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Interpenetrating Polymer Network: Biomedical Applications

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Preface

The book focuses on novel interpenetrating polymer networks (IPN)/semi-IPN technology for drug delivery and biomedical engineering. Interpenetrating polymer networks (IPN)/semi-IPN technology is a simple and feasible method to fabricate nanomedicine, hydrogels, nanoparticles, microparticles, etc. in which hydrophilic polymer chain permeates another cross-linked polymeric network without any chemical bonds between them. Generally, IPNs are synthesized for the purpose of combining individual properties of two or more polymers. In some cases, entirely new properties are exhibited by the IPN that are not observed in either of the two single networks alone. The design and development of interpenetrating network polymers is attractive because IPNs provide free volume space for the easy encapsulation of drugs in the three-dimensional network structure, which are obtained by cross-linking of two or more polymer networks. Natural polymer-based IPNs can deliver drugs at a controlled rate over an extended period of time. In majority of the cases, single polymer cannot regulate the drug release rate and therefore, the formation of interpenetrating network appears to be a better approach. It is reported that the formation of IPN can preserve the characteristics of each network structure and improve the stability of materials because of the interlocked structure in the cross-linked networks, thus ensuring better mechanical strength and sustained/controlled drug delivery properties.

The purpose of the book is to highlight recent advancements in some novel IPN/semi-IPN-based systems, potential for drug delivery and biomedical applications.

Nowadays, natural/synthetic biopolymer-based particulate systems are gaining huge attention from researchers due to their immense applicability by exploiting a green approach with minimum toxicity.

The book contains different chapters emphasizing drug delivery and biomedical perspectives of different kinds of novel IPN/semi-IPN-based systems.

In Chap. 1, micro-particular IPN-based systems for drug delivery application have been highlighted.

Nanoparticles play an important role in many engineering and industrial fields, including nanomedicine, for the development of novel drug delivery systems, biotechnology and tissue engineering. Nanoparticle IPN systems are discussed in Chap. 2.

Chitosan is a biocompatible and biodegradable polymer and a very good candidate for preparation of interpenetrating polymer networks (IPN) for drug delivery

application. Chapter 3 is focused on fabrication technology, characterization procedures and applications of chitosan-based IPN systems.

Alginate is also a naturally occurring hydrophilic anionic polymer and widely used for biomedical purposes. It has versatile properties and the ability to fabricate IPN systems. Chapter 4 discusses alginate-based IPN systems in drug delivery, wound healing and tissue engineering. This chapter also focuses on the potential use of alginate IPNs in wound dressings and regenerative medicine.

Currently, pH-sensitive IPNs have emerged as excellent drug delivery carriers controlling the release of therapeutics. Chapter 5 gives a brief overview on hydrogel, its translation to smart IPN systems and its biomedical application.

Recently, dendrimers-based drug delivery system has been popular among the scientific community. A dendrimer is typically a symmetric branching around the core, and often adopts a spherical three-dimensional morphology. In Chap. 6, the preparation of polymer-based IPN dendrimer and its use as a drug carrier material is discussed.

Protein-polymer-based IPN systems for targeted drug delivery are the focus of Chap. 7. This chapter contains IPN-based nanoparticles, synthetic process of some natural polymer especially protein which is widely used for IPN. This chapter also covers recent advancement in IPN-based nanoparticles system for pharmaceutical applications as well as in anticancer drug delivery system.

Progresses in polymer science have led to the development of several novel drug delivery systems. Semi-interpenetrating polymer network (semi-IPN) system is one of them that shows much better performance over the conventional individual polymers. This is summarized in Chap. 8, containing recent research on semi-IPNs systems that allow acquiring better understanding of potential drug delivery applications of polysaccharide-based semi-IPN systems.

Intelligent polymeric multi-component system, which is biocompatible and biodegradable, is well known for its specific drug targeting with response to a stimuli. The IPN system is widely preferred in the field of cancer therapy with zero order drug delivery method that retains a minimized fluctuation. The potentiality of this IPN system makes it a vast research area to diagnose and treat cancer and related diseases. Chapter 9 deals with preparation, mechanism of drug release and application to cancer therapy.

Chapter 10 focused on semi-interpenetrating network-based hydrogel on 2-hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), itaconic acid (IA) and poly(vinyl pyrrolidone) (PVP) and their biomedical applications.

IPN-based gels are highly versatile and have massive biomedical potential. The 3D structure of IPN hydrogels, possess the ability of holding large amounts of water. These networked gels show higher strength, are safe and biocompatible which makes them useful for a range of biomedical applications. The IPN gels are now also prepared from “smart polymers” which can be easily modified in terms of shape and volume and are sensitive to selected stimuli like temperature, pH, pressure, etc. Chapter 11 highlights the various biomedical applications of IPN gels.

The last chapter focuses on design and modification of bio-nanocomposite IPNs and their application in targeting biomedical potential.

The book is a collection of recent developments in the field of IPN technology, which is written by experts in their fields. It is very useful for the students, researcher scholars and scientists in the area of pharmaceuticals and biological and material sciences.

We express our sincere gratitude to all authors for their contribution to this book. We also thank the publisher for their continuous support in the successful completion of this reference book.

