Interpenetrating Polymer Networks polysaccharide hydrogels for drug delivery and tissue engineering

Pietro Matricardi a,*, Chiara Di Meo a, Tommasina Coviello a, Wim E. Hennink b, Franco Alhaique a

a Department of Drug Chemistry and Technologies, “Sapienza” University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
b Department of Pharmaceutics, Utrecht University, P.O. Box 80082, 3508TB Utrecht, The Netherlands

ABSTRACT

The ever increasing improvements of pharmaceutical formulations have been often obtained by means of the use of hydrogels. In particular, environmentally sensitive hydrogels have been investigated as “smart” delivery systems capable to release, at the appropriate time and site of action, entrapped drugs in response to specific physiological triggers. At the same time the progress in the tissue engineering research area was possible because of significant innovations in the field of hydrogels. In recent years multicomponent hydrogels, such as semi-Interpenetrating Polymer Networks (semi-IPNs) and Interpenetrating Polymer Networks (IPNs) have emerged as innovative bio-materials for drug delivery and as scaffolds for tissue engineering. These interpenetrated hydrogel networks, which can be obtained by either chemical or physical crosslinking, in most cases show physico-chemical properties that can remarkably differ from those of the macromolecular constituents. Among the synthetic and natural polymers that have been used for the preparation of semi-IPNs and IPNs, polysaccharides represent a class of macromolecules of particular interest because they are usually abundant, available from renewable sources and have a large variety of composition and properties that may allow appropriately tailored chemical modifications. Sometimes both macromolecular systems are based on polysaccharides but often also synthetic polymers are present together with polysaccharide chains.

The description and discussion of (semi)-IPNs reported here, will allow to acquire a better understanding of the potential and wide range of applications of IPN polysaccharide hydrogels. A quite large number of polysaccharides have been investigated for the design of (semi)-IPNs for drug delivery and tissue engineering applications. This review article however mainly focuses on two of the most studied polysaccharide-based (semi)-IPNs, namely those obtained using alginate and hyaluronic acid. An overview of the methods of preparation, the properties, the performances as drug delivery systems and as scaffolds for tissue engineering, of (semi)-IPNs obtained using these two polysaccharides and their derivatives, will be given.

© 2013 Elsevier B.V. All rights reserved.
1. Introduction

The release of drugs from an appropriate dosage form at prefixed time intervals and at predetermined rates, represents a main challenge for scientists involved in pharmaceutical studies. And, although significant advances have been made in recent years in the area of controlled/modified drug release, many objectives still need to be tackled for treating clinical pathologies. Furthermore, the rapid evolution of tissue engineering stimulated the research for new biocompatible materials suitable for adhesion and proliferation of different types of cells. Among the synthetic and natural polymers that can be used for cell culture and for formulations aimed to optimize drug targeting and/or release rate, polysaccharides represent a class of macromolecules of particular interest. This is because they are usually abundant, in most cases available from renewable sources and have a large variety of composition and properties that may allow appropriately tailored chemical modifications. Also hydrogels composed of crosslinked polysaccharides and their derivatives have been frequently studied for innovative dosage forms [1,2]. Furthermore, environmentally sensitive hydrogels have been investigated as “smart” delivery systems capable to release an entrapped drug in response to specific physiological triggers, at the appropriate time and site of action [3]. In recent years multicomponent drug delivery systems have been developed for potential therapeutic and diagnostic applications and among these, semi-Interpenetrating Polymeric Networks (semi-IPNs) and Interpenetrating Polymeric Networks (IPNs) have emerged as innovative biomaterials for drug delivery and as scaffolds for cell cultures [4]. These networks most often show physico-chemical properties that can remarkably differ from those of the macromolecular constituents. Importantly, the network properties can be tailored by the type of polymer and its concentration, by the applied crosslinking method as well as by the overall procedure used for their preparation. In many cases, polysaccharides are selected for the formation of IPN hydrogel networks, which are either chemically or physically crosslinked. Sometimes both entangled macromolecules are based on polysaccharides, but often also combinations of synthetic polymers together and polysaccharides chains are used to create (semi)-IPNs.

A quite large number of polysaccharides have been investigated for the design of (semi)-IPNs for drug delivery and tissue engineering applications. This review article however mainly focuses on two of the most studied polysaccharide (semi)-IPNs, namely those based on alginate and hyaluronic acid.

The two main chapters of this review that are focused on alginate and hyaluronic acid, will be prefaced by an introductory general discussion and will give further more detailed information on (semi)-IPNs to get a better understanding of the potential and wide range of applications of these polysaccharide-based systems.

2. IPNs, semi-IPNs and polysaccharides

Although IPNs were first described in 1914, when Aylsworth designed the first synthetic IPN for the manufacturing of phonograph records [5], in the 1950’s–early 1960’s researchers began to show their interest in these complex structures. Millar was the first who investigated the properties of these materials and introduced the term “Interpenetrating Polymer Network” [6].

From that time on many studies have been focused on the synthesis and characterization of these networks for different applications using both synthetic and natural polymers.

The IUPAC definition of IPN is as follows: “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” [7]. The last sentence is added as a note to underline the difference between IPNs and polymer blends.

If only one component is crosslinked, the resulting network is defined as semi-IPN of which the IUPAC definition is the following: “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” [7]. Also in this case, a note was added to stress the difference between semi-IPNs and IPNs as well as polymer blends: “Semi-interpenetrating polymer networks are distinguished from interpenetrating polymer networks because the constituent linear or branched polymers can, in principle, be separated from the constituent polymer network(s) without breaking chemical bonds; they are polymer blends”.

The most commonly applied procedure to form an IPN is by an in situ preparation where the reactants (monomers or polymers) are mixed in a solution before crosslinking is carried out, as depicted in Fig. 1. In the case of IPN, the two networks can be formed either simultaneously or sequentially, depending on the crosslinking reactions adopted for the two systems [8,9]. In the case of the simultaneous pathway, the reactions leading to the two networks must be orthogonal, because otherwise cross reactions, i.e. copolymer formation, will most likely occur.

An alternative procedure is based on the sequential formation of the semi-IPNs. Firstly, a polymer network is prepared and subsequently the monomers or the second polymer are loaded into the swollen network, thus leading to a semi-IPN. When the loaded polymer is crosslinked to form the second network the semi-IPN is converted into an IPN. It is worth to note that when large scale samples are prepared, it is possible that the interpenetration is not fully established in the whole material, and consequently blend regions interspersed within true IPN domains can be present [10].

