Chapter 4

Natural Polymers: Polysaccharides and Their Derivatives for Biomedical Applications

Aja Aravamudhan[*,†,‡], Daisy M. Ramos[*,†,‡,§], Ahmed A. Nada[*,†,‡], Sangamesh G. Kumbar[*,†,‡,§]

[*]*Institute for Regenerative Engineering, University of Connecticut Health Center, Farmington, Connecticut, USA*

[†]*Raymond and Beverly Sackler Center for Biological, Physical and Engineering Sciences, University of Connecticut Health Center, Farmington, Connecticut, USA*

[‡]*Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, Connecticut, USA*

[§]*Department of Chemical, Materials and Biomedical Engineering, University of Connecticut, Farmington, Connecticut, USA*

Chapter Outline

4.1 INTRODUCTION

Polymers of both natural and synthetic origin have been used for a variety of biomedical applications. Polysaccharides, proteins, and polyesters derived from both plant and animal kingdoms constitute the family of natural polymers. Several of these polymers are part of our diet and have been used in a variety of human applications in pharmaceutical excipients, prosthetics, drug delivery, and imaging applications. These polymers are known to be recognized by the biological environment and channeled into metabolic degradation. Due to the similarity that natural polymers share with the extracellular matrix (ECM) components, these materials

may also avoid the stimulation of chronic immunological reactions and toxicity, often detected with synthetic polymers [1].

Polysaccharides consist of monosaccharides (sugars) linked together by *O*-glycosidic linkages. Differences in the monosaccharide composition, linkage types and patterns, chain shapes, and molecular weight dictate their physical properties, such as solubility, viscosity, gelling potential, and/or surface and interfacial properties. Polysaccharides are derived from renewable resources, like plants, animals, and microorganisms, and are therefore widely distributed in nature. In addition, polysaccharides perform different physiological functions and hence have great potential applications in the fields of tissue engineering and regenerative medicine [1] (Figure 4.1).

There are hundreds of known polysaccharides. A list of polysaccharides from varying sources is given below:

1. Examples of polysaccharides from higher plants include starch, cellulose, and exudate gums like arabinogalactan, guar gum, and gum arabic.
2. Examples of algal polysaccharides: alginates, galactans, and carrageenan.
3. Examples of polysaccharides from animals: chitin, chitosan, glycosaminoglycans (GAGs), and hyaluronic acid (HA).
4. Examples of polysaccharides from microorganisms: dextran, gellan gum, pullulan, xanthan gum, and bacterial cellulose.

Their monomer composition and biological source provides these polysaccharides with different sets of physicochemical properties. Often, polymers of natural origin have limitations in terms of their solubility and industrially acceptable processability factors such as high temperature of melting, which are commonly applied to synthetic polymers. For instance, the majority of polysaccharides are water-soluble and oxidize at elevated temperatures beyond their melting point. These limitations have to be overcome prior to designing any products using polysaccharides. For instance, techniques to cross-link polymer chains have been developed to stabilize polysaccharide structures in order to give structural stability in aqueous environments [2,3]. Polysaccharide chitin in its native form cannot be produced into desired sizes and shapes due to its inability to dissolve in most common industrial solvents. Thus, chitosan, the deacetylated form of chitin, was produced and applied widely instead of native chitin itself, as chitosan is a water-soluble polymer at low pH. Due to its properties, chitosan is widely used for biomedical applications [4]. The following sections will summarize these efforts in the context of biomedical applications (as shown in Figure 4.1)

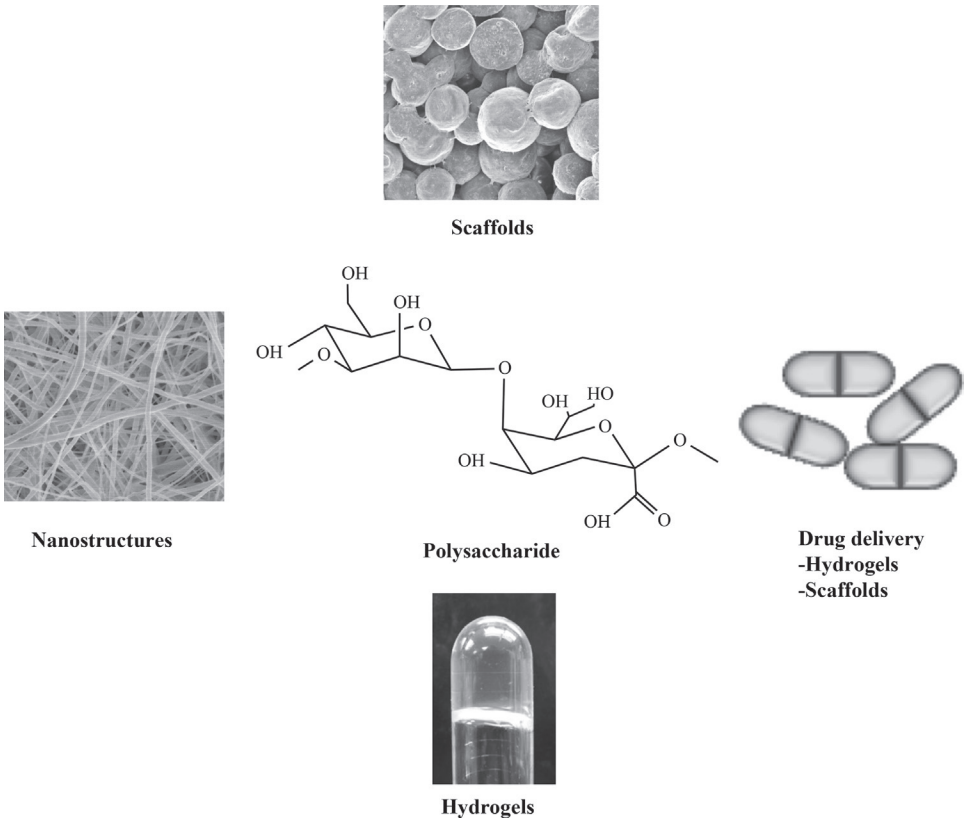

FIGURE 4.1 The use of polysaccharides in biomedical applications.

of polysaccharides, invoking native polysaccharides, semi-synthetic polysaccharide derivatives, and their blends with other synthetic polymers.

4.2 HYALURONIC ACID

4.2.1 Chemical Structure, Properties, and Sources

Chemically, HA is a linear polysaccharide made up of D-glucuronic acid and *N*-acetyl-D-glucosamine that are linked to one another by a β-(1→3) linkage. There could be 250-25,000 such basic disaccharide units in a polymer chain of HA, connected by β-(1→4) linkage (Figure 4.2). The disaccharide units of HA are extended, forming a rigid molecule whose many repelling anionic groups bind cations and water molecules. In solution, hyaluronate occupies a volume approximately 1000 times than in its dry state. Hyaluronate solutions exhibit clear viscoelastic properties that make them excellent biological absorbers and lubricants. These properties also attribute to its preferred form of fabrication into hydrogels. Because of its hydration properties, HA has the ability to bear compressive loads *in vivo* and provide lubrication at the same time. *In vitro*, HA has been shown to facilitate cell migration and pericellular matrix formation [5].

Biologically, HA is an important GAG component of connective tissue, synovial fluid (the fluid that lubricates joints), and the vitreous humor of the eye in mammals [6]. The biological roles of HA are widespread and widely appreciated [7–14]. They range from development, angiogenesis, cellular migration, and receptor-mediated signaling through receptor CD44 and receptor for HA-mediated motility in ECM remodeling and mediation of inflammatory responses [15,16]. HA chain length plays an essential role in the biological functions elicited in native and hence the engineered tissues. Therefore, the molecular weight of HA is an important consideration for the response elicited. For instance, while the low-molecular-weight HA (less than 3.5×10^4 Da) is known to be involved in cytokine activity implicated in inflammatory responses [17], the higher-molecular-weight HA (above 2×10^5 Da) is known to inhibit cell proliferation [18]. Similarly, (1-4 kDa) smaller fragments of HA have a positive effect in promoting vascularization during injury, whereas the (1-9 kDa) large fragment showed no significant effects [18,19]. Not only is the usage of HA at the correct molecular weight and chain length, but also the hyaluronidase (HAS) isozyme (Figure 4.3) that is responsible for degradation of HA in the tissue determines the chain length of the degradation product. Therefore, the enzymes in the tissue where the HA material is to be implanted are a factor for consideration while using the material in tissue engineering [20].

4.2.2 Attempts Made in Tissue Engineering and Drug Delivery

4.2.2.1 HA Alone

HA is highly water-soluble at room temperature and at acidic pH values and exhibits high rates of turnover *in vivo* (half-life varies from only minutes in the blood to weeks in cartilage) [7,21,22]. These properties pose challenges for the material's integrity *in vivo*. Therefore, the usage of HA in its native form in tissue engineering and drug delivery applications is pretty limited. Several cross-linking methods are used to increase the stability of HA in such applications. Some of them are as follows: water-soluble carbodiimide cross-linking, polyvalent hydrazide cross-linking, divinyl sulfone (DVS) cross-linking, disulfide cross-linking, and photo-cross-linking hydrogels through glycidyl methacrylate-HA conjugates [20]. Hence, these techniques of covalent cross-linking provide the opportunity to combine HA with more mechanically stronger polymers.

4.2.2.2 HA Derivatives and Combinations with Other Polymers

The early usage of HA was in ophthalmic drug delivery systems where it provided an ideal matrix for covalent

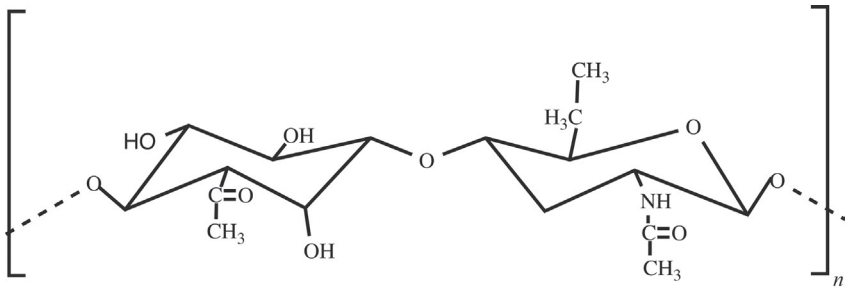

FIGURE 4.2 The structure of hyaluronic acid (HA).