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Contents

About the Editors

Sougata Jana is a B.Pharm. (Gold Medalist) from West Bengal University of Technology, Kolkata, and M.Pharm. (Pharmaceutics) from Biju Patnaik University of Technology, Odisha, India. He worked as an Assistant Professor at Gupta College of Technological Sciences, Asansol, West Bengal, India. Currently, he is working at the Department of Health and Family Welfare, Directorate of Health Services, Kolkata, India. He is engaged in pharmaceutical education and research for the last 11 years. IPA Bengal branch, Kolkata, India conferred upon him the "M.N Dev Memorial Award" for securing the highest marks in the state of West Bengal in 2005. He bagged "Best Poster Presentation Award" at the 21st West Bengal State Science and Technology Congress-2014, and "Outstanding Paper Award" at the 1st Regional Science and Technology Congress-2016, organized by DST, Govt. of West Bengal, India. He has 30 publications in different national and international peer-reviewed journals. He edited books in Springer, Elsevier and Pharmamedix India Publication Pvt. Ltd. He has contributed more than 35 book chapters with Elsevier, Springer, Wiley VCH, CRC Press, and Taylor & Francis Group. His research area of interest includes modification of synthetic and natural biopolymers, microparticles, nanoparticles, semisolids and interpenetrating network system for controlled drug delivery.

Subrata Jana is presently working as Associate Professor at the Department of Chemistry, Indira Gandhi National Tribal University (Central University), Amarkantak, Madhya Pradesh, India, and his current research focuses on design and synthesis of artificial receptors for the recognition of anions, cations and *N*-methylated protein residue. He is also working on biodegradable polymeric-based carrier systems for the delivery of drug molecules by collaboration with pharmaceutical scientist. So far he has published ~40 research papers in peer-reviewed international journals and contributed more than 10 book chapters in different edited books published by internationally renowned publishers. He also serves as an editorial board member for the *Journal of PharmaSciTech* (ISSN: 2231 3788) and *International Journal of Scientific and Engineering Research* (ISSN: 2229-5518) as well as reviewer for *International Journal of Biological Macromolecule* (Elsevier), *Journal of PharmaSciTech* and *Current Pharmaceutical Design* (Bentham).

He has obtained his PhD in organic chemistry from the Indian Institute of Engineering Science and Technology (IIEST), Shibpur, India. Then he moved to the University of Victoria, Canada, to work with Professor (Dr.) Fraser Hof on supramolecular and medicinal chemistry as a post-doctoral fellow. He then worked further with Dr. Kenneth J Woycechowsky at the University of Utah, USA, on protein engineering and enzyme catalysis as a post-doctoral research associate. Overall, he extensively studied supramolecular behaviour of the host-guest interaction and synthesis of heterocyclics like pyrimidines, naphthyridines, quinoline and diazepines, etc. by exploiting microwave protocol for green chemical synthesis.

1 Interpenetrating Polymer Network in Microparticulate Systems: Drug Delivery and Biomedical Application

Sreejan Manna, Manasa Manna, and Sougata Jana

Abstract

The advancement in polymer grafting has played an important role in the evolution of interpenetrating polymer network (IPN) systems. Microparticle-based interpenetrating IPN systems are important for delivering the drug at target site in a controlled manner. The improved properties of IPN microparticles such as stability, swelling ability, non-toxicity and biodegradability have gained attention in drug delivery and biomedical fields. In recent past many study reports showed that IPN-based microparticles have emerged as a drug carrier to deliver drugs at different bio-targets. Different stimuli response delivery system was also developed which can protect the drug from surrounding biological environment. The application in IPN microparticles in biomedical domain is growing. It has been successfully applied as functional tissue substitution. The notable application of IPN microparticles has been reported in the field of tissue engineering, heart valve regeneration, blood capillary regeneration and for ophthalmic implants.

Keywords

Interpenetrating polymer network · Microparticles · Bio-targets · Tissue engineering

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1.1 Introduction

Interpenetrating polymer networks are a novel approach of polymeric system in which two polymers firmly combine to form a network and not less than one polymer is either cross-linked or synthesized in the intimate presence of another polymer (Sperling 1994; Chern et al. 1994; Jana et al. 2014; Jana and Sen 2017). Characteristically an interpenetrating polymer network (IPN) is different from polymer blends, grafts or blocks in both ways – swelling without dissolvation and suppressed flow (Aklonis and MacKnight 1983).

Fabrication of interpenetrating polymer networks is a method of blending polymers to create a polymer mixture with reduced phase separation ability. Interpenetration generally improves the compatibility between the polymers present in the blend and thus able to control phase separation as cross-linking of polymer makes permanent entanglement between them (Chen and Chen 2006; Matsuo et al. 1970). The property of an IPN is significantly affected by its morphology which is controlled by chemical compatibility between polymers, method of polymerization, interfacial tension, network density of cross-linking and composition of IPN (Harani et al. 1999). Simple polymer mixture results multiphase morphology due to thermodynamic incompatibility which is obtained from small entropy gain during mixing.