Depending on the nature and the intrinsic properties of the components, the interpenetration of the polymer chains can result in the formation of a hydrogel. The main question is why IPN and/or semi-IPN hydrogels are so successfully used for biomedical and pharmaceutical applications? The answer is that the combination of favourable properties of each constituent polymer of the IPN leads to new systems with improved properties, which quite often are substantially different from those of the individual polymers. Importantly, in several systems synergism of properties is also observed [11–13].

The combination and synergism of properties can be exploited to modify and tailor the characteristics of the resulting material to meet specific needs. Moreover, by combining natural and synthetic polymers, as well as by grafting of natural polymers on synthetic ones, the range of reachable properties can be broadened. Because of these features, IPN hydrogels have attracted substantial interest and many studies have been carried out on the development of IPNs suitable for various applications, particularly in the biomedical and pharmaceutical fields [8,14–16].

To briefly summarize the literature, applications of (semi)-IPNs for the design of hydrogels may lead to significant improvements of:

1) stimuli responsive behaviour, exploiting the ‘smart’ properties of at least one of the polymeric component
2) bioadhesion as well as drug/protein release rates by an appropriate tuning of the network properties, and
3) cell compatibility.
Fig. 1. Schematic representation of the semi-IPN and IPN formation. Polymer A and Polymer B are generic polymers (such as linear, comb or grafted). IPNs can also be obtained as a combination of the various pathways that are not included in the scheme for the sake of clarity. $\alpha$ and $\beta$ are generic either chemical or physical crosslinkers.
Generally, drawbacks of classical hydrogels are their low mechanical strength and, sometimes, the heterogeneity of the network structure \[17,18\]. When a force is applied on a heterogeneous gel, the stress is concentrated around the shortest chains, which subsequently can result in failure of the sample, even at very low forces. Many efforts have been made to synthesize hydrogels with a homogeneous network structure, exploiting slide-ring (SR) gels (a hydrogel with sliding crosslinking points) \[19\], and tetra-PEG gels (a hydrogel from tetrahedron-like macromonomers) \[20\]. These gels exhibit a high stretching ratio without fracturing because of their homogeneous structure. Nanocomposite (NC) hydrogels (e.g., hydrogels obtained from a mixture of a polymer and clay), microgels, microparticles and voids effectively improve the mechanical strength \[21\].

While the above reported studies are focused on the development of hydrogels with – as good as possible – homogeneous structures, J. P. Gong and her research group \[22\] exploited the heterogeneity of hydrogel networks to improve their mechanical properties. In this new class of IPN hydrogels, named double-network hydrogels (DN gels), a neutral polymer network is incorporated within a swollen heterogeneous polyelectrolyte network. The mechanical properties of DN gels, prepared from many different polymer pairs, were much better than those of the individual components. The most frequently studied system of this new class of gels is based on the polyelectrolyte poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) as the first network and the neutral polymer polyacrylamide (PAAm) as the second network (see Fig. 2A and B).

These IPNs are generally synthesized via a two-step sequential free-radical polymerization process. A hydrogel composed of a tightly crosslinked network of a rigid polyelectrolyte is obtained in the first stage and the obtained gel is then swelled in an aqueous solution of a neutral monomer that is finally polymerized thus leading to a loosely crosslinked network within the first gel \[23\]. The obtained DN gels that may contain up to 90% by weight of water, possess good mechanical properties (elastic modulus of 0.1–1.0 MPa, failure tensile stress 1–10 MPa, strain 1000–2000%, failure compressive stress 20–60 MPa, strain 90–95%), and toughness (tearing fracture energy of 100–1000 Jm\(^{-2}\)). These excellent mechanical characteristics have so far not been achieved with classical hydrogels, and are comparable with, and even exceed, those of soft load-bearing tissues, such as tendon, dermis, connective tissue, contracted muscle and human heel pad that have an elastic modulus in the range 0.1–10 MPa \[24,25\]. The excellent mechanical properties of DN hydrogels originate from the specific combination of two networks with contrasting structures. During a deformation, the network based on the rather brittle polyelectrolyte polymer, breaks into small clusters that efficiently disperse the stress around the crack tip into the surrounding damage zone, thus serving as sacrificial bonds. Additionally, the second more ductile neutral network extends extensively thereby sustaining large deformations \[26\] (Fig. 3).

DN gels have attracted much attention in the soft matter community in recent years and several research groups designed and developed innovative hydrogels with significantly enhanced mechanical strength and toughness. Some tough DN hydrogels also exhibit good biocompatibility and low friction resistance, and consequently have promising perspectives for a wide range of industrial applications, in particular in the biomedical field, for the development of load-bearing artificial soft tissues such as artificial cartilage \[21\].

Polysaccharides are an important class of polymers that can be used as building blocks of IPN hydrogels \[1,27,28\]. The reasons that polysaccharides are utilized for the development of IPN structures with specific characteristics are their abundance, low cost of production and peculiar and complex properties. Moreover, the functional groups present along the backbone of polysaccharides can be used for chemical derivatization aimed to introduce new properties to result in new polymer systems, such as networks obtained by crosslinking of chains. In particular hyaluronic acid, alginate, cellulose, chitosan and, to a lesser extent other polysaccharides, received attention for the development of (semi)-IPN, used for the development of several biomedical applications such as

![Fig. 2. Structure of synthetic polymers used in the IPN preparation: A) poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS); B) polyacrylamide (PAAm); C) poly(N-isopropylacrylamide) (pNIPAAm); D) poly(hydroxyethylmethacrylate) (pHEMA); E) poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO); F) poly(HEMA-co-METAC); G) PEGMEMA-MEO2MA-PEGDA.](image-url)
Stress, PAMPS fractured to clusters and the PAMPS clusters behave as a sliding crosslinker of PAAm. The DN gel becomes soft after the necking. See details in Ref. [22]. Figure 3. A) Loading curve of a PAMPS/PAAm DN gel under uniaxial elongation at a strain rate of 0.13 s\(^{-1}\), and the pictures demonstrate the necking process. The inset alphabets represent the correspondence between the pictures and the arrowed data points. B) Illustration of the network structure of the DN gel before (a) and after (b) necking. Above a critical stress, PAMPS fractured to clusters and the PAMPS clusters behave as a sliding crosslinker of PAAm. The DN gel becomes soft after the necking. See details in Ref. [22]. Figure reproduced with permission.