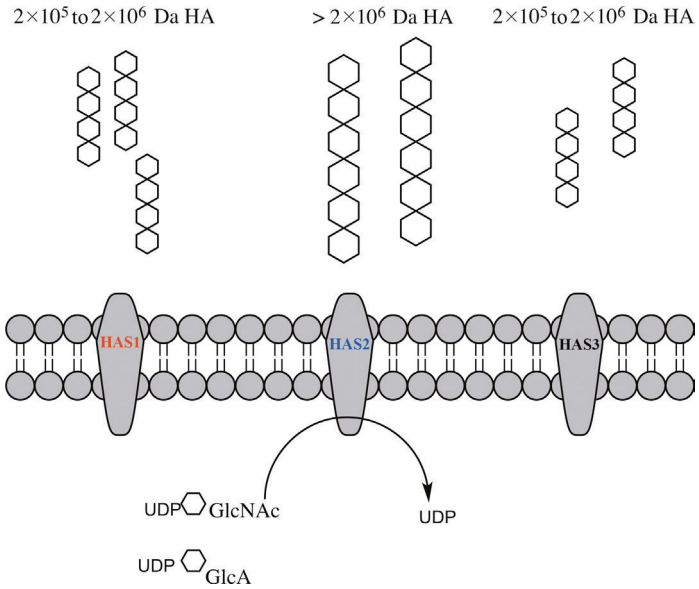

FIGURE 4.3 The three HAS isozymes produce distinct chain lengths of HA. HAS1 and HAS3 can produce chains of 2×10^5 to 2×10^6 Da, while HAS2 produces HA of chain length greater than 2×10^5 Da. Modified from ref. [23].

attachment of drugs and showed as much as twice the retention in contrast to free drug (methylprednisolone esters of HA) [24,25]. Different formulations such as gels, solutions, and hydrogels with several model drugs such as pilocarpine and tropicamide showed that HA ester systems were effective ophthalmic drug delivery systems [25]. HA has also been employed in liposomal dermal drug delivery. HA was conjugated to the surface of liposomes by carbodiimide cross-linking of its surface carboxyl residues to the amine residues on the liposome. The epidermal growth factor showed an encapsulation efficiency of >87% in these HA-conjugated liposomes. Avid binding of these HA-conjugated liposomes to a cellular monolayer in culture was seen that did not occur with the unmodified liposomes [26]. Sodium butyrate used as an antiproliferative drug in treatment of cancer has an extremely short half-life of 5 min *in vivo*. In order to bypass this constraint, butyric ester derivatives of HA were synthesized by stepwise chemical treatment of HA. The degree of substitution (DS) varied from 0.1 to 2.24 (1.8-28.4%). MCF breast cancer cell lines showed maximum antiproliferative response with DS of 0.2. It was seen that complete internalization of the HA vehicle occurred in 2 h through CD44 receptors that are frequently overexpressed on cancer cells [27]. From these and several other systems where HA and its derivatives have been used to deliver drugs, its innate role that facilitates binding of HA to receptors/specific cell types helps achieve efficient targeted delivery of intended drug to a tissue while its activity is preserved.

Another major application of modified HA is in the formulation of tissue engineering scaffolds. Radiation-mediated cross-linked HA networks have been used as scaffolds for cell growth with positive outcomes [28]. HA modified with methacrylic anhydride (MA) was photopolymerized to produce HA-MA hydrogels containing porcine chondrocytes [29]. The chondrocytes within the HA scaffolds were viable and were able to produce neo-cartilage within the porous networks. Photopolymerizable HA-MA hydrogels have also been used in heart valve applications in which the HA hydrogels were designed to mimic the cardiac ECM from which the heart valves develop [30]. HA has been combined with other natural/synthetic polymers to produce scaffolds. For example, HA has been combined with polypyrrole to create a multifunctional copolymer [31,32]. When implanted into rats, there was a marked early increase in local vascularization [32]. On the other hand, it was observed that polypyrrole-HA polymers were subsequently sulfonated, which was shown to significantly reduce platelet and cell adhesion [31].

Benzyl derivatives of HA (Hyalograft C and HYAFF-11) are used as polymeric scaffolds for tissue engineering of cartilage [33]. Though laboratory tests have given mixed results, human clinical results show normal cartilage formation when implanted into previously damaged tissue [34]. Hence, benzyl esters of HA have a great potential as scaffolds and drug delivery vehicles for chondrocytes in tissue engineering. Another HA-based scaffold examined for tissue engineering-based cartilage regeneration is auto-cross-linked polysaccharide polymer (ACP). While comparing ACP with HYAFF-11, poly(L-lactic acid) (PLLA) and poly(DL-lactic-glycolic acid) (PLGA) in an osteochondral defect, faster degrading scaffolds of ACP, PLAGA had greatest regeneration, while it was slower in HYAFF-11, PLLA that had slower degradation rates. These *in vivo* data

revealed that the scaffolds degraded within 4 months and were able to repair the osteochondral lesions, again emphasizing the selection of the type of HA and the consideration of HAS system in the tissue that would degrade it [35,36]. HYAFF-11 scaffolds have shown very positive effects as scaffolds for engineering of vascular and hepatic tissue and showed great ability for maintenance of cell phenotype, indicating that it could be used as scaffolds for many tissue engineering applications [37,38].

Combining chitosan and high-molecular-weight HA (2.4×10^6 Da) in a three-dimensional copolymer system promoted chondrocyte adhesion. The production of aggrecan and the native rounded morphology of seeded chondrocytes increased with the concentration of added HA and the scaffolds with HA performed better than scaffolds of chitosan alone [39]. Incorporation of chondrocytes and HA into orthopedic implants by cross-linking HA to amine-terminated PLGA-poly(ethylene glycol) (PEG) scaffolds allowed the attachment and proliferation of donor chondrocytes. It was also observed that collagen II expression, a marker of healthy cartilage phenotype, and DNA synthesis were significantly increased in polymers that incorporated HA [40]. Combining HA with DVS, and cross-linking with ultraviolet light, created suitable surfaces for cell adhesion. When a DVS-cross-linked HA scaffold was dehydrated before seeding with smooth muscle cells, gels were more porous and conducive to cell migration and infiltration, but did not lose their nonimmunogenic properties [41–43]. Follow-up studies conducted on the same material showed that smooth muscle cells increased the synthesis of ECM components elastin and collagen of aortic valve tissues, over cells cultured on tissue culture plastic; this synthesis was controlled by HA fragment size and dose [42,44]. Such studies have shown that HA is a suitable material for vascular and cardiac tissue engineering. HA was also introduced directly to engineered structures exogenously. For example, collagen matricides implanted in rabbits have shown greater numbers of chondrocytes (1.5 times control values) after the addition of soluble HA. The addition of HA fragments increased the amounts of proteoglycans, a desirable component of remodeling [45]. However, small HA oligosaccharides (4-16 disaccharides), on the other hand, can prevent cell proliferation *in vivo* [46]. These findings could have implications for improving the potency of implanted small-diameter vascular grafts and preventing stenotic lesions after graft implantation in cardiovascular tissue engineering.

With knee osteoarthritis (OA) patients increasing to a staggering 19 million in 2010, treatment by visco-supplementation has become popular. Visco-supplementation refers to the concept of synovial fluid replacement with intra-articular injections of hyaluronan mainly for the relief of pain associated with OA [47,48]. Intra-articular injections of hyaluronan ameliorate pain and function, generally for up to 3 months with no serious adverse events. In the United States, the first single-injection hyaluronan for visco-supplementation, Synvisc-One®, was approved in early 2009. The global market for dermal fillers is constantly increasing with 100 different dermal fillers on the market for aesthetic plastic surgery and about half of them are based on hyaluronan [49]. Thus, it can be summed up that HA has already stepped into clinical treatments in tissue engineering and will gain greater importance in the future.

4.2.3 Promises and Challenges with HA

HA has had a profound impact on the field of tissue engineering. The incorporation of HA into biomaterials and scaffolds has yielded a new class of biocompatible, controllable, and readily degradable materials. These new scaffolds have been tested with multiple types of cells and have been shown to promote beneficial remodeling of engineered tissues, as well as the gross preservation of cell phenotypes. However, harnessing the endogenous activity of the hyaluronan synthases to stimulate endogenous HA production is yet another useful strategy for the future. Recent research developments regarding HA as a molecular delivery vehicle for pharmacological and oncological applications, as well as in the orthopedic and cardiovascular arenas, have the potential to transform the clinical future of tissue engineering.

4.3 CHONDROITIN SULFATE

4.3.1 Chemical Structure, Properties, and Sources

Chemically, chondroitin sulfate (CS) is a sulfated GAG derivative. Alternating units of *N*-acetylgalactosamine and glucuronic acid are the monomers that constitute the basic unit of CS (Figure 4.4). Hundreds of these alternating units could be present together in a CS polymer chain. CS is a GAG seen associated with proteins in living systems termed proteoglycan. CS is classified according to the position of sulfation of the monomer unit. The types of CS and their chemical structure are listed in Table 4.1.

Biologically, CS is a major structural component of the ECM of several tissues of the body, like cartilage. The biological function and efficacy of CS seems to be highly

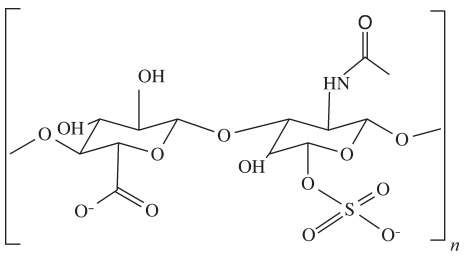

FIGURE 4.4 The structure of chondroitin sulfate (CS).