There are different types of IPN (Fig. 1.1):

Sequential IPN: Initially polymer network A is synthesized by reacting monomer, initiator and cross-linking agent together. Afterwards network A is allowed to swell in the presence of monomer B which contains initiator and cross-linking agent resulting in the formation of network B (Bauer and Briber 1994). Here, the sequential IPN morphology is controlled by cross-linking density of the network.

Simultaneous interpenetrating network (SIN): Synthesis of both polymer networks occurs simultaneously. For both the components, the monomers, catalysts and cross-linker are mixed together followed by individual monomer polymerization reactions. It is commonly observed that in this polymerization process the degree of entanglement is highest with very little phase separation.

Gradient IPN: The polymer network A is partially swelled by a monomer B and then followed by quick polymerization before the occurrence of diffusional equilibrium. It may enhance the structural solidarity for lamination application.

Pseudo-IPNs: These are a kind of simultaneous IPNs where one polymer forms the cross-linked network and another polymer is linear (Allen et al. 1973).

Full IPNs: In this type of IPN the components are individually cross-linked without any induced cross-linking between individual polymers.

Graft or joined IPNs: In this type, without homopolymer cross-linking, polymer A is grafted to polymer B. Interfacial bonding can be improved by slight grafting of polymers. If the morphology of IPN is unaffected and the cross-linker concentration is much more than graft site concentration, then grafting can be neglected.

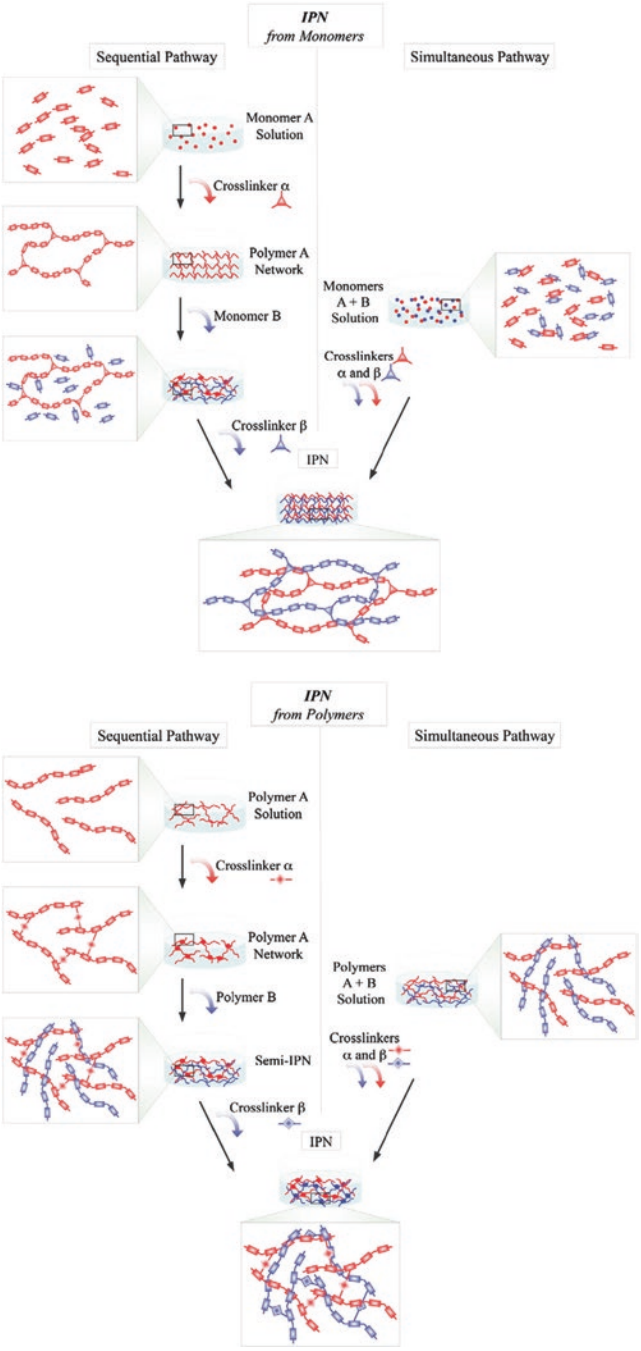


Fig. 1.1 Schematic diagram of IPN and semi-IPN formation. (Reprinted from Matricardi et al. 2013; Copyright 2013, with permission from Elsevier)

Semi-IPNs: In this type, one polymer is present as cross-linked network, whereas other polymers are either in linear form or branched. There are two types of semi-IPNs:
(a) In first type polymer A forms a network and polymer B is linear.
(b) In second type polymer A is in linear form and polymer B is cross-linked.

Thermoplastic IPN: They are a combination of IPNs and polymer blend and the materials can flow at higher temperature when physical cross-linkers (e.g. ionomers) are used. The physical cross-linking agent joins the polymeric chains together. They often behave like thermosets at lower temperature but at higher temperature, the physical cross-links break and they behave like thermoplastics (Sperling 1981).

Functional silicone fluids with thermoplastic resin: They are thermoplastic IPNs prepared by blending molten thermoplastic resin and functional silicone fluid together (Crosby and Hutchins 1985).