3. Alginate-based semi-IPNs and IPNs

Alginate (Alg) is a well known polysaccharide (Fig. 4A) produced by bacteria or extracted from marine brown algae. It is composed of a polymer backbone of 1,4 linked \(\beta\)-D mannuronic acid (M) and \(\alpha\)-L-guluronic acid (G) residues. Alg contains homopolymeric M- and G-blocks, that are joined by regions of alternating structures [37,38].

The physico-chemical properties of Alg strongly depend on the M/G ratio as well as on the structure of the alternating zones. Also, the gelling ability of this polysaccharide in aqueous solutions, arising from the interactions between the carboxylic acid moieties and divalent counter ions (i.e. calcium, lead, and copper), is highly dependent on the M/G ratio. It has been found that the G-blocks are responsible for the typical “egg-box” structure formation [39]. The Alg gel formation driven by divalent ions is generally very fast and the resulting hydrogels have been investigated for many industrial and biomedical applications.

Alg hydrogels are widely used in pharmaceutics as drug delivery vehicles for drug and protein release in the form of films, microspheres, depot matrices, and in tissue engineering as scaffolds for cartilage, bone and soft tissue regeneration [40–44]. Because of its high versatility and tailorable mechanical properties, Alg has been used as one of the most studied building blocks of interpenetrating polymer networks.

3.1. Temperature-responsive alginate IPNs

Stimuli-responsive synthetic polymers, and in particular thermosensitive ones, have been frequently combined with Alg hydrogels to yield (semi)-IPNs. These “smart” materials, which are characterized by peculiar rapid responses to thermal stimuli [45], undergo a transition from a hydrophilic to a more hydrophobic material triggered by small changes in environmental temperature. Two major classes of physical thermosensitive IPN hydrogels can be distinguished:

- hydrogels that show an Upper Critical Solution Temperature (UCST), i.e. that show a transition from a gel to a sol state when the temperature is raised above this temperature,
- hydrogels that show a Lower Critical Solution Temperature (LCST), i.e. that show a transition from a hydrogel to sol when the temperature is lowered below this temperature.

The hydrophilic/hydrophobic (sol/gel) transition is usually reversible, meaning that the material returns to its original initial state by a temperature variation in the opposite direction.

Hydrogels composed of LCST polymers have been extensively studied for biomedical and pharmaceutical applications, and the attention was particularly focused on systems that show an LCST value near body temperature [46]. It should be pointed out that, for the formation of temperature-responsive hydrogels, the most extensively used polymer is poly(N-isopropylacrylamide) (pNIPAAm, see Fig. 2C) that has its phase-transition near body temperature (32–37 °C) depending on the copolymer composition and molecular weight [47–49]. The use of this “smart” polymer in interpenetrating structures has been particularly investigated in order to improve its mechanical properties and its response to temperature for tailoring the release of drugs [50–52].

Such improvements can be achieved by preparing IPNs composed of pNIPAAm and a natural crosslinkable polymer, and Alg has been most frequently used for this purpose. Muniz and co-workers [53–56] studied the effect of temperature on the mechanical properties and permeability of semi-IPNs and IPNs membranes composed of chemically crosslinked pNIPAAm and physically calcium-crosslinked Alg (CaAlg). The authors evaluated the IPN hydrogel strength and observed a synergism of the mechanical performances of the Alg and pNIPAAm networks, both below and above the LCST. The effect was more pronounced above LCST where the hydrogel uniaxial compressive modulus was much higher than that of plain Alg and pNIMAam hydrogels. Also the permeability of the IPN membrane (orange II was used as a model drug) was strongly affected by the temperature and a remarkable decrease of orange II diffusion was observed when the temperature was above the LCST. The authors suggested that at
temperatures above the LCST, PNIPAAm chains shrink and pull the Alg networks back. Under these conditions, IPN hydrogels have a more tight structure with smaller pore sizes and these IPN-based hydrogels have a great potential as bio-membranes. Moreover, the same hydrogels were also investigated as matrices for the release of proteins and it was found that the release BSA (used a model protein) was dependent on the temperature as well as on the polymer network density [57].

Several studies were carried out on CaAlg/pNIPAAm (or its derivates) IPN hydrogels [58] in the form of microspheres and beads for the controlled release of drugs. In some studies homogenous IPNs were prepared by water-in-oil (w/o) emulsification method using glutaraldehyde as Alg crosslinker instead of Ca2+ ions [59]. 5-fluorouracil was loaded in the semi-IPN hydrogel and the drug release was studied at temperatures below and above the LCST of pNIPAAm. The obtained results evidenced a slower release rate from the microspheres above the critical temperature of 37 °C, as only 60% of entrapped drug was released after 12 h whereas within the same time interval, the drug was quantitatively released from the semi IPN Alg microspheres.

Inhomogenous IPN particulate systems that showed an interesting reversible change in morphology in response to temperature have also been prepared [60]. The beads were formed by dropping an aqueous solution of Alg, NIPAAm and the chemical crosslinker N,N′-bis(acryloyl)cystamine into an aqueous CaCl2 and radical polymerization initiator solution, thus immediately and simultaneously the physical gelation of Alg and the polymerization and chemical crosslinking of NIPAAm occurred. The formed beads had a higher concentration of CaAlg hydrogel on the outer part as compared to the core, because the diffusion of Ca2+ ions into the droplets is slowed down as the gelation occurs, whereas pNIPAAm was more abundant in the inner core of the beads where reactants were confined during the formation of the beads. Consequently, the resulting IPN was inhomogeneous and could be microscopically visualized by raising the temperature above the LCST of pNIPAAm. Actually, when the temperature was raised up to 37 °C the pNIPAAm network collapsed evidencing the formation of beads with a core–shell structure (Fig. 5).

This special structure was exploited to control the release of the model drug indomethacin. A study of Raz et al. [61] reported on the preparation of IPN microgels with a narrow size distribution based on ionically crosslinked Alg and chemically crosslinked pNIPAAm with size 100 ± 10 μm, using the microfluidic device as shown in Fig. 6. The mechanical properties of the microgels, such as Young’s modulus and the characteristic relaxation time, were studied using atomic force microscopy (AFM). The lower limits of the elasticity were within the range of the elasticity reported for neutrophils, making these microgels a promising model system to study the flow of cells through constrained geometries.