TABLE 4.1 Types of Chondroitin Sulfate (CS)

S. No.	Name (Type)	Synthetic Name (Sulfation of GalNAc)
1.	Chondroitin sulfate A	Chondroitin-4-sulfate
2.	Chondroitin sulfate C	Chondroitin-6-sulfate
3.	Chondroitin sulfate D	Chondroitin-2,6-sulfate
4.	Chondroitin sulfate E	Chondroitin-4,6-sulfate

dependent on the chain length of the polymer. For instance, CS extracted from the trachea is usually shorter ca. 20-25 kDa and considered to be of lower quality. On the other hand, CS polymer extracted from shark is considered more bioactive and of higher quality with longer chain length ca. 50-80 kDa. Even CS extracted from a single source could have variable polymer chain length [50]. Hence, the type and chain length of CS is likely to determine its biological functions, such as interaction with growth factors and proteins in GAG complexes of the ECM, and its ability to influence cellular function [51,52]. CS is also an indispensible component of tissues for maintaining their mechanical properties. The resistance of cartilage to compression is attributed to the tightly packed, charged sulfate groups of CS. This leads to osmotic water retention and swelling of cartilage and hence endows it with the weight-bearing mechanical properties [53]. Apart from its structural role, CS has also been an important player in basic biological processes including cell division and development of the nervous system. Such biological roles are mediated by the binding of CS to growth factors and cytokines and regulating signaling pathways in neurons [54,55]. Age-related changes in sulfation of the CS chains indicate their fundamental biochemical role in tissues [56] during age-related pathological changes. Loss of CS has been implicated in pathological conditions. For example, OA in cartilage is seen to occur due to the loss of CS leading to cartilage degeneration. Many studies on the impact of CS administration in patients suffering from OA along with *in vitro* results suggest that CS could improve pathology of OA through promoting proteoglycans synthesis, usually lost during cartilage degeneration [57], inhibiting elastase [58–60] and cathepsin G activity [61] and reducing gene expression for a number of proteolytic enzymes [62].

The reason for high variability in the outcomes of treating OA with CS is due to the variation in the chain length of the polymer used in these studies. While the high-quality, long-chain CS is more effective, the low-quality, short-chain CS has minimal effects on the pathogenesis of OA. Apart from its effects of improving OA, the anti-inflammatory effects of CS have led to improvement in conditions such as psoriasis and inflammatory bowel disease. These effects seem to be related to the inhibition of cytokines such as TNF-α [63], and IL-1β-induced translocation of NF-κB [64]. However, most of the effects are dependent on the type of CS determined by degree and position of sulfation, as well as the chain length of the polymer [65].

4.3.2 Attempts Made in Tissue Engineering and Drug Delivery

4.3.2.1 CS Alone

CS is often administered orally as it is seen to improve joint-related pathologies [50]. The intact polymer is often consumed, and several studies have examined the levels of the CS in blood plasma after consumption [65,66] in humans, mice, and horses. The degree of sulfation and chain length of the polymer were seen to determine the rate of uptake, retention, and clearance from these biological systems. For instance, tracheal CS with lower molecular weight is absorbed quickly within 1-5 h and reached a peak plasma concentration at around 10 h. On the other hand, shark CS of high molecular weight is seen to have a slower rate of uptake at around 8.7 h and was retained in the system for as long as 16 h [66]. It was also observed that desulfated chondroitin had quick uptake rate (15 min) and was also cleared within 3 h [67]. Further, *in vitro* experiments showed that there was a direct correlation between the polymer size and the rate at which it crossed the gut wall [68]. This in turn reflected as the rate of uptake and retention of the polymer in the biological system. Therefore, smaller CS polymer is taken up and cleared quickly, while the larger CS polymer takes longer time to be absorbed and cleared from a system.

4.3.2.2 CS Derivatives and Combination with Other Polymers

CS is a water-soluble polymer. Early attempts of using CS as a vehicle for colonic drug delivery cross-linked the polymer to different degrees to achieve a biodegradable system for controlled release of model drugs, indomethacin. A linear correlation between degree of cross-linking and rate of drug release emerged, indicating that the level of cross-linking of CS can be used to regulate the kinetics of drug delivery [69,70]. Still, cross-linking of CS is used as a strategy to decrease the dissolution of CS in water. For example, treatment of water-soluble CS polymer with different proportions of trisodium trimetaphosphate achieved cross-linking of CS and reduced solubility for the usage of the polymer in drug delivery applications [71].

Mostly due to its highly water-soluble nature and lack of mechanical stability, CS has been modified with or used along with other polymers for drug delivery and tissue engineering applications. Porous sponge of CS-chitosan was used for the delivery of platelet-derived growth factor

(PDGF), aimed at achieving greater bone regeneration. Aqueous CS-chitosan solution was subjected to freeze-drying followed by cross-linking to form a porous sponge with 100-150 μm pore size. PDGF-BB was incorporated into the CS-chitosan sponge by soaking CS-chitosan sponge into the PDGF-BB solution. The amount of CS could act as a factor to control the release of PDGF-BB from the sponge [72]. It was also seen that the presence of CS increased the osteoconductive characteristics of the material. Hence, the bioactive CS could be used in combination with other polymers in the formulation of materials for delivery of growth factors.

As CS has biological roles that are advantageous and is a water-soluble and enzymatically biodegradable polysaccharide, it has been used in combination with materials such as collagen in preparation of scaffold matrices. Addition of CS to collagen type I matrix was achieved by using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide as a cross-linking agent. The CS-bound matrix had increased water-binding capacity, with decreased tensile strength and temperature of denaturation [72]. Apart from imparting advantageous biological properties to the scaffold, CS also presented the opportunity to control the scaffold's mechanical and degradation characteristics.

As explained in the previous section, CS is an important component of joint ECM and known to play crucial roles in development and amelioration of joint/cartilage pathologies. Hence, it is used extensively in osteochondral tissue engineering. Here again, a number of CS variants such as modified CS or CS in combination with other natural and/or synthetic polymers have been utilized for the formulation of scaffolds. Scaffolds of different formats were utilized. Both fibrous spongy scaffolds and hydrogel scaffolds have been formulated utilizing CS. While employing CS in combination with collagen type I in the form of fibrous sponge matrix, a clear advantage in chondrocyte proliferation and phenotype maintenance was seen [73]. Similarly, sponges with a pore diameter of 180 μm were synthesized using natural polymers gelatin, CS, and HA together. When these scaffolds were seeded with chondrocytes, they maintained their morphology for up to 5 weeks and showed higher levels of aggrecan production than scaffolds that did not contain CS and HA components. These *in vitro* studies clearly point to the potential of scaffolds incorporating CS for cartilage tissue engineering [74,75].

A number of hydrogel scaffolds have also been formulated using CS for cartilage. Chondrocytes have a rounded morphology and this is seen to be best preserved in hydrogels, and therefore, hydrogel scaffolds are an ideal option for cartilage regeneration. Polymers such as PEG, polyvinyl alcohol (PVA), and poly(3-hydroxybutyrate) (PHB) have been used with CS to formulate hydrogels. While PVA hydrogels alone could not support cell adhesion, the addition of CS lowered the extreme hydrophilicity of the hydrogel and facilitated greater cell attachment in the scaffold [76]. When CS containing collagen hydrogel was conjugated to a fabric of PHB, it showed greater osteogenic potential than the parent fabric, on the seeded osteoblasts [77]. The presence of CS in PEG hydrogels promoted the chondrogenic differentiation of seeded bone marrow-derived mesenchymal stem cells, but did not allow them to proceed onto a hypertrophic state. This was an optimal outcome suitable for chondrocyte differentiation, to achieve cartilage regeneration [78]. In a recent study, the polysaccharide backbone was modified with methacrylate and aldehyde groups to form an adhesive gel. This modification led to better integration of the hydrogel with the implanted tissue *in vivo*, as seen in a rat model. This was due to greater scaffold-protein interaction brought about by the addition of modified CS [79]. Efforts have been made to deliver CS using scaffolding principles to joints affected by OA. For instance, a scaffold with 40% chitosan and 60% CS was designed to serve as a carrier of CS proved effective [80]. Hence, CS by itself after certain modifications and in combination with other synthetic and natural polymers has a great potential in scaffolding and drug delivery for osteochondral regeneration.

4.3.3 Promises and Challenges with CS

There have been many promising outcomes with CS as a drug and as a matrix for tissue engineering, both *in vitro* and *in vivo*. However, clinical outcomes have been inconsistent and have variable results. Better experimental design and ways to determine the polymer length and level of sulfation would be crucial to determining the fate of exogenously administered CS. Better experimental design and a greater understanding of the biological role of CS are important factors for determining the polymer's role and the most suitable type of CS polymer that lend the desirable biological outcomes.

4.4 CHITIN AND CHITOSAN

4.4.1 Chemical Structure, Properties, and Sources

Chitin is a structural polysaccharide found in nature. Chemically, chitin is made monomer units of 2-acetamido-2-deoxy-b-D-glucose connected through β (1→4) linkages (Figure 4.5). The C2 position in the glucose ring in the monomers has an acetamido group. The N-deacetylation of this chitin leads to the formation of chitosan. The degree of conversion of the acetamido group to amine group is never really complete. This is given by the degree of deacetylation (DD) of the chitosan. The DD of chitosan can vary from 30% to 95%. This conversion of chitin to chitosan renders the material more readily soluble and processable for various applications (Figure 4.6). Though crystalline chitosan

is insoluble in aqueous solution at a pH > 7.0, in dilute acid where pH < 6.0, the positively charged amino group facilitates its solubility [82,83]. There are three reactive functional groups in chitosan—the amino group at C2 and primary and secondary −OH groups at C3 and C6 positions, respectively.