Some IPN-Related Materials

Latex interpenetrating elastomeric network: They are prepared by mixing two elastomeric lattices of previously mixed with catalysts and cross-linkers. The linear polymers are coagulated together with subsequent cross-linking by different reactions. Unlike other IPNs, the major interpenetration occurs between phases instead of molecules. Thus, the degree of interpenetration for IENs is expressed in respect of phases rather than molecules (Thomas and Sperling 1978).

AB-cross-linked copolymers or conterminously grafted copolymers: This is a specially grafted copolymer whereas grafted network is formed by attaching two ends of polymer B with individual polymer A molecules. Free radicals are generated at particular site on a prepolymer and the resulting microradicals are used to start polymerization of monomer B. The copolymer structure is very similar to ABA block copolymers apart from instead of following a certain pattern of chain linking (A-B-A); the attachment point may be present anywhere of the prepolymer chains. If the cross-linking density is low, there is a tendency of microphase separation depending on the chemical incompatibility of the polymers (Sperling 2001).

Non-bonded cross-linked IPNs: It consists of a double bond which is polymerizable and a large ring which can be linked via rotaxane formation with the help of a novel cross-linker (Zada et al. 2000). It allows more freedom of movement of different segments of polymers which results better swellability and oxygen transport.

Latex IPN: Latex-based polymers are prepared where a micro-IPN is present in each particle. The morphology of core shell and degree of entanglement depends on monomer addition and polymerization rate (Sperling 1981).

Organic-inorganic IPNs: They are different in terms of cross-link type and numbers. They are majorly classified into two types:

(a) Non-covalent IPNs – Inorganic material is incorporated into organic network. Reduced molecular interaction between dissimilar phases is a setback for non-covalent IPNs. The phase interaction can be improved by formation of hydrogen bonding (organic polyacrylates with silicon alkoxides give better phase interaction during IPN development) (Sperling 1981).
(b) Covalent IPNs – Silicon alkoxides are incorporated along with polymer backbone through free radical reaction for synthesis of polymers. The interfacial interaction is better here due to the incorporation of silicon alkoxide.

Anionically polymerized sequential IPNs: They are sequential IPNs, anionically polymerized from living polymeric substances (Dean et al. 2001).

Among pharmaceutical drug delivery technologies, microparticulate-based delivery system has been preferred due to its controlled drug release and site-specific ability. Polymeric nanoparticles have numerous advantages over other drug delivery strategies such as: (i) better in vivo stability; (ii) application or removal without invasive procedure, wide range of drug incorporation; (iii) possibility of avoiding certain part of GI tract to provide effective protection to drug; (iv) possible to administer directly to the target tissue, (v) highly reproducible method of preparation; (vi) increased surface area for drug absorption from gastrointestinal tract gives them an added benefit to increase bioavailability and reduction of adverse effects (Siepmann and Siepmann 2006; Mathiowitz et al. 1997; Gupta and Fung 1990; Onal and Zihnioglu 2002).

For polymeric microparticles, there are few uncertain areas, such as: (i) fate of polymeric matrix in vivo, (ii) fate of different additives used in microparticle preparation, (iii) various process parameters that can affect the stability of the particles and (iv) effect of degradation products of polymeric microparticle due to environmental factors (Linder and Markus 2005).

Microspheres are spherical shape microscopic particles ranging between 1 and 1000 μm (Freiberg and Zhu 2004). The microspheric particles are having very wide application in the area of drug delivery. Selected biocompatible polymers with tailored physical and chemical characteristics can be effectively utilized to deliver the drug at target site. Microspheres are preferred for the delivery of proteins and nucleic acids with adequate protection from the biological environment (Berkland et al. 2004; Xia et al. 2013). The spherical shapes of microspheres are suitable for vaccine delivery due to the cellular uptake nature. The physical-chemical properties of the polymers used for the microsphere preparation become an important factor for releasing the active ingredient, morphology and structural properties of the sphere (Cai et al. 2013). Polymeric porous microspheres are advantageous to be used as a carrier for a wide variety of therapeutic agents and hormones for targeted delivery due to their large surface area and porous nature. On the other hand glass and ceramic microspheres are investigated for tissue engineering, orthopaedic and dental application, radionuclide therapy and other biomedical applications (Choi et al. 2012; Bohner et al. 2013).

The spherical shape of particles is an important characteristic for injectable biomaterials. In a suitable vehicle suspended non-spherical particles may aggregate and

hence create difficulty in administration (Laeschke 2004). Drug loading techniques are another important features for microspheres. A stable and biocompatible microsphere displays desired characteristics with predictable degradability. Designing of the microsphere and the surface modification becomes very critical for preferred drug release pattern and degradation kinetics. A biodegradable polymer which possesses predictable in vivo degradability can be used in controlled release drug delivery system for delivering large biomolecules (Williams 2008; Christensen 2009).