Besides pNIPAAm, also other synthetic polymers have LCST values near body temperature. Among them are several pluronics and some biopolymers such as gelatin [62], agarose and cellulose derivatives [63–65] that, when dissolved in water, show a reversible sol/gel temperature driven transition.

Pluronics are block copolymers of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO, see Fig. 2E) that are characterized by an LCST that depends on their composition (e.g. ratio and molecular weight of the PEO/PPO blocks and overall molecular weight) [66,67]. Some pluronics exhibit thermo-reversible gelation below body temperature and particularly these types are often applied in the field of pharmaceutics. However, to reach the appropriate stiffness, high polymer concentrations have to be used which represents for some applications, such as ophthalmic drug delivery, a major drawback. In order to decrease the polymer concentration, and at the same time retain the stiffness that is necessary for this type of pharmaceutical applications, viscosity increasing agents can be added to the pluronic solution. Vong and co-workers have successfully developed an in situ gelling device based on an Alg and Pluronic F127-based system for the ophthalmic release of pilocarpine [68,69].

The rheological properties of the (semi-)IPN Alg/Pluronic F127 hydrogel were substantially better as compared to systems composed of Pluronic F127 only. In vitro and in vivo studies assessed a slower release of pilocarpine from the semi-IPN network as compared to that from the corresponding gels, as clearly evidenced from the data reported in Fig. 7. Liu et al. reported on an in situ gelling vehicle for the ophthalmic delivery of gatifloxacin, an antibiotic of the fluoroquinolone family, which was obtained by mixing Alg with hydroxypropyl methyl cellulose (HPMC). The formed IPN showed a sustained release of the drug for 8 h [70].

Semi-IPNs based on Alg and cellulose derivatives, i.e. ethyl hydroxyethyl cellulose (HMEHEC) or HPMC, prepared using an emulsification method, were also used for the controlled release of BSA as model protein and the polysaccharide drug heparin [71,72]. A key point that should be mentioned is the advantage of these systems with respect to the previously described ones, due to the biodegradability and non-toxicity of the building blocks of the matrices. As far as the protein delivery is concerned, just like the IPNs containing synthetic thermosensitive polymers, also for these gels the authors evidenced that the delivery of proteins was controlled by the environmental temperature and slowed down because of the hydrophobic character of the cellulose derivatives above their LCST (Fig. 8).

The authors investigated in depth the morphology of the IPN microbead surfaces, evidencing the porosity as the controlling factor of the release of the entrapped compounds. In particular, the developed systems are suitable for a long lasting delivery (more than 10 days) of heparin [72].
A detailed rheological investigation on the IPN hydrogels based on Alg and hydrophobically modified ethyl hydroxyl ethyl cellulose (HMEHEC) [73] showed that the mechanical strength of this type of IPN was strongly dependent on the relative ratio of the two polymers and that the high molecular weight HMEHEC gave hydrogels with improved mechanical properties. These IPNs were able to entrap highly hydrophobic drugs, such as the prodrug NSAID sulindac, increasing their solubility because of the presence of hydrophobic HMEHEC domains in the hydrogel matrix. It was further shown that the drug was released in a controlled and delayed manner due to its partition in the hydrophobic domains of the gels.

The use of these thermosensitive polysaccharides leads to the temperature-triggered increase of hydrophobicity of the formulation that, in turn, can influence its in vivo behaviour. For example, microspheres aimed for the oral delivery of a vaccine/antigen must be taken up by the M-cells in the Peyers patches present in the upper tract of the intestine which process is strongly dependent on size, hydrophobicity and charge of the particles. Bowersock et al. [74] studied the in vitro fate of IPN microspheres, produced by the emulsification–crosslinking technique, composed of CaAlg and different thermosensitive polymers (methylcellulose, HPMC and Pluronic L61). The hydrophobicity of the used polymers influenced the mean diameter of the microspheres and the surface hydrophobicity (measured at body temperature—above the LCST) of the different IPN hydrogels. The effect of coating of the microspheres with poly- l-lysine, usually adopted to increase the stability of Alg microspheres in body fluids, was also investigated. The obtained dosage forms were all non-toxic but, and it was shown that the cellular uptake of these IPN formulations depended on composition, size and hydrophobic character of the particles.

Other examples of thermosensitive semi-IPNs and IPNs entirely based on natural polymers are those of Alg and gelatin [75]. It was observed that Alg chains accelerated the gelatin gelation kinetics, without affecting the gelatin triple helix formation which was explained by an excluded volume effect due to the solvation of the polysaccharide chains. This solvation reduces the water activity, favouring an increase of local gelatin concentration thus increasing the rate of association of the protein helices. The rheological properties of the hydrogels were also determined and a slight synergistic effect between the two biopolymer networks was observed. Moreover, oligomeric Alg chains, obtained by the entrapment of Alg lyase (an enzyme able to hydrolyze glycosidic bonds in Alg) in the semi-IPN, enhanced the kinetics of the sol/gel transition of gelatin. Alg/gelatine IPN beads were used as matrices for the intestinal release of the poorly soluble drug pindolol, a β-adrenoreceptor antagonist used in the treatment of hypertension, angina pectoris and other cardiovascular disorders. To slow down the release properties of the IPNs, chemical crosslinking of the polymer chains, using formaldehyde or glutaraldehyde, was carried out, thus freezing the IPNs’ structure and reducing erosion rate of the dosage form in the gastric and intestinal environments. These IPNs successfully delayed the release of pindolol and a suitable single dose formulation administered per day was obtained by adjusting the amount of crosslinking agent added to the formulation [76].

3.2. pH-responsive alginate IPNs

Electrolyte and ampholyte polymers can exhibit a sol/gel transition in response to environmental pH changes. This sol/gel transition is due to hydrogen bonding or electrostatic repulsions of the polymer
chains and is dependent on the $pK_a$ of the ionizable moieties. As a consequence, pH-sensitive hydrogels based on polyelectrolyte/polyampholytes exhibit swelling or de-swelling behaviour depending on the environmental pH. These hydrogels have been applied in pharmaceutics for the development of formulations suitable for intestinal drug delivery after oral administration. A pH sensitive semi-IPN hydrogel was developed by Chen et al.\[77\] and in their study a chemical network was obtained by crosslinking N,O-carboxymethyl chitosan, (NOCC) with genipin in the presence of Alg. The obtained semi-IPNs were stable and their swelling was dependent on both pH and polymer concentration. As a result, also the release of an entrapped model protein (BSA) was affected by environmental pH, as depicted in Fig. 9.