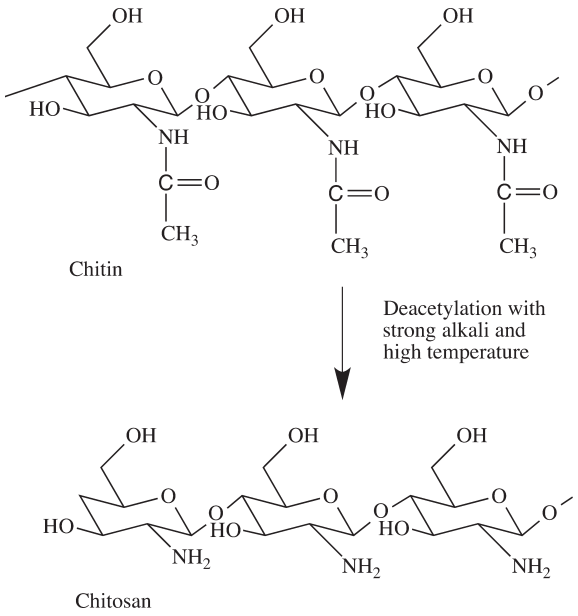

FIGURE 4.5 Structure of chitin and chitosan.

These reactive groups allow for chemical modification of chitosan such as covalent and ionic modifications.

Chitosan is one of the highly studied and utilized polysaccharide for tissue engineering. A process called "internal bubbling process" can be used to form porous structures from chitosan by freezing and lyophilizing a solution of chitosan. The addition of $CaCO_3$ to chitosan is used in this process to create porous chitosan gels [84]. This processability of chitosan is attributed to its cationic nature. The cationic property also leads to interaction of the material with negatively charged small molecules and proteins in biological systems. This is structurally very similar to native GAGs and hence plays crucial role in stimulating favorable responses in biological systems. Chitosan's mechanical properties are determined by pore sizes, the molecular weight, and crystallinity of the polymer. High-porosity, lower molecular weight, and less crystalline polymers are mechanically less competent and vice versa. Apart from these properties, chitosan is biodegradable. The acetylated residues of chitosan are targeted by lysozyme *in vivo* and this seems to be the major mechanism of chitosan degradation. Therefore, DD and crystallinity of the polymer are inversely related to degradation. Thus, the higher DD (>85%), the more crystalline the chitosan polymer and the slower its degradation in the body [85–87].

Chitosan is known to have antibacterial properties that [88] are attributed to the attack of negatively charged

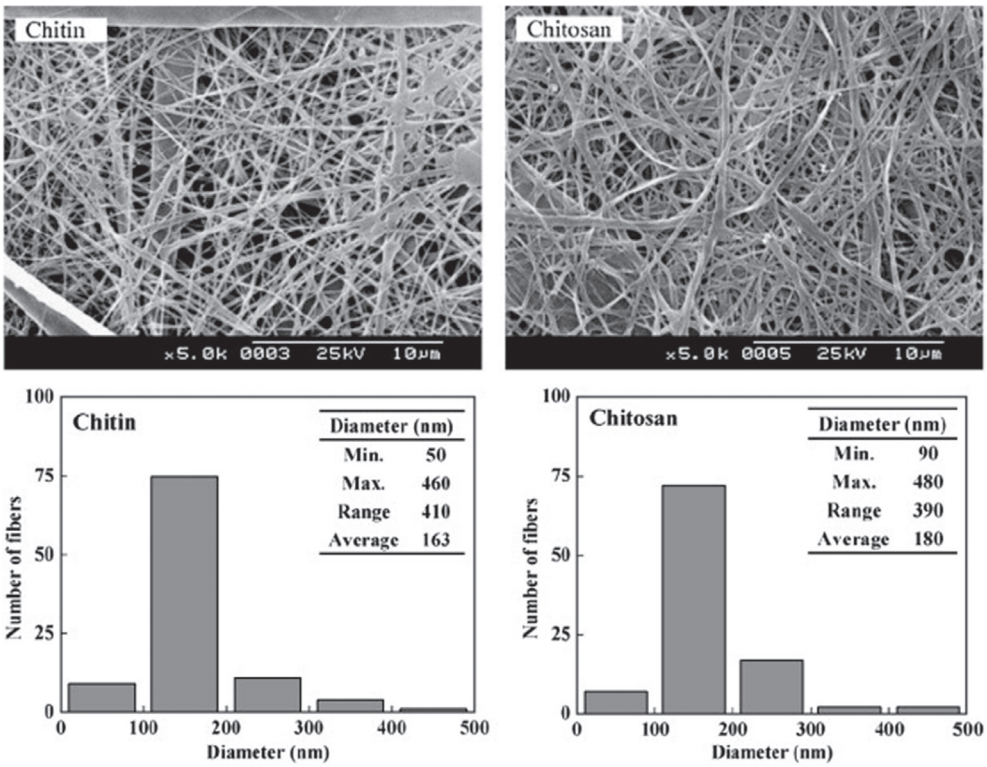

FIGURE 4.6 Chitin (on left) and chitosan after deacetylation (on right) nanofibers [73].

groups on the cell wall by the positively charged chitosan polymer. This leads to lysis of bacterial cell wall and hence its bactericidal activity. Inhibition of bacterial growth by chitosan is also attributed to its binding of bacterial DNA and interference of bacterial transcription [89]. Chitosan is also seen to have minimal immune rejection. Chitin is the major source for chitosan (Figure 4.6). It is the most abundant polymer to undergo biosynthesis, next to cellulose. It is a constituent of the exoskeleton in animals, like crustaceans, mollusks, and insects. It is also a polymer found in the cell wall of certain fungi. Most of the polymer used for commercial source comes as a by-product of the fishery industry [90].

4.4.2 Attempts Made in Tissue Engineering and Drug Delivery

4.4.2.1 Chitosan Alone

The various biological properties of chitosan have been discussed earlier. Chitosan has also been used as a dietary supplement. It is seen to lower low-density cholesterol and is helpful in weight loss [91]. Apart from its direct consumption, chitosan is used in drug formulations of different types such as microparticles, liposomes, granules, and gels for oral and parenteral drug delivery. In most of these applications, chitosan is physically or chemically cross-linked to obtain stability. The degree of cross-linking and drug loading are parameters used to control drug delivery [92,93]. In tissue engineering, chitosan alone was used initially. Since the mechanical and dissolution properties of the polymer make it tough to work with and formulate scaffolds, chemical modifications and their combinations with other natural and synthetic polymers are at present the more popular strategy. Common strategies are discussed in the following section.

4.4.2.2 Chitosan Derivatives and Combination with Other Polymers

Some of the limitations chitosan suffer from are those of insolubility at neutral pH and high water absorption by the polymer at a rapid rate. These factors pose problems of processability and also lead to rapid drug release from chitosan. Hence, chitosan is modified to overcome these limitations. Most modifications are brought about by reactions with the amine or hydroxyl groups of the glucosamine unit in chitosan. Using the reactive amine group, a number of modifying reactions are carried out. A simple example is a reaction in which an aldehyde functional group reacts with $-NH_2$ group of chitosan by reductive amination [94]. The introduction of *N*-cyanoethyl groups into the side chain of glucosamine in chitosan is a good example of this process. This reaction produced some cross-linking through a reaction between the nitrile group and the amine group of chitosan [95]. Examples of covalent modifications of chitosan include acylation and quarternization. When two oppositely charged polymers (a polycation and a polyanion), in a solution phase, separate out in a solution, a dense polymer phase called coacervate and a supernatant with low polymer content separate out. This process is termed polyelectrolyte complex formation. Polyelectrolyte complex formation has been used in a number of chitosan drug delivery systems [96,97] where controlled release of the loaded drug was desired. Modified chitosan and its blends with other polymers have been used in different formats. Nanoparticles and microparticles of chitosan and its derivatives have been formulated using techniques such as emulsification/solvent evaporation [81], spray drying [98], ionotropic gelation and coacervation [99], emulsion cross-linking [100], and sieving [101]. Thin films have been produced using solution casting, while cross-linking and gelation processes have been applied to produce hydrogels of chitosan for drug delivery [102]. Chitosan drug delivery vehicles in the form of tablets and gels are applied in dental, buccal, gastrointestinal, colon-specific, and gene delivery applications due to their favorable biological properties [102]. In tissue engineering, chitosan had been used mostly in minimally modified forms. The focus presently has shifted to improving the properties by introducing chemical modifications to form derivatives of chitosan for specific tissue regeneration purposes. Some of them are listed below.

4.4.2.2.1 Introduction of Sugars

Synthesis of chitosan bound to sugar has many applications in drug delivery and tissue engineering. This is due to the fact that cells, viruses, and bacteria recognize these sugar moieties and hence render these polymers good agents for targeting several target components in tissue engineering. For example, galactosylated chitosan worked as a good ECM for hepatocytes [103]. Specific antigen presenting B cells were recognized by mannosylated chitosan [104].

4.4.2.2.2 Graft Polymerization

Chemical grafting of chitosan can be used to functionalize chitosan and obtain important derivatives. Ceric ion, Fenton's reagent, gamma irradiation, various radicals, and ring opening reactions are the various routes used to achieve graft polymerization of chitosan [105]. Cell morphology and function were controlled by chitosan graft polymerized onto poly(L-lactide) (PLA) by plasma coupling reaction [106]. Further cooperative complementation through graft copolymerization or blend with poly(α-hydroxy acids) using a photosensitive cross-linking agent led to attachment of chitosan onto PLA films. These films showed improved cell attachment [107]. On the other hand, copolymerization of chitosan with heparin inhibited

platelet adhesion [108]. Therefore, graft polymerization can help modulate chitosan's properties to elicit a desired cellular response.

