cell inoculation, and pattern of loading also impact the ECM synthesis (Drury and Mooney, 2003).

20.4.3.2 Natural hydrogels

20.4.3.2.1 Collagen

Collagen is a natural polymer and widely studied for its role in scaffold development. Collagen type I and II are commonly used for developing hydrogels scaffold for regeneration of cartilage. Collagen hydrogels have high swelling ratio and catalyzes cartilage formation in hydrogels (Yamaoka et al., 2006). However, it also shows some limitation due to its potential to trigger immune response, low mechanical strength and being expensive. The formation of gel is mediated chemically either by aldehyde or carbodiimide method (Lee et al., 2001). Collagen efficiently interacts with chondrocytes and promotes the production of ECM (Yamaoka et al., 2006). Furthermore, the MSCs are more effectively differentiated into chondrocytes in type II collagen hydrogel as compared to type I collagen hydrogels (Lu et al., 2010). Porous collagen scaffold (pore size 150−250 μm) showed high content of type II collagen ad aggrecan with better mechanical properties (Zhang et al., 2014). Recombinant type II collagen/polylactide scaffold was implanted in domestic pigs and it showed promising future for further study in articular cartilage regeneration (Muhonen et al., 2016). In another study, a multilayered scaffold of collagen type I, type II, hydroxyapatite, and HA was designed to promote differentiation of cells for cartilage and osteochondral-defects repair (Levingstone et al., 2014). This in vitro study showed promising results for cartilage repair.

20.4.3.2.2 Agarose

Agarose is a natural polymer synthesized by the marine algae and is one of the oldest hydrogels explored in tissue engineering (Guo et al., 1989). It forms reversible gel and gelation is rapid due to hydrogen bonding (Mauck et al., 2000). Agarose has been reported in synthesis of cartilage under dynamic loading and defined environmental conditions and mimic the mechanical properties of natural cartilage (Mauck et al., 2000, 2007; Lima et al., 2006). Pore size of the 3 D network of agarose gels is controlled by regulating the concentration of agarose and their performance could be improvised after it is attached with cell adhesion molecules (Borkenhagen et al., 1998).

20.4.3.2.3 Hyaluronic acid

HA is a linear polymer of disaccharide subunits of β-D (1 → 3) glucuronic acid and β-D (1 → 4) *N*-acetyl-β-D-glucosamine units. It is highly prevalent as GAGs in

synovial fluid of joints (Drury and Mooney, 2003) and play crucial role in wound healing, ECM organization, and cell differentiation (Fraser et al., 1997). A rapid synthesis of cartilage using HA has been reported as compared to fibrin and PEG hydrogels entrapped with chondrocytes and MSCs (Chung and Burdick, 2009). Further, the HA might enhance chondrogenesis after establishing interaction with chondrocytes (Spiller et al., 2011).

20.4.3.2.4 Chitosan

Chitosan, a linear polymer, represents structural similarity to natural GAGs. The subunit of this polymer is 1, 4-linked D-glucosamine and *N*-acetyl-D-glucosamine residues derived from chitin (VandeVord et al., 2002). Notably, the chitosan is derived from *N*-deacetylation; however, the extent of deacetylation varies from 50% to 90% (Francis Suh and Matthew, 2000). It is easily degraded by chitosanase and lysozyme (Singh and Ray, 1998). Hydrogels of chitosan are synthesized by using ionic/chemical cross-linking with glutaraldehyde, UV irradiation, and thermal variation (Drury and Mooney, 2003). Chitosan has also been modified to enhance cellular interactions, increase structural similarity to natural cartilage and prepare injectable hydrogels (Spiller et al., 2011).