3.3. pH/temperature responsive alginate IPNs

Semi-IPNs as well IPNs based on Alg and pNIPAAm have also been investigated as dual stimuli responsive hydrogels. These semi-IPNs, in contrast to pNIPAAm hydrogels, displayed a swelling profile that depends on the charge of Alg which in turn is governed by the pH of the medium. The properties of the Alg/pNIPAAm semi-IPN hydrogels were, due to the presence of pNIPAAm, affected not only by pH but also by temperature\[78\]. In another study, Lee and co-workers compared the properties of Alg/pNIPAAm semi-IPNs with those of a comb-graft hydrogel of the same composition that was obtained by grafting Alg with pNIPAAm followed by crosslinking with Ca$_2^+$ ions. This comb-graft hydrogel exhibited faster temperature responses than the semi-IPNs, because of the higher mobility of the grafted PNIPAAM chains as compared with those in the semi-IPN. Furthermore, the comb-graft hydrogel showed an increase in swelling with an increase of pH, whereas the semi-IPNs showed a decrease in swelling in response to both stimuli (pH and temperature, see Fig. 10), as a result of the more structured network\[79\].

Mano and co-workers studied the effect of pH and temperature on the release of indomethacin, a non-steroidal anti-inflammatory drug, from semi-IPN beads based on CaAlg and pNIPAAm\[80\]. At pH 2.1 and 37 °C the drug was mainly retained in the beads and less than 10% of the drug was released in 7 h (burst release), while it was fully released within 3 h at pH 7.4. Also the temperature influenced the drug delivery profiles of the same gel, leading to a faster release at 37 °C than at 25 °C. The authors attributed this behaviour to a “squeezing out” of the drug from the pNIPAAm hydrogel, assuming a demixing of the semi-IPN when the temperature of the system was raised above the LCST. This behaviour is not in contrast with findings of other authors, because in their systems, pNIPAAm chains were not crosslinked and consequently demixing was possible, depending on the chain length and environmental conditions\[81,82\]. Coating these beads with chitosan led to an increase of the drug delivery...
loading efficiency, due to the reduction of the porosity of the bead surface, and a slight decrease of the release rate without altering the dual responsiveness of the beads [83].

3.4. Physically crosslinked alginate IPNs

An interesting application of CaAlg based IPN networks for the in vitro growth of ovarian follicles intended to support women facing premature infertility due to cancer therapies or other disorders, was reported by Shea et al. [84]. They designed an interpenetrating matrix based on Alg and fibrin aimed to tailor the mechanical properties of the hydrogel to fit the needs of the follicles during their development. The two biopolymers were simultaneously crosslinked by addition of Ca\(^{2+}\) ions and thrombin, respectively leading to IPNs with mechanical properties suitable for tissue regeneration. After preparation, both polymer networks contribute to the mechanical properties of the matrix and the elastic modulus was 300 Pa. After fibrin degradation, due to plasmin and various other proteases secreted by the cells, the Alg network assured the mechanical support to the hydrogel and the modulus was about 40 Pa. Mechanical properties of the matrix emerge as significant regulators of follicle development and the authors supposed that small two-layered follicles, cultured in a mechanically dynamic environment, could be able to mimic the in vivo environment and increase the rate of oocyte maturation. Previous studies using CaAlg hydrogels [84 and references therein] could not recapitulate this dynamic environment, leading to poorer results.

3.5. Chemically crosslinked alginate IPNs

In numerous studies on IPNs, one of the networks is formed by introducing cofalent bonds between the polymer chains. Usually this approach leads to an increased resistance to failure and mechanical strength of the hydrogels compared to gels in which the networks are held together by physical/ionic interactions as in CaAlg gels. In this section some IPNs based on Alg interpenetrated with chemical networks are discussed, focusing particularly on hydrogels based on poly(hydroxyethylmethacrylate) (pHEMA) and its derivatives. pHEMA (see Fig. 2D) is a hydrophilic and neutral polymer that is capable to form synthetic hydrogels as first described by Wichterle [85], that have a large variety of applications, such as medical devices and drug delivery systems [86].

Among various systems that have been developed, we report on a semi-IPN with antibacterial activity based on Alg and pHEMA that functions as template for the in situ synthesis of silver nanoparticles. It should be pointed out that embedding colloidal nanoparticles into polymer matrices is an effective method for enhancing their functions [87]. In this study, the semi-IPN was obtained by free radical polymerization of HEMA using N,N’-methylenebisacrylamide as crosslinker, in a solution containing sodium Alg. The resulting IPN, after purification, was dried and swollen in a silver nitrate solution. Silver ions embedded in the hydrogel were reduced by addition of sodium borohydride, thus obtaining silver nanoparticles of about 20 nm uniformly distributed within the semi-IPN hydrogel matrix. The advantage of using such a hydrogel system relies on the ability of the polysaccharide chains to hold large amounts of metal ions in their network by anchoring the ions through the carboxylic and hydroxyl groups of Alg. Moreover, the carbohydrate chains in the semi-IPN hydrogel network limit the agglomeration of silver nanoparticles. This hydrogel system showed a very good antibacterial activity against *Escherichia coli*, thus indicating its potential suitability for wound repair applications [88]. La Gatta et al. [89] developed polyelectrolyte materials based on a semi-IPN matrix composed of Alg and a pHMA. In more detail, after dissolution of the monomers in an aqueous solution of Alg, HEMA was copolymerized with the cationic monomer 2-methacryloyloxy ethyltrimethyl ammonium chloride (METAC, see Fig. 2F). The mechanical properties as well as the swelling behaviour of the resulting semi-IPN hydrogels were dependent on both charged polymers. In the studied systems, Alg chains adversely affected the hydrogel strength, since this polymer lowered its Young modulus. On the other hand, the Alg chains had a very positive effect on the biocompatibility of the systems as the gels showed better cell viability and cell adhesion properties than the control p(HEMA-co-METAC) hydrogels.