4.4.2.2.3 Immobilization of Specific Sequences

Specific amino acid sequences promote cell adhesion. The most commonly used sequence is the RGDs from adhesion proteins. Photo-cross-linking RGD peptides to chitosan improved the adhesion of human endothelial cells, compared to unmodified chitosan scaffolds [109]. In another approach, the −COOH group of amino acids such as lysine, arginine, aspartate, and phenylalanine reacts to the $-NH_2$ group of chitosan. These functionalized chitosan polymers were entrapped onto the surface of PLA to improve cellular responses [110].

4.4.2.2.4 Production of Nanofibers

Nanofibers mimic the structure of natural ECM closely. Hence, enhanced cellular responses are achieved on electrospun nanofiber scaffolds. Chitosan nanofibers ranging from several down to a few nanometers have been produced by electrospinning technique [111,112].

4.4.2.2.5 Thermal Gelation

Thermal gelling is a technique of injecting a polymeric aqueous solution while keeping the temperature above the polymer's sol-gel transition temperature and allowing the polymer to form a gel as it reaches the body temperature. A thermal gelling chitosan polymer was formed by neutralizing highly deacetylated chitosan solution with glycerol phosphate (GP). Chitosan remained in solution at physiological pH. Chitosan/GP solution gelled at body temperature and hence was an attractive, injectable hydrogel drug delivery system local delivery of antineoplastic drugs like paclitaxel [113]. Derivatives of chitosan have been used in skin, bone, cartilage, and liver tissue engineering. A detailed description of chitosan is given elsewhere in this book.

4.4.3 Promises and Challenges with Chitosan in Tissue Engineering

Chitin and its derivatives have a number of applications in drug delivery and tissue engineering. While being an abundantly available polymer, chitosan is also biodegradable inside the body, mostly by the enzymatic activity of lysozyme. However, chitosan/chitin by itself lacks good mechanical properties required in certain structural applications. Chemical modifications of chitosan and blending the polymer with other natural and synthetic polymers are done to overcome this limitation. On the whole, chitosan has a huge potential in tissue engineering and drug delivery applications.

4.5 ALGINIC ACID

4.5.1 Chemical Structure, Properties, and Sources

Alginic acid is an anionic, strictly linear (unbranched) copolymer of mannuronic acid (M block) and guluronic acid (G block) units arranged in an irregular pattern of varying proportions of GG, MG, and MM blocks [114] (Figure 4.7). C5 epimerization and flipping of sugar ring to $^1C_4$ position [115] is seen to occur for steric stability in the polymer. The M residues are linked at $^4C_1$ (diequatorial links). The G residues are the C5 epimers of M. These G residues are linked at $^1C_4$ with diaxial links [116]. The G and M blocks are present as similar or strictly alternating (GG, MM, or GM). Due to the diaxial linkage, G blocks (GG) are stiffer than alternating blocks (GM) and hence more soluble at lower pH. The content of G in alginates varies from 40% to 70%, depending on the source, and determines the quality of the alginate polymer. The molecular weight of alginate can vary widely between 50 and 100,000 kDa. It is generally seen that alginates with high G block content are highly suitable for biomedical application due to the ease of processability and low immunogenicity in the body. Hence, the content of G and M blocks is a crucial factor that determines the properties and applications of the resultant alginate [115].

Alginates are polysaccharides produced by a wide variety of brown seaweeds (*Laminaria* sp., *Macrocystis* sp., *Lessonia* sp., etc.). Additionally, bacteria also synthesize alginates and these can be used as tools to tailor alginate production, by understanding the biosynthesis of the polymer in these bacteria. A family of enzymes termed mannuronan-C5-epimerases convert M into G at the polymer level. By genetically selecting and engineering *Pseudomonas* strains that contain only a single epimerase for the production of high G containing alginates has been possible. Using such strategies, alginates with

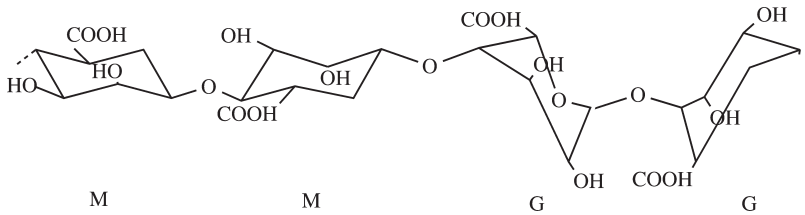

FIGURE 4.7 Structure of alginic acid.

up to 90% G content and extremely long G blocks have been produced [117–119].

Though such strategies are useful to engineer alginates, most of the alginates extracted for large-scale applications originate from natural sources such as seaweeds. The quality is determined by the species and even the seasonal variations. The alginate could contain from 10% to 70% G. Techniques of separation such as fractionation and precipitation in calcium can help separate the G block- and M block-rich alginates. The molecular weight of alginate is a critical factor to influence its viscosity in solution, besides the concentration of the polymer. The most important property of alginate is its ability to gel in the presence of cations (like Ca^{2+} and Ba^{2+}) (Figure 4.8). The carboxylic acid groups of sugars in G blocks of adjacent polymer chains cross-link with multivalent cations to form a gel. Factors that influence the stiffness of the gel are molecular weight distribution of the alginate polymer (dependent on M/G ratio) and the stoichiometry of alginate with the chelating cation [120,121].

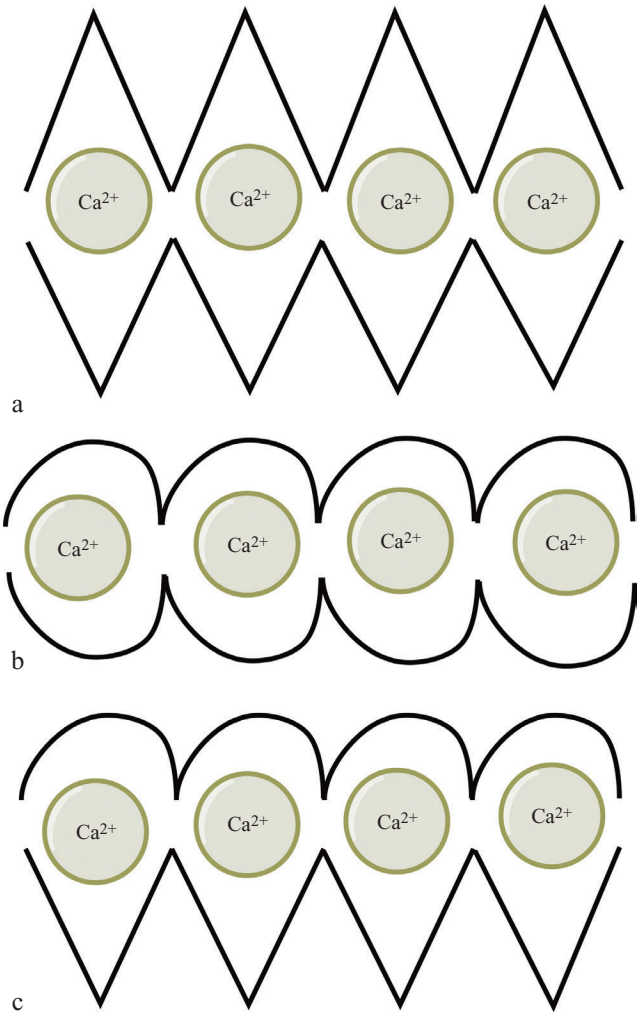

FIGURE 4.8 Gelation of alginic acid in the presence of Ca^{2+}. Possible junctions: (a) GG/GG junctions, (b) MG/MG junctions, and (c) mixed GG/MG, with Ca^{2+}.

4.5.2 Attempts Made in Tissue Engineering and Drug Delivery

4.5.2.1 Alginate Alone

The usage of polymers in biomedical applications such as drug delivery and tissue engineering is highly dependent on its ability to degrade in the body [122]. There are enzymes that can degrade alginate in the human body. The mechanism of degradation of alginate in the body happens in multiple ways as described below:

1. Disintegration of the alginate material by exchange of gelling calcium ion with sodium.
2. Acid hydrolysis and alkali hydrolysis. At a physiological pH of 7.4, β-elimination by alkali hydrolysis is the predominant mechanism of alginate polymer length reduction. Oxidation of the gels, by agents such as peroxides [123], not only helps hasten the process but also weakens the gel, in a ring opening reaction of the polymer.
3. Degradation by reactive oxygen species. Most polymers including alginates undergo "free-radical depolymerization" or "oxidative-reductive depolymerization." Water molecules or molecular oxygen generates free radicals in living systems. Exposure of polymer to gamma radiation increases this process and can be used to enhance the rate of degradation [124].

Thus, the major disadvantages of alginate are the lack of enzymatic degradation and its inert nature that makes it nonadherent for cells. To overcome these limitations, alginate is widely used as its derivative in combination with other polymers in drug delivery and tissue engineering applications. Alginate by itself is used widely in many industries. It is used as a stabilizer and emulsifier in food industry, as it interacts with proteins, fats, and fibers. Alginate-pectin mixtures are used as gelling agents independent of sugar content in foods. Hence, alginate is used in many low-calorie substitute foods. The high hydrophilicity of alginates renders the material biocompatible and nonimmunogenic. Therefore, it is used widely in pharmaceutical industry as drug excipient [116], as dental impression material [125], and as a material for wound dressing [126].