20.4.3.2.5 Fibrin

Fibrinogen is the precursor of fibrin, which is mainly isolated from the patient's own blood; therefore, it mitigates the chance of immune rejection within the host body (Spiller et al., 2011). Fibrinogen is enzymatically polymerized in presence of thrombin to form hydrogels of fibrin. This hydrogel promotes differentiation of MSCs into chondrocytes and promotes formation of cartilage (Dare et al., 2009, van Susante et al., 1999). However, this hydrogel shows inferior mechanical characteristics (Chang et al., 2008).

20.4.3.3 Synthetic hydrogels in cartilage engineering

Synthetic hydrogels have garnered interest as their properties could be easily modulated depending on requirements, and these are also produced at large scale with reproducible quality (Lee et al., 2001).

20.4.3.3.1 Polyvinyl alcohol

Structural similarity between polyvinyl alcohol (PVA) hydrogels and cartilage is well established, and its behavior is controlled by regulating the molecular weight and level of hydrolysis (Lee et al., 2001). These hydrogels are prepared by chemical cross-linking, repeated freezing/thawing process, and electron or gamma irradiation (Lee et al., 2001; Spiller et al., 2011). The major drawback of hydrogels synthesized by the above method is nonbiodegradability under physiological environment, so these polymers are only used when a permanent support biomaterial is required (Lee et al., 2001). Further, because PVA hydrogels also show poor integration with surrounding cartilage, the cell and scaffold interaction is improvised by attaching oligopeptide sequence (Spiller et al., 2011).

20.4.3.3.2 Polyethylene glycol

PEG hydrogels have been used to entrap chondrocytes, MSCs, and ESCs to trigger the synthesis of cartilage. Moreover, the PEG is modified to understand the effects of hydrogel's characteristic on cartilage formation and properties (Bryant et al., 2004; Bryant and Anseth, 2003; Kloxin et al., 2009; Martens et al., 2003). Cell-retention capacity of hydrogels could be improvised through attaching the collagen-mimetic peptide compounds (Lee et al., 2006; Liu et al., 2010), whereas, the ECM synthesis might be enhanced by adding the chondroitin sulfate to PEG hydrogel entrapped with MSCs (Bryant et al., 2005; Kisiday et al., 2002). This hydrogel is mainly prepared by UV photopolymerization and frequently used in clinical study and is approved by FDA for tissue engineering applications due to biocompatible and low toxic behavior.

20.4.3.3.3 Polypeptides

The peptide contains both hydrophobic and hydrophilic groups and self-assembles to form β-sheet in presence of water (Kisiday et al., 2002). At low concentrations (0.1%−1%), these peptides assemble themselves into stable hydrogels and provide specific and unique characteristics to the scaffold and promotes cellular interaction, which promote cellular differentiation formation and reduce the chance of microbial infections (Zhang et al., 1993; Holmes, 2002). In a seminal report, the peptide hydrogel−containing chondrocytes have also been reported to promote cartilaginous matrix synthesis (Kisiday et al., 2002).

20.5 Conclusion and future prospects

Recent developments in the field of tissue engineering have explored its potential to repair diseased or traumatized articular cartilage. Yet, this systemic approach needs to be tailored as per need to overcome major barriers to restore structure and function. The articular cartilage is avascular and possesses limited regeneration ability in diseased or injurious conditions. Further, aging also adversely affects healing capacity of cartilage, leading to significant changes in normal gate pattern. Unfortunately, the conventional treatments for improving the articular cartilage have proven ineffective due to complexity of its zones and regions. The chondrocytes are seeded in scaffold and transferred to the knee joint of patient. However, the current tissue engineering approaches, combining cells, scaffolds, and growth factors have demonstrated a significant development of cartilaginous tissues. Hydrogels scaffolds, both the natural and synthetic, are most promising for fabrication of cartilaginous tissues due to their biocompatibility, biodegradability, and closeness to native tissues. These quintessential properties of hydrogels are also attributed to porous nature and efficient regulation of mechanical and chemical signals. However, the future developments in 3D printing and microfabrication technology will significantly improvise the design of hydrogels to develop zonal scaffolds for cartilage regeneration.

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