1.2 Applications of IPN Microparticles in Drug Delivery

1.2.1 Delivery of Anticancer Drugs

Capecitabine, an oral chemotherapeutic agent used for metastatic breast cancer and colorectal cancer, was encapsulated in semi-IPN matrix of chitosan, poly(ethylene oxide) and polyacrylamide. Free radical polymerization method was used for grafting of poly(ethylene oxide) and polyacrylamide by using ceric ammonium nitrate as an initiator. Grafting variables were changed to study their effect in the developed IPN matrix. The study confirmed an amorphous dispersion of drug in polymer matrix. The in vitro drug release study was carried out in simulated gastric buffer solution for first 2 h and then followed by simulated intestinal buffer solution until complete drug release (Agnihotri and Aminabhavi 2006). Rokhade et al. have successfully developed 5-fluorouracil-loaded IPN microsphere composed of pluronic F-127 and chitosan prepared by emulsification cross-linking method by using glutaraldehyde as cross-linker. The average particle size of the hydrogel microsphere was found to be between 110 and 382 μm. The concentration of pluronic F-127, cross-linking agent and drug loading was found to have an impact on drug release which was extended up to 24 h and followed non-Fickian release mechanism (Rokhade et al. 2007a).

Another stimuli-sensitive semi-IPN microspheres of 5-fluorouracil were prepared by using N-isopropylacrylamide and sodium alginate by W/O emulsification technique using a surfactant Tween 80. The study results confirmed a molecular level dispersion of drug within the semi-IPN matrix. The swelling study and drug release study was carried out at 25 ° C and 37 ° C in different buffer solution and the results confirmed the thermoresponsive behaviour of IPN matrix (Reddy et al. 2008).

Drug-eluting semi-IPN microspheres of succinyl-modified chitosan and poly(2-acrylamide-2-methylpropanesulfonic acid) were developed by inverse suspension method with cross-linking. This method has been used by Sang et al., to administer an anticancer agent doxorubicin hydrochloride. The percentage of chitosan grafting was determined by the relative mass gain of the final product. The micrographic images of microspheres showed spherical shape with smooth surface. The insertion of –COOH and SO_3H groups has benefitted rapid drug loading and sustained release. The biodegradable nature of chitosan is helpful for designing drug-eluting microspheres of anticancer drug (Sang et al. 2019). 6-Thioguanine is used in the treatment of lymphoblastic leukaemia in children. Methyl cellulose and PVA were

used to develop IPN microsphere and 6-thioguanine was incorporated into the microspheres by in situ method. The drug loading efficiency was found up to 72%. In vitro dissolution results showed that the release of 6-thioguanine from microspheric core was dependent on cross-linker concentration, drug loading and the concentration of PVA in formulation. The drug release was sustained up to 12 h and found to follow non-Fickian type of diffusion (Siraj et al. 2014; Vora et al. 2006).

1.2.2 Delivery of Antiviral Drugs

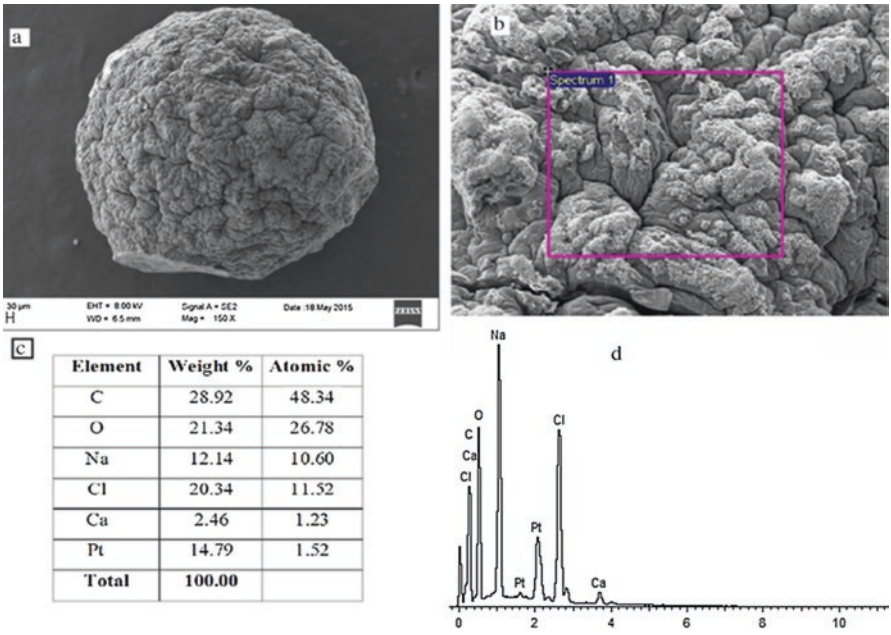
A temperature- and pH-sensitive drug delivery system was investigated for delivering antiretroviral drug zidovudine. Sodium alginate and guar gum-g-poly(N-vinyl caprolactam) were used together to develop hydrogel microbeads for colon-specific delivery system (Prasad et al. 2012). A maximum of 68% drug entrapment was observed and the drug release was found to follow non-Fickian transport. It was confirmed from study reports that the drug release was increased at pH 7.4 at colonic temperature (Rao and Eswaramma 2017).