3.6. Photopolymerized alginate IPNs

Photopolymerization is an alternative approach for the formation of chemically crosslinked hydrogels and offers the possibility to obtain in situ formed systems by means of UV or visible light irradiation. 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 so-called photoinitiator undergoes homolytic bond cleavage upon exposure to UV/visible light [90]. At present, several photoinitiators are available that have a good cytocompatibility allowing their in vivo use [91]. UV curing, which is generally a fast process, can be used at body temperature and in aqueous medium to generate hydrogels. Because of these advantages, photopolymerization has been used in many studies for the preparation of a large number of hydrogels, including IPNs, with potential application in the biomedical field [92,93]. A combination of a photocrosslinkable PEG acrylate, and Alg was used to form after UV-polymerization IPNs beads for the encapsulation of Langerhans islets [94]. In more detail, Langerhans cells were dispersed in an Alg/PEG acrylate solution, also containing a photoinitiator, which was subsequently dropped into a CaCl\(_2\) solution to obtain a semi-IPN. The subsequent exposure to UV light resulted in photocrosslinking of the acrylate moieties to yield stable IPNs in which the cells were entrapped. By varying the ratio between Alg and PEG acrylate, the porosity/permeability of the IPNs could be appropriately tuned. In vitro viability tests on the encapsulated human islets demonstrated the cytocompatibility and non-toxicity of these systems since the encapsulated islets retained both their viability and insulin secretory activity.

An injectable and homogenous in situ crosslinkable IPN hydrogel was obtained by combining a CaAlg network with a dextran methacrylate derivate [95,96]. It was found that the presence of this dextran derivative disturbed the formation of the CaAlg “egg-box” structure, leading to a semi-IPN that was easily injectable. After UV photopolymerization of the methacrylic moieties on the dextran chains, a full IPN was obtained that showed synergistic properties compared with those of
the single networks. The prepared IPNs were also tested as drug delivery systems and it was shown that the model drug theophylline was completely released within 10 h whereas roughly 50% of the loaded model protein BSA was released in the same time frame. Expanded chondrocytes were entrapped in the IPN and it was demonstrated that the cells remained viable and were able to re-differentiate. In two follow-up studies, IPNs in the form of macroscopic gels and beads were obtained from partially oxidized Alg and methacrylated dextran and it was reported that these gels showed a faster degradation kinetics as compared to systems based on no oxidized Alg [42,97,98]. Recently Fan and coworkers published a paper [99] on an IPN based on methacrylated Alg and collagen suitable for 3D pre-osteoblast spreading and osteogenic differentiation. Compared to a methacrylated Alg hydrogel, this IPN possessed higher mechanical strength, lower swelling ratios and denser network structures.

4. Hyaluronic acid

Hyaluronic acid (or hyaluronan, HA) is a linear, nonsulphated glycosaminoglycan composed of β-1,4-linked disaccharide units of β-1,3-linked glucuronic acid and N-acetyl-d-glucosamine (Fig. 4B). HA is one of the major components of the extracellular matrix (ECM) and is present at high concentrations in all connective tissues (cartilage, in the vitreous humour and in synovial fluids), where it performs a rheological/structural function. Moreover, due to its capacity to interact with some cell receptors, HA plays an important role in processes such as cell proliferation, migration and differentiation [100].

In the past HA was obtained by extraction from rooster combs, but nowadays it is preferentially obtained as a product with improved properties (molecular weight, polydispersity) but with some impurities by
fermentation of *Streptococcus* strain. More recently, commercial HA has been obtained by recombinant *Bacillus subtilis* sp. that is recognized as a GRAS (safe) microorganism [101].

The properties of HA can be modified and tailored in many ways in order to obtain materials with new physico-chemical and biological characteristics, as hydrophobicity, amphiphilicity and particular biological activities. The most frequently used chemical modifications of HA target three functional groups, namely the glucuronic acid group, the primary and secondary hydroxyl groups, and the amine group (after deacetylation of N-acetyl group). In particular, carboxylates are frequently modified by esterification and amidation reactions mostly established using carbodiimide assisted coupling reactions. The hydroxyl groups have been modified by etherification and esterification reactions as well as by bis-epoxide and divinylsulfone crosslinking, resulting in linear and cross-linked HA-based products, respectively [102–104].

Because of its excellent biocompatibility and biodegradability, HA is one of the most frequently used biopolymers used in the biomedical field and industry. Actually, numerous HA linear or crosslinked derivatives have been synthesized that are employed for tissue repair, wound healing, treatment of joint diseases, anticancer drug delivery, and as scaffolds for tissue engineering.

### 4.1. Temperature-responsive hyaluronic acid semi-IPNs

A pH and temperature-sensitive semi-IPN system was developed by Santos and co-workers [105] by combining crosslinked thermosensitive pNIPAAm and HA. pNIPAAm was crosslinked by addition of N,N'-methylenebisacrylamide as cross-linking agent and tetramethylethylenediamine as catalyst, in solutions of HA with different concentrations. DSC analysis showed that the LCST of the systems was not affected by the presence of HA, whereas the transition enthalpy $\Delta H$, decreased with increasing concentrations of HA, most likely because HA interferes with the hydrophobic/hydrophilic interactions of the pNIPAAm network. Water uptake measurements at pH = 7.4 and 2.1 were carried out on the systems at 25 °C (measuring the swelling process) and 37 °C (measuring the de-swelling process), i.e., below and above LCST. It was shown that the swelling increased at pH 7.4, due to the anionic form of the polysaccharide, while no changes occurred at pH 2.1 where HA is in its neutral form. HA in its anionic form at pH 7.4 also influenced the de-swelling process, leading to an almost complete dehydration of the semi-IPN, whereas the pNIPAAm system alone de-swelled in a fast but incomplete manner, probably due to the formation of a hydrophobic coating on these gels above the LCST. Gentamicin was loaded in the hydrogel before crosslinking and the presence of HA led to an increased fraction of released drug, probably because of the more efficient de-swelling process.

Another semi-IPN with HA was investigated by Dong and co-workers [106]. In their system, a hyperbranched thermoresponsive and photo-crosslinkable PEG-based copolymer, PEGMEMA-MEO$_2$MA-PEGDA (see Fig. 2G), obtained by the co-polymerization of poly(ethylene glycol) diacrylate (PEGDA), poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) and 2-(2-methoxyethoxy) ethyl methacrylate (MEO$_2$MA) via an in situ DE-ATRP (Deactivation Enhanced Atom Transfer Radical Polymerization) approach, was crosslinked by Michael addition using pentaerythritol tetras (3-mercaptopropionate) as thiol-functional crosslinker. By tuning the hydrophobic/hydrophilic ratio of the copolymer, the LCST was varied from 26 to 31 °C. When the crosslinking process was carried out in the presence of HA, a semi-IPN was obtained and it was found that both the porosity (SEM analysis) as well as the swelling of the gels increased with increasing HA content. HA was slowly released (20% in two days) from the gels, thus demonstrating the semi-IPN nature of the system. The HA-containing semi-IPNs showed good adhesion and proliferation of adipose-stem cells.