4.5.2.2 Alginate Derivatives and Combinations with Other Polymers

In general, alginates are formed into gels either by using multivalent cations or by covalent cross-linking. These modifications will be discussed further, here. The major

purpose in subjecting alginates to chemical and physical modifications is to tailor their physical properties such as degradation, mechanical strength, and biological properties such as enhanced interaction with cells. Mechanical properties like stiffness and strength of alginate gels can be controlled by physical factors. Concentration of polymer [127] and its molecular weight [121] can be used to determine the density of polymer solution for formulation of gels. Increase of both these factors leads to higher viscosity of the polymer solution and hence a stiffer and mechanically strong gel. Cationic poly(ethyleneimine) (PEI) [128] addition leads to improvement of alginate gel's mechanical properties. High-molecular-weight PEI increases the resistance of the gel to de-cross-linking agents and thus improves the gel's stiffness. Gelling conditions such as temperature, type, and concentration of cross-linker also affect the mechanical properties of alginate gels. Low-temperature cross-linking [129] leads to slow cross-linking, due to reduced rate of calcium ion diffusion. This results in the formation of gels with enhanced mechanical properties. Apart from these factors, the presence of cells in the gels is also shown to improve its mechanical strength [130].

As discussed earlier in the previous section, there are many methods used to control the degradation of alginates. Gama irradiation [131] and partial oxidation [132] can be used to reduce the molecular weight of polymer and accelerate degradation rates. These techniques also affect the mechanical strength of alginate gels. Gels with bimodal molecular weight distribution have been formulated with one molecular weight polymer being oxidized and the other left untreated. This approach also accelerated gel degradation rate. In gels that are covalently cross-linked, the linker density [133] determines the rate of degradation as well as the strength of the gel. As alginates are not conducive to cell adhesion, covalent modification of the polymer, coupling whole (like fibronectin and collagen) [134,135] or parts of cell adhesion molecules (like RGD peptides), is a popular strategy used to increase cellular responses. Here, the concentration [136] of these adhesion molecules and the composition of the gel (M/G ratio) determine the effectiveness of the gel in inducing cell adhesion.

Alginate-based hydrogels have been used as drug delivery vehicles for low-molecular-weight (small) molecule drugs as well as proteins such as growth factors. Drug-alginate interactions play a crucial role in determining the rate of drug release from alginate gel matrices. Drug release rate can be completely controlled by charge polarity (hydrophilic molecules will be released quickly and hydrophobic ones more slowly), when there is no chemical interaction between the drug and the alginate matrix. Carbodiimide chemistry is used to link hydrophilic drugs [137] to alginate matrices to delay their release. In such a scenario, the rate of drug release is determined by polymer degradation. Here, a linker such as AAD could be used to attach the drug to alginate and the rate of release would be controlled by the concentration of the spacer. Ionic complexes can also be used to attach drugs to alginate [138]. Proteins such as growth factors have been successfully delivered by alginate gel systems. It was seen that such deliver systems preserved the bioactivity of the factors. Hence, angiogenic growth factors like β-fibroblast growth factor [139] and vascular endothelial growth factor (VEGF) loaded into alginate beads were successful in inducing angiogenesis to a greater extent than free administration of the growth factors. Ionic complexes were used to link VEGF [140,141] to alginate matrix and the dissolution of this complex along with diffusion acted to control the release of active VEGF from the gels over several weeks *in vivo*.

In most tissue engineering applications, alginates with modified physical, chemical, and biological properties are desirable. These characteristics can be achieved by chemically modifying alginate to form its derivatives and blends with other polymers. Alginate has a number of hydroxyl and carboxyl groups along its polymer backbone and these are ideal candidates for its chemical modification. Chemical modifications that can be carried out using the hydroxyl group are as follows:

- **i.** Oxidation: Oxidation of alginate polymer chain produced a decrease in the stiffness of the polymer by breaking C_2–C_3 bond. Here, sodium alginate is usually the substrate to be reacted with sodium periodate. This leads to oxidation on the −OH group at C2 and C3 positions of the uronic acid on sodium alginate. Two aldehyde groups result in each oxidized monomeric units. The resultant oxidized alginate has reactive groups on its backbone and large rotational freedom of the molecule. This renders the polymer more amenable to further chemical modifications and greater biodegradation [142,143].
- **ii.** Reductive amination of oxidized alginate: Oxidized alginate can be used as a substrate for chemical reactions such as reductive amination. Reductive amination is performed with alkyl amine by using $NaCNBH_3$ as reducing agent. This reaction is favorable at a pH of 6-7 and by-products such as aldehyde/ketone are negligible under the reaction conditions [144].
- **iii.** Sulfation: On sulfation, alginate structurally resembles heparin and attains anticoagulant properties, alongside high blood compatibility [145]. Reacting sodium alginate with formamide and chlorosulfuric acid ($ClSO_3H$) at 60 °C can sulfate it.
- **iv.** Copolymerization: Microwave irradiation of sodium alginate and acrylamide led to synthesis of various grades of grafted polymers [146]. Alginate-*g*-vinyl sulfonic acid prepared by employing potassium peroxydiphosphate/thiourea redox system has also been reported [147]. These synthesized graft copolymers

exhibit better results for swelling, metal ion uptake, and resistance to biodegradability in comparison to parent alginates themselves.

v. Linking cyclodextrins: α-Cyclodextrin can be covalently linked to alginate. This reaction can be targeted to the hydroxyl groups of the alginate by cyanogen bromide (CNBr) method to prevent reaction at the carboxyl group. This specificity was necessary to form the calcium-alginate beads that have great potential in encapsulating bacteria for environmental remediation [148].

Chemical modifications that can be carried out using the carboxyl group are as follows:

i. Esterification: Alkyl group is attached to a molecule during esterification. Addition of alkyl group to the backbone of alginate results in increasing the hydrophobicity of alginate. Alginate can be modified by direct esterification using several alcohols in the presence of catalyst. The alcohol is present in excess to ensure that the equilibrium is in favor of product formation. Propylene glycol ester of alginate was obtained by esterification of alginate with propylene oxide. This is a commercially useful derivative of alginate [149].
ii. Ugi reaction: Hydrophobically modified alginate can be prepared by the Ugi multicomponent condensation reaction. The Ugi reaction is a multicomponent reaction in organic chemistry involving a ketone or aldehyde, an amine, an isocyanide, and a carboxylic acid to form a bis-amide [150].
iii. Amidation: In amidation reaction, a coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, is reacted with alginate to form amide linkages between amine-containing molecules and the carboxylate moieties on the alginate polymer backbone. This results in hydrophobic modification of the alginate [151].

A general outline of reactions used to modify alginate for biomedical applications was summed up earlier. Though mostly hydrogel of alginate is the most popular form of application of the polymer, other formats have also been formulated. Alginate foams [152], fibers [153,154], and nanofibers [155] are other forms in which alginates are fabricated [115]. Bioartificial pancreas, bone [156], vasculature, and liver [157] are some of the tissue-engineered organs where alginate materials have been used successfully.

4.5.3 Promises and Challenges with Alginates in Tissue Engineering

Alginates are a versatile class of polysaccharides that present a great tool as materials for tissue engineering. They have been formulated as gels, microspheres, foams, and fibers in tissue engineering and for delivery of drugs and biological factors. Some constraints posed by the material are its nonenzymatic degradation in the human body and its extremely hydrophilic nature that discourage cell anchorage. However, these limitations have been overcome by subjecting its −OH and −COOH functionalities to chemical modifications. Understanding the biosynthetic pathways leading to alginate biosynthesis in bacterial systems has been very useful in tailoring their polymer chain composition and the molecular weight of the polymer. Yet, more work needs to be done in improving these systems. Alginate hence serves as a low-cost biopolymer that is a good tool in biomedical engineering.

4.6 CELLULOSE

4.6.1 Chemical Structure, Properties, and Sources

Cellulose, the "sugar of plant cell wall," is the most abundant biopolymer in the biosphere.

The basic monomer unit of cellulose is β-D-anhydroglucopyranose. These units are joined together covalently by acetal functions between the equatorial group of the C4 carbon atom and the C1 carbon atom (β-1, 4-glycosidic bonds). These β-1,4-glycosidic linkages bestow cellulose with its resistance to chemical/enzymatic attack [158] (Figure 4.9). Therefore, cellulose is a linear-chain polymer with a large number of hydroxyl groups (three −OH groups per anhydrous AGU unit). This linear structure can be extended to molecules containing 1000-1500 β-D-glucose monomer units, in a cellulose polymer chain. This chain length of cellulose is expressed in terms of number of constituent AGUs, termed degree of polymerization. The degree of linearity and the presence of extensive −OH groups throughout the cellulose chain are responsible for formation of inter- and intramolecular hydrogen bonds throughout the polymer chain. This causes cellulose chains to organize in parallel arrangements into crystallites and crystallite strands, the basic elements of the supramolecular structure of the cellulose fibrils and the cellulose fibers. This arrangement of fibers in the polymer is termed its supramolecular structure and it in turn influences its physical and chemical properties. Cellulose is known to exist in at

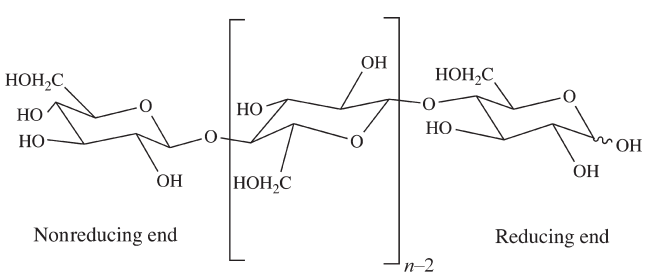

FIGURE 4.9 Structure of cellulose.

least five allomorphic forms. Cellulose I is the form found in nature. Cellulose may occur in other crystal structures denoted celluloses II, III, and IV. Cellulose II is the most stable structure of technical relevance. This structure can be formed from cellulose I by treatment with an aqueous solution of sodium hydroxide. This leads to regeneration of native cellulose from solutions of semi-stable derivatives. This crystalline structure is modified from cellulose I. A parallel chain arrangement of cellulose in cellulose I form undergoes a change to form cellulose II. This renders the cellulose II more accessible to chemical treatments and hence more reactive. A fringe fibrillar model is used to describe the microfibrillar structure of cellulose polymer. It describes the structure to be made of crystalline regions of varying dimensions called crystallites and noncrystalline regions. This structure explained the partial crystallinity and reactivity of cellulose in relation to its microfibrillar structure [159].