Semi-IPN microspheres of poly(vinyl alcohol) and dextran-grafted-acrylamide were prepared by emulsification cross-linking technique to deliver an anti-HIV drug abacavir sulphate. pH-dependent control release of drug was observed from the IPN matrix (Sullad et al. 2011). Hydroxyl propyl cellulose and chitosan-based hydrogel microspheres were developed for delivering an anti-HIV drug valganciclovir hydrochloride. The developed IPN microspheres were biodegradable in nature and were cross-linked by glutaraldehyde (Soppinath and Aminabhavi 2002). The microscopic study results revealed a smooth surface. The increasing concentration of glutaraldehyde was found to reduce the size of the microspheres due to shrinkage of polymer matrix. The in vitro drug release was investigated in pH 7.4 buffer solution and the results showed controlled release of drug (Mallikarjuna et al. 2013).

Rokhade et al. have investigated acyclovir-loaded semi-IPN microspheres of chitosan and acrylamide-g-dextran by emulsification cross-linking technology with the help of glutaraldehyde.

The entrapment efficiency was up to 79.6%. The in vitro drug release indicated its dependence on degree of cross-linking and % of acrylamide grafted dextran in the formulation. The drug release was sustained up to 12 h and the release behaviour was of non-Fickian pattern (Rokhade et al. 2007b).

Jana et al. investigated acyclovir-loaded IPN hydrogel microparticles in the combination of carboxymethyl tamarind polysaccharide and alginate by ionic cross-linked gelation method in the presence of Ca^{2+}. The fabricated hydrogels were characterized by different instrumental techniques. Surface morphology and elemental composition of IPN hydrogel microparticles were characterized by field emission scanning electron microscopy (FESEM) and the images showed spherical microparticles with rough surfaces (Fig. 1.2). In vitro dissolution study demonstrated sustained drug release in alkaline medium.



Element	Weight %	Atomic %
C	28.92	48.34
O	21.34	26.78
Na	12.14	10.60
Cl	20.34	11.52
Ca	2.46	1.23
Pt	14.79	1.52
Total	100.00	

Fig. 1.2 Field emission scanning electron microscopy (FESEM) image (**a** and **b**). Surface morphology, (**c**) elemental composition and (**d**) energy dispersive X-ray (EDX) analysis of IPN hydrogel microparticles. (Reprinted from Jana et al. 2016; Copyright 2016, with permission from Elsevier)

1.2.3 Delivery of Antibiotics

A chitosan-based pH-sensitive microgel system was used to achieve controlled release of an antibiotic drug cefadroxil. Microgel particles are polymeric intramolecularly cross-linked gel particles which are homogenously distributed in a solvent system. Along with chitosan, other polymers used to develop the system are hydrolysed acrylamide grafted PVA and acrylamide grafted PVA. The IPN matrix of microgel system was found to retard the complete dissolution of drug above 10 h (Thorne et al. 2011; Rao et al. 2006). Reddy et al. have developed cefadroxil-loaded semi-IPN microsphere of chitosan and guar gum using W/O emulsification cross-linking method. The SEM study showed non-uniform microspheres with rough surface. XRD and DSC study confirmed molecular dispersion of cefadroxil in IPN matrix. In vitro dissolution was performed in pH 7.4 buffer solution and was found a controlled release over 10 h. The percentage of drug entrapment and polymer ratio was found to alter the drug release from polymer matrix (Reddy et al. 2012).

Xanthan gum-based poly(vinyl alcohol) and superabsorbent polymer was used to design IPN microspheres of ciprofloxacin hydrochloride. Hydrolyzed superabsorbents and cross-linkers were used in different ratios with PVA to develop hydrogel microspheres by W/O emulsification cross-linking technology. The release

profile of ciprofloxacin hydrochloride from IPN matrix showed sustained release of drug release through non-Fickian diffusion (Bhattacharya et al. 2013).

A widely used antibiotic ofloxacin hydrochloride was entrapped in sodium alginate-chitosan IPN microbeads. Microscopic study indicated spherical shape of microbeads with smooth surface. The in vitro dissolution study showed a sustained release of ofloxacin hydrochloride up to 24 h. A higher sodium alginate concentration and higher cross-linking time was found to retard the percentage of drug release (Kulkarni and Keshavayya 2010).

1.2.4 Delivery of Antihypertensive Drugs

An antihypertensive drug atenolol was entrapped in a semi-IPN polymer matrix made of N-isopropylacrylamide and gellan gum. The developed microspheres were in the range between 34 and 76 micron. Thermal analysis showed that the drug is dispersed at molecular level. A thermoresponsive pulsatile drug release was observed from the microsphere (Kuckling et al. 2003; Mundargi et al. 2010).

An investigation reported successful delivery of carvedilol through IPN microspheres developed by emulsification cross-linking method with gellan gum and PVA. The study results confirmed a crystalline dispersion of drug in the IPN matrix. The prepared microsphere was of spherical shape with smooth surface morphology. Simulated gastric buffer solution and simulated intestinal buffer solution was utilized for drug release study. The drug release from the polymer matrix was found to sustain up to 12 h (Agnihotri and Aminabhavi 2005). Delivery of chlorothiazide was investigated by a chitosan and N,N'-dimethylacrylamide-based semi-IPN microspheres developed by W/O emulsification technique. Molecular dispersion of drug was confirmed by DSC and X-RD method. The drug release from the microsphere matrix has been controlled up to 12 h (Babu et al. 2008). An antihypertensive drug felodipine was encapsulated in IPN microparticles by ionic cross-linking of chitosan and tripolyphosphate. A rise in the concentration of tripolyphosphate decreases the release of drug from microparticle matrix. Increased degree of cross-linking was found to retard the swelling and drug release from IPN matrix (Bodmeier et al. 1989).