### 4.2. Chemically crosslinked hyaluronic acid semi-IPN

La Gatta and co-workers [107] prepared a semi-IPN composed of HA and a network of poly(2-hydroxyethylmethacrylate-co-2-methacrylicoxyethyltrimethylammonium) (p(HEMA-co-METAC)) crosslinked by ethylenglycol dimethacrylate (EGDMA). Because of the partial neutralization of the positive charges of the synthetic networks by HA, the water uptake of this IPN decreased within increasing weight fraction of the polysaccharide in the matrix. This phenomenon was even stronger by replacing HA with chondroitin sulphate, a polysaccharide with a higher charge density because of the presence of sulphate groups. The p(HEMA-co-METAC)/HA semi-IPN showed good cytocompatibility with mouse fibroblasts and the net positive charge of the IPN gels improved the cell adhesion compared to that of gels composed of only HA.

A semi-IPN system suitable for bioprinting was developed by Peschosoldo and co-workers [108] using a photopolymerizable dextran derivative, dex-HEMA (hydroxyethyl-methacrylate-derivatized dextran) as crosslinkable component and high molecular weight HA. Dex-HEMA dissolved in an aqueous solution of Alg was crosslinked upon UV exposure using liraguce 2559 as photoinitiator. Mechanical characterization of these semi-IPN hydrogels with different HA contents were carried out evidencing, in particular, that the crosslinking kinetics were almost instantaneous, as shown by the rapid increase of the storage modulus $G'$ after 10 s of UV exposition. The system showed good viability of chondrocytes after 3 days of incubation. Bioprinting [109] was carried out using a bioscaffold pneumatic dispensing system. The polymer solution was extruded through a needle on a stationary platform following a layer-by-layer deposition protocol, and stabilized by photocuring (Fig. 11). The results showed that the obtained 3D construct had a high porosity with a well-defined strand spacing and that the overall architecture could be easily tuned by controlling the process parameters, such as fibre spacing and orientation, demonstrating the suitability of the HA/dex-HEMA systems for bioprinting applications in tissue engineering.

### 4.3. Photopolymerized hyaluronic acid IPNs

The industrial interest for HA-based semi-IPNs and IPNs is demonstrated by a world patent dated 1994 filed by the Italian Industry Fidia Farmaceutici SpA, that describes IPN biomaterials based on native HA or semi-synthetic HA derivatives and a non-toxic, non-carcinogenic synthetic polymer as second IPN component. The patent also claims HA derivatives with pharmacologically active molecules for IPN applications in a wide range of sanitary fields, from dermatology, urology, orthopaedics up to plastic and cardiovascular surgeries, in the form of films, hydrogels, membranes, sponges, non-woven tissues, etc. [110].

The most important chemically modified HA polymers for the IPN formation are the methacrylated or acrylated derivatives, because of the mild conditions needed for their preparation [111,112]. Methacryl moieties can be easily introduced on the polysaccharide chains by exploiting the reactivity of carboxy or hydroxy groups of HA, and the properties of the obtained networks can be appropriately modulated by tuning the polysaccharide derivatization degree (see e.g. [113]).

Many examples are reported in the literature of IPN systems based on HA and synthetic biocompatible polymers. Park and co-workers prepared and studied interpenetrating hydrogels based on methacrylated HA (HA-Ac) and diacrylated PEG (PEG-DA) for applications in the field of tissue engineering [114]. High molecular weight HA was enzymatically depolymerized using hyaluronidase and the obtained HA fragments were subsequently chemically functionalized with an amino derivative of methacrylamine. Furthermore, commercially available PEG-DA was derivatized with RGD peptides exploiting Michael addition between cysteine residues of the synthesized peptides and acrylate moieties on PEG, in order to provide a matrix suitable for
cell adhesion and proliferation. IPN hydrogels, obtained after photopolymerization in the presence of eosin Y, as visible light sensitizer, were studied for their rheological, swelling and degradation properties. The obtained results showed that, as expected, the complex modulus $G^*$ of the IPN gels increased and the degree of swelling decreased as the derivatization degree of HA-Ac and the PEG-Da concentration in the mixture increased. RGD-modified IPNs showed good adhesion and proliferation of human dermal fibroblasts and behaved differently from HA-Ac hydrogels without RGD peptides and IPN hydrogels modified with an inactive form of the peptide.

Another interesting IPN system of methacrylated HA (PHA) and N, N-dimethylacrylamide (DAAm), was described by Weng and coworkers [13]. This PHA/DAAm IPN was prepared by photopolymerization of methacrylated HA in the presence of 2-oxo-ketoglutaric acid as photoinitiator, followed by immersion of the formed hydrogel (PHA) in DAAm solutions of different concentrations in the presence of different amounts of N,N-methylene bisacrylamide (MBAAm) as crosslinking agent, and followed by UV-exposure to synthesize the DAAm network. The equilibrium water content of the IPNs was inversely dependent on the DAAm and MBAAm contents, due to stronger hydrophobic interactions that in turn led to the formation of denser polymer networks. SEM micrographs confirmed that the PHA hydrogels possessed larger pores (50 μm) compared to IPN systems in which the second DAAm network conferred a more compact and less porous structure (10–20 μm). The stress–strain behaviour of the PHA/DAAm systems under uniaxial compression showed that, due to synergistic effects of the double network structure, the resulting PHA/DAAm hydrogels possessed significantly enhanced mechanical properties in comparison to those of the PHA hydrogels (Fig. 12). The loosely crosslinked second network dissipates stress during compression, thus contributing to the high mechanical strength of the double network hydrogel. Compared to PHA hydrogels, which are generally very brittle and easily fracturable, the PHA/DAAm hydrogels are ductile.

By comparing the mechanical properties of the DN structure with those of PHA and DAAm networks, the authors suggested that the PHA network contributed mainly to the strength of the gel (elastic stress), while the DAAm component was mainly responsible of the gel ability to elongate without breaking (elastic strain).