Cellulose behaves as an active chemical due to the three hydroxyl groups in each glucose unit. The hydroxyl groups at the second and third positions behave as secondary alcohols, while the hydroxyl group at the sixth position acts as a primary alcohol (numbering as shown in Figure 4.10). These −OH groups are responsible for the reactivity of cellulose. DS is the term used to indicate the average number of −OH groups substituted in an anhydroglucose unit of a cellulose molecule. That is, a DS of 3 indicates that all the three −OH groups have been substituted in the anhydroglucose units of the cellulose derivative. In general, the relative reactivity of the hydroxyl groups can be expressed as $OH\text{-}C_6 \gg OH\text{-}C_2 > OH\text{-}C_3$ [160].

Cellulose, like the polysaccharides above, has certain drawbacks. These include poor solubility in common solvents, poor crease resistance, poor dimensional stability, lack of thermoplasticity, high hydrophilicity, and lack of antimicrobial properties. To overcome such drawbacks, the controlled physical and/or chemical modification of the cellulose structure is essential [160]. Introduction of functional groups into cellulose can alleviate these problems while maintaining the desirable intrinsic properties of cellulose. Apart from the conventional plant source, cellulose is also obtained from bacteria, termed bacterial cellulose.

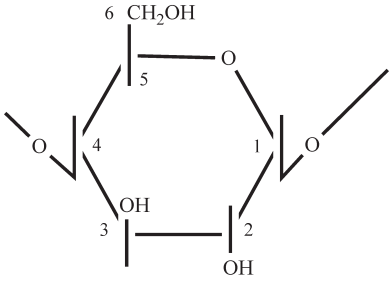

FIGURE 4.10 Numbering of carbon atoms in anhydroglucose unit of cellulose.

Understanding and engineering these biological systems has opened further doors for bringing in desired modifications into cellulose.

4.6.2 Attempts Made in Tissue Engineering and Drug Delivery

4.6.2.1 Cellulose Alone

As cellulose in its native form has extensive hydrogen bonds, it is not very processable. Most of the early attempts resorted to viscose process of regenerating (restoring cellulose structure back) cellulose from its derivatives. Regenerated cellulose has been used as a matrix for wound dressing. Early results indicated that implanted viscose cellulose sponges led to increased granulation tissue formation over a period of time and that the pore structure of the scaffold could influence cell infiltration within a certain limit [161]. On the other hand, it was seen that a lower content of cellulose and lower pore diameter induced greater tissue invasion of the implant, and later proved to be a matrix conducive for bone formation in a rat model [162]. However, cellulose sponges were seen to be slow-degrading matrices taking up to 60 weeks for them to be degraded [163]. Cellulose also was used successfully as an enzyme carrier by dissolving cellulose in ionic liquid and regenerating it. Usage of a hydrophobic ionic liquid preserved the enzymatic activity to a greater extent [164]. While biodegradation remains a challenge for the absorption of cellulose, it was seen that treatments that markedly reduced crystallinity led to degradation as well as high biocompatibility of regenerated cellulose [165]. In recent years, microbial cellulose (MC) produced by bacterial species such as *Acetobacter xylinum* has gained much attention as a material for use in biomedical appliances. Though chemically similar to plant cellulose, MC has a microfibrillar and nanostructured arrangement that enables higher water retention by the material. This property is conducive in its application in wound dressing, production of vascular conduits, etc. [166]. This cellulose was also seen to have a high degree of biocompatibility [167].

4.6.2.2 Cellulose Derivatives and Combination with Other Polymers

The supramolecular structure of cellulose and the extensive hydrogen-bonded chemical structure of cellulose render it insoluble in water and organic compounds. Therefore, most of the reactions involving cellulose are carried out in solid or swollen state as heterogeneous reactions. Here, the limiting factors for the heterogenous reaction are the breakage of hydrogen bonds (by alkaline treatment) and degree of interaction with the reaction media (by swelling). Therefore, specific solvents that disrupt the hydrogen

bonds in cellulose such as *N*,*N*-dimethylacetamide/lithium chloride (DMA/LiCl) and dimethyl sulfoxide/tetrabutylammonium fluoride (DMSO/TBAF) [168] are used widely for the purpose. Hence, even though using such strategies, cellulose alone has been employed for different purposes as a material, its derivatives are easier to work with as they overcome the limitations posed by cellulose. The following section gives an overview of derivatives of cellulose and its combination with other polymers:

4.6.2.2.1 Cellulose Esters

Esters of cellulose with interesting properties such as bioactivity and thermal and dissolution behavior can be obtained by esterification of cellulose with nitric acid in the presence of sulfuric acid, phosphoric acid, or acetic acid. Commercially important cellulose esters are cellulose acetate, cellulose acetate propionate, and cellulose acetate butyrate. Cellulose esters of aliphatic, aromatic, bulky, and functionalized carboxylic acids can be synthesized through the activation of free acids *in situ* with tosyl chloride, *N*,*N*′-carbonyldiimidazole, and iminium chloride under homogeneous acylation with DMA/LiCl or DMSO/TBAF. A wide range of cellulose esters that vary in their DS, various substituent distributions, and several desirable properties can be obtained through these reactions. Recently, a number of enzymes that degrade cellulose esters have been reported. Some of them are acetyl esterases, carbohydrate esterase (CE) family 1, and esterases of the CE 5 [169–172] family.

Cellulose esters have been put to use in many biomedical applications. Hemodialysis membranes used in purification of blood, for patients with renal failure have employed melt-spun cellulose diacetate membrane. These membranes have been produced by Altin (company) and used successfully. They were advantageous and had less toxicity than synthetic polymer-based membranes [173]. Cellulose acetate is seen as a preferred material for the fabrication of blood filtration devices that are used to separate a fraction of the blood such as red blood cells and leukocytes [174,175]. Cellulose acetate and regenerated cellulose fibrous matrices have been successful in supporting the growth of cardiac myocytes and present a potential scaffold platform for cardiac regeneration [176]. Our lab has been successful in the formulation of mechanically competent cellulose acetate- and ethyl cellulose-based scaffolds [177]. Further, these scaffolds could maintain the growth of osteoblasts (bone cells), *in vitro* [178] (Figure 4.11), indicating their potential application as scaffolds for bone regeneration. Hence, cellulose esters have a huge potential in tissue engineering and drug delivery applications.

4.6.2.2.2 Cellulose Ethers

Carboxymethyl cellulose (CMC) is the major cellulose ether. By activating the noncrystalline regions of cellulose, selective regions of alkylating reagents can attack the cellulose. This is termed the concept of reactive structure fractions and is used widely for the production of CMC. Another route for carrying out the same reaction is by derivatization of cellulose in reactive microstructures, formed by induced phase separation. This process involves the usage of NaOH in anhydrous state in combination with solvents like DMA/LiCl. These CMC products have a distribution of substituents that deviate significantly from statistical prediction of the product theoretically.

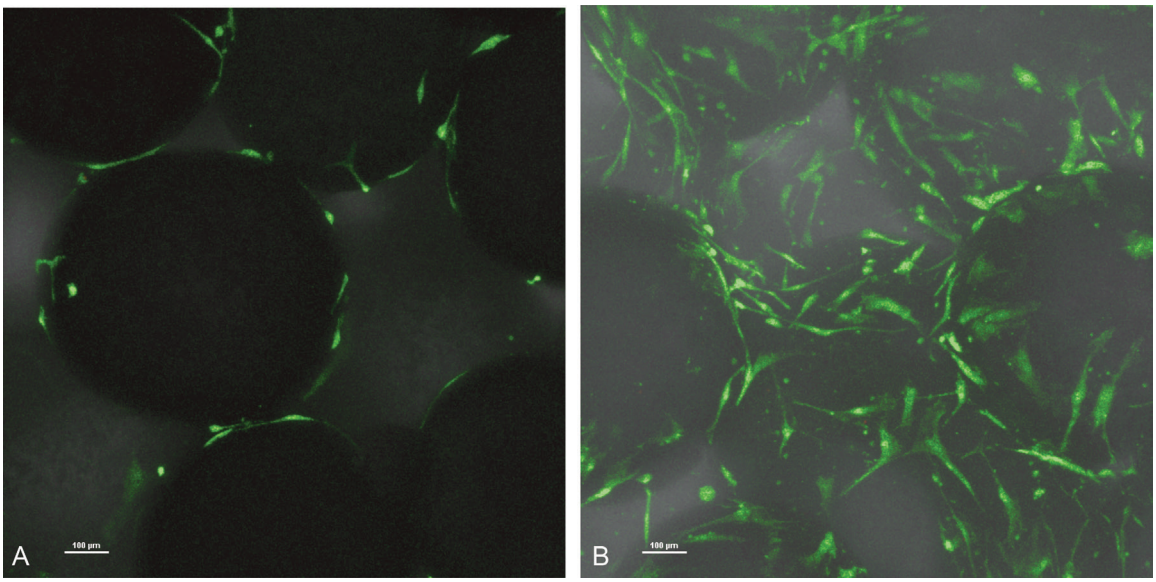

FIGURE 4.11 Cellular viability of osteoblasts on (a) cellulose acetate scaffolds and (b) cellulose acetate collagen scaffolds. The green cells are viable and the red ones are nonviable (Live/Dead staining) [170].

CMC is used in several drug delivery and tissue engineering purposes. The release of apomorphine, a drug used to regulate motor responses in Parkinson's disease, was successfully incorporated into CMC powder formulation and exhibited a sustained nasal release, and performed better than starch-based delivery vehicle [179]. Sodium CMC has been used successfully in gastrointestinal drug delivery [180]. Hence, CMC is seen as a successful drug delivery system for mucosal tissue [181]. Apart from drug delivery, CMC is useful as a scaffold in tissue engineering. CMC hydrogels having pH-dependent swelling characteristics were capable of releasing entrapped drug at the right pH present in the tissue of interest and showed great potential as a wound dressing material [182]. CMC hydrogels could be used for encapsulating cells of nucleus pulposis and hence are a potential replacement for intervertebral disk degeneration [183]. CMC has been combined with chitosan [184] and hydroxyapatite [185] for bone and dental regeneration purposes too.