1.2.5 Delivery of NSAIDs

pH-sensitive gastroprotective IPN microbeads have been developed by using sodium alginate and polyacrylamide grafted gum ghatti (PAAm-g-GG) (Fig. 1.3). PAAm-g-GG copolymer was synthesized by using ceric ammonium nitrate as an activator with the help of microwave radiation (Rani et al. 2012). Then the PAAm-g-GG was subjected to alkaline hydrolysis to make it pH sensitive. A widely used NSAID, ketoprofen, was loaded in the developed IPN microbeads to study the gastroprotective nature. The swelling index of microbeads was increased in phosphate buffer pH 7.4 in respect of HCl buffer pH 1.2 (Boppana et al. 2015).

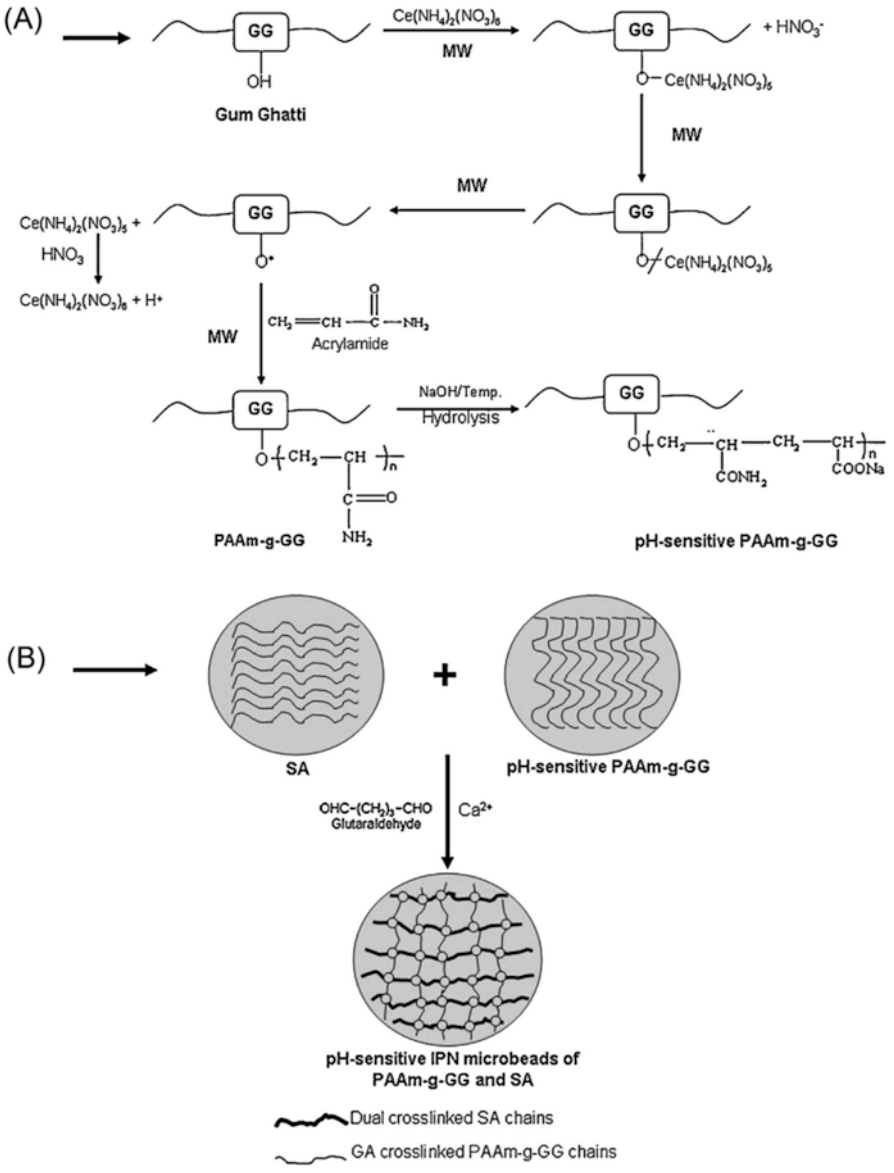


Fig. 1.3 (**a**) Schematic representation of pH-sensitive PAAM-g-GG copolymer and (**b**) preparation of pH-triggered gastroprotective IPN microbeads. (Reprinted from Boppana et al. 2015; Copyright 2015, with permission from Elsevier)

Aceclofenac-loaded chitosan-tamarind seed polysaccharide IPN microparticle was developed for sustained release of drug. Glutaraldehyde was used as a cross-linking agent and it was confirmed that the unreacted glutaraldehyde was not present in the formulation by negative test of aldehyde. The in vivo anti-inflammatory

activity was observed by carrageenan-induced paw oedema study on rats. The study showed a prolonged anti-inflammatory activity in vivo (Nayak and Pal 2011; Jana et al. 2013).