The PHA/DAAm IPN hydrogels showed good cytocompatibility with mouse dermal fibroblasts but lacked cell adhesion properties until the surface of the gel was covered by ECM produced by the cells. Collagen, along with HA, is a major component of ECM and this protein forms gels without chemical modification. A class of photocrosslinkable semi-IPN gels made of methacrylated HA (MeHA) and collagen was developed [12,16] by photopolymerization of HA with various degrees of methacrylation in the presence of collagen, using Irgacure 2959 as photoinitiator, followed by incubation at 37 °C to promote the formation of a collagen network. The distribution of collagen in the IPN hydrogel was investigated using fluorescein isothiocyanate-labelled collagen (FITC-collagen) and observed using an inverted microscope. The results of this analysis, as well as those of morphological analysis carried out by SEM microscopy, showed a homogeneous structure and fibrous/flaky morphology of the semi-IPNs (Fig. 13). Rheological analysis demonstrated that collagen caused a synergistic effect on the compression modulus of the IPNs at different MeHA contents, whereas it did not contribute to the fracture stress of the gels. Cell adhesion on MeHA/collagen semi-IPNs was tested using the mouse embryonic fibroblast cell line NIH-3T3 and the obtained results showed that, due to the adhesive properties of collagen and mechanical stiffness of the MeHA network, these systems were suitable as tissue scaffold materials. Furthermore, microscale tissue engineering applications were investigated using micropatterned stamps where collagen-MeHA semi-IPN was formed inside microwells and microchannels in the presence of NIH-3T3 cells.

5. Conclusions and perspectives

For more than 60 years, IPN and semi-IPN technologies have been studied in polymer science and used for the development of industrial applications to result in new materials and consumer products by combination of properties, sometimes synergistic, of the constituents. About 20 years ago (semi)-IPNs were also introduced also in the pharmaceutical and biomedical fields and, since then, several dosage forms and devices have been successfully developed. Many of these systems are based on biocompatible and biodegradable natural polymers and, in this respect, the gelling polysaccharides Alg and HA (able to form gel after appropriate chemical derivatization) have been widely exploited. Both Alg and HA, polysaccharides on which this review is mainly focused, have been used to prepare (semi)-IPN hydrogels by combination with synthetic stimuli-responsive polymers or, as in the case...
of Alg, exploiting its gelling ability with divalent cations, to prepare new biomaterials which adjust their mechanical and drug release properties in response to an external stimulus.

Numerous semi-IPNs and IPNs have also been obtained by the combination with other natural or synthetic network forming polymers to yield new dosage forms for pharmaceutical applications or new biomaterials with improved rheological properties that resemble those of natural soft tissues, making them particularly suitable for tissue engineering applications.

Although there is a wide choice of polymers that can be combined with Alg and HA to obtain semi-IPNs or IPNs, beyond the chemical difficulties in obtaining a true semi-IPN or IPN instead of a polymer blend, the major concerns that must be addressed for effective applications of new systems are the biocompatibility and biodegradability of the used polymers and their networks. Therefore, efforts should be given to develop new interpenetrated systems based on the combination of either Alg or HA with other natural polymers and, in particular, other polysaccharides. Moreover, it is worth noting that some polysaccharides intrinsically possess a bioactivity that can be exploited to improve the performances of the prepared hydrogels. Special attention should also be devoted to the synergistic behaviour that can be obtained by combining two polysaccharide networks and a more

Fig. 13. (A) Visualization of collagen and methacrylated hyaluronic acid (MeHA) mixtures. The homogeneity of well-mixed collagen-MeHA (a, e), unmixed collagen-MeHA (b, f), MeHA only (c, g), and collagen only (d, h) were examined by mixing a small fraction of fluorescein isothiocyanate-conjugated collagen into the collagen stock. Top views (a–d) demonstrate that the well-mixed collagen and MeHA resulted in a more-uniform collagen distribution than that of the unmixed collagen and MeHA, comparable with that of the collagen-only control. The same trend is observed in cross-sectional views (e–h) (views are 2.5 mm × 2.5 mm). As expected, the MeHA-only hydrogels (b, f) did not fluoresce. Also, the network microstructures were examined using scanning electron microscopy at 100× (i–k) and 2000× (l–n). Collagen-only networks (i, l) displayed a highly fibrous structure, MeHA-only networks (j, m) were made of flaky porous sheets, and elements of the fibrous collagen structure and the MeHA flakes were present in the well-mixed collagen-MeHA IPNs (k, n). (B) Encapsulated cell viability and micromolding of collagen–methacrylated hyaluronic acid (MeHA) semiinterpenetrating networks (IPNs). After ultraviolet (UV) exposure and 2-h collagen gelling time, cell-laden polymer networks were soaked in calcein/homodimer live/dead stain to determine the viability of NIH-3T3 cells after encapsulation (a). To demonstrate the micromolding of cell-laden collagen and MeHA IPN hydrogels, mouse embryonic fibroblasts were mixed with 5.0 wt.% MeHA with 4.1 mg/mL of collagen prepolymer and moulded using a poly(dimethylsiloxane) stamp. Microwells with a diameter of 250 mm were fabricated with encapsulated mouse embryonic fibroblasts and analysed for cell viability (b). The mouse embryonic fibroblasts exhibited high viability in the IPNs (88.1 ± 5.4%) (c). Collagen-MeHA microchannels were moulded and sealed using a two-part UV cross-linking method (d). The gels were incubated, and the channel was subsequently perfused with trypan blue dye (e). After perfusion, a cross-section was taken, and the diffusion of the dye into the hydrogel was observed (f). See Ref. [12]. Figure reproduced with permission.
detailed and systematic approach should be promoted to generate the basic and fundamental knowledge that is required to develop polysaccharide IPN systems as effective tools for drug delivery and tissue regeneration applications.

We can conclude that many successful systems based on (semi)-IPN polysaccharide hydrogels have been developed so far, but there is still room for fundamental studies to understand and tailor their physico-chemical and biological properties, as well as for development of systems that fulfill unmet medical and pharmaceutical needs.

Acknowledgements

This work was financially supported by the Sapienza University of Rome “Ricerche Universitarie” 2011, n. C26A119N2S. Dr. Claudia Cencetti and Dr. Laura Pescosolido are acknowledged for their contribution.

References