4.6.2.2.3 Sillyl Cellulose

Silyl ethers of cellulose are characterized by a remarkable increase in thermal stability, lipophilic behavior, and a lack of hydrogen bonds. They can be used as selective protecting groups in organic synthesis, due to the simple cleavage of the silyl ethers under acidic conditions or through nucleophilic attack. Therefore, the silyl ethers of cellulose are very attractive for engineering polysaccharide chemistry [186]. The silylation of polar protic −OH groups of cellulose with chlorosilanes and silazanes leads to these silyl ethers. The degree (DS) and position of silyl substitution is determined by the reaction condition. All the three −OH groups will be substituted when trimethylsilylation with hexamethyldisilazane in liquid ammonia is used in the reaction. Dissolution of cellulose in DMA/LiCl (homogeneous reaction) makes the −OH groups more accessible. Following this, if the synthesis takes place in the presence of imidazole, the bulky silylation reagent thexyldimethylchlorosilane leads to complete silylation at O_6 and O_2 (DS value = 2.0). Here, the primary and the most reactive secondary −OH groups are converted. If silyl ether formation starts with the same reagent in cellulose suspension in aprotic dipolar media like *N*-methylpyrrolidone, which contain gaseous ammonia, silylation of all primary C_6-OH groups takes place. This state does not permit any further reaction of the secondary hydroxy groups [187].

4.6.2.2.4 Cellulose Sulfonates

The most frequently synthesized and used cellulose sulfonates are the *p*-toluenesulfonates (tosylates), methanesulfonates (mesylates), *p*-bromobenzenesulfonates (brosylates), and trifluoromethanesulfonates (triflates). The synthesis of sulfonates through simple esterification of the −OH groups of cellulose with the corresponding sulfonic acid chlorides or anhydride is a way to attach nucleofuge groups to cellulose [188]. By varying the solvent and reaction conditions, the DS of the polymer can be controlled. For instance, at temperatures of 7 °C, cellulose tosylate with a maximum DS of 2.3 can be formed with tosyl chloride in the presence of triethylamine. The DS of sulfonated cellulose can also be controlled by the molar ratio of tosyl chloride to glucose units of the cellulose.

4.6.2.2.5 Aminocellulose

Aminocellulose is an aminodeoxy derivative bearing the nitrogen function directly on the cellulose skeleton. These are useful in the immobilization of enzymes and other proteins, by having a specific structural design based on cellulose tosylates. Aminodeoxycellulose is synthesized with corresponding halogen derivatives and sulfonates as starting materials. PDA cellulose is a material used successfully for immobilization of enzymes like oxidoreductases, glucose oxidases, and peroxidases using glutaraldehyde. Diazo coupling and redox coupling have also been utilized to link ascorbates and dyes. Here too, the solvent composition and reaction conditions can determine the reaction chemistry (S_N2) and hence the DS of the product formed [189].

4.6.2.2.6 Resinification of Cellulose

Resinification of cellulose can impart crease resistance termed "durable press" properties to cellulose. The reaction of cellulose with bi- or polyfunctional compounds leads to formation of cross-linked cellulose matrix, called resinified cellulose [158].

4.6.2.2.7 Graft Polymerization of Cellulose

A new approach to modification of cellulose is by graft polymerization. A graft copolymer generally consists of a long sequence of one monomer, referred to as the backbone polymer (main chain) (cellulose in this case) with one or more branches (grafts) of long sequences of a different monomer [190]. Graft copolymerization permits the combination of the best properties of two or more polymers in one physical unit [160]. The aim with cellulose graft polymerization is to retain the inherent properties of cellulose and incorporate qualities from the polymer grafted onto it. Depending on the nature of the grafted polymer, properties such as dimensional stability, resistance to abrasion and wear, wrinkle recovery, oil and water repellence, elasticity, sorbancy, ion exchange capabilities, temperature responsiveness, thermal resistance, and resistance to microbiological attack can be incorporated into cellulose [191–195].

The methods for graft polymerization of cellulose can be generally classified into three major groups such as (i) free-radical polymerization, (ii) ionic and ring opening polymerization, and (iii) living radical polymerization.

Strategies used in cellulose graft polymerization can be divided into three categories:

(i) The "grafting to" approach—here, the functional preformed polymer with its reactive end-group is coupled with the functional groups located on the backbone of cellulose, the major polymer. The inherent weakness of this approach is a hindrance to diffusion caused by crowding of polymer chains on the surface [196].

(ii) The "grafting from" approach—here, the growth of polymer chains occurs from initiating sites on the cellulose backbone. This is the most commonly used approach. Easy access of the reactive groups to the chain end of the growing polymer is achieved in "grafting from" approach. This makes it possible to attain high graft density [196].

(iii) The "grafting through" approach—in this approach, a vinyl macromonomer of cellulose is copolymerized with a low-molecular-weight comonomer. Though this approach is more convenient, a cellulose-derived macromonomer has to be synthesized. This poses a limitation to this technique [196].

4.6.3 Promises and Challenges with Cellulose

Cellulose and other polysaccharides discussed earlier have been gaining importance as polymeric materials. Increasing the knowledge of organic, polymer chemistry and the chemistry of low-molecular-weight polysaccharides can greatly help us understand more about the chemistry of cellulose and control the different processing techniques for cellulose to a greater extent. Having a multidisciplinary approach will further help us utilize the polymer more in biomedical applications. The development of derivatives and grafted polymers of cellulose has been an important step toward the utilization of cellulose, which is considered as a renewable resource. Processes such as lyocell processing of cellulose are environment-friendly techniques and promise a safer polymer processing technology. New insights are still being obtained on the process of wood pulping and biosynthetic pathways in cellulose synthesis. By using this knowledge, engineering cellulose and utilizing bacteria for production of the polymer are the advances we could expect in the future.

4.7 CONCLUSIONS

Polymers derived from plants and animal kingdoms have been widely researched as biomaterials for a variety of biomedical applications including drug delivery and regenerative medicine. These polymers have biochemical similarity with human ECM components and hence are readily accepted by the body. Additionally, these polymers inherit several advantages including natural abundance, relative ease of isolation, and room for chemical modification to meet the technological needs. In addition, these polymers undergo enzymatic and/or hydrolytic degradation in the biological environment with body-friendly degradation by-products. Natural polymers include the list of polysaccharides (carbohydrates) and animal-derived proteins. Polysaccharides are an important class of biomaterials with significant research interest for a variety of drug delivery and tissue engineering applications due to their assured biocompatibility and bioactivity. Polysaccharides are often isolated and purified from renewable sources including plants, animals, and microorganisms. Essentially, these polymers have structural similarities, chemical versatilities, and biological performance similar to ECM components, which often mitigate issues associated with biomaterial toxicity and host immune responses. The building blocks of carbohydrate monosaccharide are joined together by *O*-glycosidic linkages to form a polysaccharide chain. Polysaccharides offer a diverse set of physicochemical properties based on the monosaccharide that constitutes the chain, its composition, and source. The popular list of polysaccharides used for a variety of biomedical applications includes cellulose, chitin/chitosan, starch, alginates, HAs, pullulan, guar gum, xanthan gum, and GAGs. In spite of many merits as biomaterials, these polysaccharides suffer from various drawbacks including variations in material properties based on the source, microbial contamination, uncontrolled water uptake, poor mechanical strength, and unpredictable degradation pattern. These inconsistencies have limited their usage and biomedical application-related technology development.

Numerous synthetic polymers with well-defined mechanical and degradation properties have been developed to meet the technological needs in the biomedical applications. However, these polymers from the biological standpoint lack much-desired bioactivity and biocompatibility and may cause toxicity and immune response. Polysaccharide structure offers freely available hydroxyl and amine functionalities that make it possible to alter its physicochemical properties by chemically modifying polysaccharide structure. For instance, grafting synthetic monomers on the polysaccharide chain offers an easy way to control polymer solubility in desired solvents, water uptake, and degradation. These semi-synthetic polymers offer best features of the both natural and synthetic polymers. Various cross-linking techniques to restrict the polysaccharide chain movement to control their water uptake, degradation, and mechanical properties have also been developed. Polysaccharide-based porous scaffolds, fiber matrices, hydrogels, and micro- and nanoparticles have been developed for a variety of tissue regeneration and drug delivery applications. In the recent years, glycochemistry has gained research momentum for understanding carbohydrate biological functions and

development of carbohydrate-based drugs and vaccines. Engineered carbohydrate-based polymeric structures may serve as an alternative material platform for a variety of regenerative medicine and drug delivery applications.

A new nonpetroleum-based biomaterial platform to meet the versatile needs in biological science and biomedical engineering could be achieved by collaborative efforts between academia, government, and industry partnership. The collaborative efforts should include bringing scientists working in different disciplines of chemistry, biology, polymers, materials sciences, and engineering to work toward these activities. These collaborative efforts could lead to the development of a methodology for synthesis of natural polymer-based semi-synthetic polymers and provide a greater depth of understanding of carbohydrate biological functions, polymer structure, material properties, degradation, and mechanical properties. Further, the development of modeling tools to predict structure, property and biological activity of carbohydrates for biomedical applications is a step in this direction. The goal of the new initiatives should focus on the development of natural polymer-based orthopedic fixation devices, biomedical implants, drug delivery vehicles, carbohydrate-based drugs, hydrogels, surfactants, coagulants, and absorbents for a variety of biomedical applications. The research activities in this area could generate commercially available technologies and products from the renewable resources and contribute immensely toward economic development.

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