Banerjee et al. have developed diclofenac sodium-loaded IPN hydrogel microsphere prepared by emulsification cross-linking technology by using sodium carboxymethyl cellulose and poly(vinyl alcohol) (Banerjee et al. 2010). The drug release from microsphere matrix followed a non-Fickian mechanism. Glutaraldehyde was used as cross-linking agent and the cross-linking density was found to vary with varying ratio of polymers (Banerjee et al. 2012). An intestinal delivery of diclofenac sodium was reported based on chitosan and gum ghatti IPN microparticles. The surface morphology was characterized by scanning electron microscopy. The mean particle size varied between 294 and 366 μm. The in vitro dissolution study showed non-Fickian release of drug from polymer matrix. Rat intestine was used to study simultaneous dissolution-absorption profile for the optimized formulation. The study results were similar without significant difference ($p < 0.05$) which confirmed the site-specific delivery of drug (Reddy et al. 2014).

Another NSAID, ketorolac tromethamine, was delivered through IPN microsphere of sodium carboxymethyl cellulose and gelatin. Glutaraldehyde was used as a cross-linking agent. The water transport diffusion coefficient through the microsphere matrix was calculated by the use of empirical equation. The in vitro drug release study showed that the NaCMC concentration and the concentration of cross-linker played an important role for sustaining the release up to 10 h (Rokhade et al. 2006).

Naproxen sodium was successfully encapsulated into IPN microsphere of sodium alginate and PVA. Microspheres were prepared by W/O emulsification cross-linking technique using glutaraldehyde as cross-linker. The cross-linking density was found to vary with the time of cross-linking. The release characteristics were studied in three different buffer systems (pH 1.2 Hcl buffer and 6.8 and 7.4 phosphate buffer solution) which showed controlled release of naproxen sodium (Solak 2011).

Madhavi et al. have investigated locust bean gum-sodium alginate IPN microbeads for developing controlled release delivery of nimesulide. The hydrogel beads were developed by extrusion method by using a cross-linker glutaraldehyde. SEM study revealed spherical shape of microspheres with rough surfaces. The study results showed the presence of polymorphic nimesulide in molecular dispersion in hydrogel IPN matrix. The drug release study was carried out in pH 7.4 buffer medium and the study results showed the drug release was sustained up to 48 h (Madhavi et al. 2017).

A glutaraldehyde cross-linked pH-sensitive sequential IPN microspheres were prepared by emulsification cross-linking technology of poly(vinyl alcohol) and poly(methacrylic acid). Ibuprofen was selected as a model anti-inflammatory drug for site-specific oral delivery. XRD study confirmed the polymorphism of drug. The mean particle size was found to range between 51 and 176 μm. the swelling study showed a pulsatile swelling characteristics when the buffer solution was changed. The drug release was found to vary depending on the pH of the buffer solution, cross-linking density and drug entrapment (Mundargi et al. 2008).

1.2.6 Delivery of Antidiabetic Drugs

Repaglinide is an oral antidiabetic agent which was successfully entrapped in IPN microparticles of sodium alginate and sterculia gum prepared by inotropic gelation and emulsification cross-linking method. Glutaraldehyde-treated microparticles were administered in diabetic rats and resulted significant reduction of blood glucose level within 3 h (Raghavendra et al. 2014).

1.2.7 Delivery of Anticonvulsant Drugs

Delivery of an anticonvulsant drug, oxcarbazepine, was studied by IPN beads using sodium alginate and egg albumin (Fig. 1.4). The IPN microbeads were developed by ionic gelation technique with calcium chloride as a cross-linking agent. The beads exhibit slow swelling rate which ensured a slow drug release pattern. The in vitro drug release was examined which showed a controlled release of drug with non-Fickian diffusion (Vipul et al. 2015).

1.2.8 Delivery of Antiasthmatic Drugs

Theophylline, an antiasthmatic drug, was successfully entrapped in glutaraldehyde cross-linked IPN microsphere by using methyl cellulose and chitosan. The cross-linking density of the hydrogel microsphere was found to vary with the changing concentration of methyl cellulose and cross-linker. DSC and XRD study showed crystalline characteristics of drug in IPN matrices. In vitro drug release was found to extend up to 12 h and the release characteristic was Fickian mechanism (Rokhade et al. 2007c).

1.2.9 Delivery of Antihistaminic Dugs

pH-sensitive IPN bead was synthesized by using chitosan, glutamic acid, glycine and glutaraldehyde as cross-linker. An antihistaminic drug chlorpheniramine maleate was loaded into microbeads for controlled delivery. XRD study showed amorphous dispersion of chlorpheniramine maleate throughout the polymeric matrix. The study showed that the swelling nature and drug release is dependent on the pH of the buffer solution, cross-linking and composition (Rani et al. 2011).

In vitro investigation on chitosan, alanine-based semi-IPN microbeads was done for controlled delivery of an antihistaminic drug chlorpheniramine maleate. In acetic acid environment chitosan, alanine solution was prepared and extruded in the form of droplets with the help of a syringe to sodium hydroxide-methanol solution followed by cross-linking with glutaraldehyde. A pH-dependent swelling behaviour was reported. The in vitro release profile suggested that the developed IPN microbeads are a suitable delivery system for controlled release of antihistaminic drugs

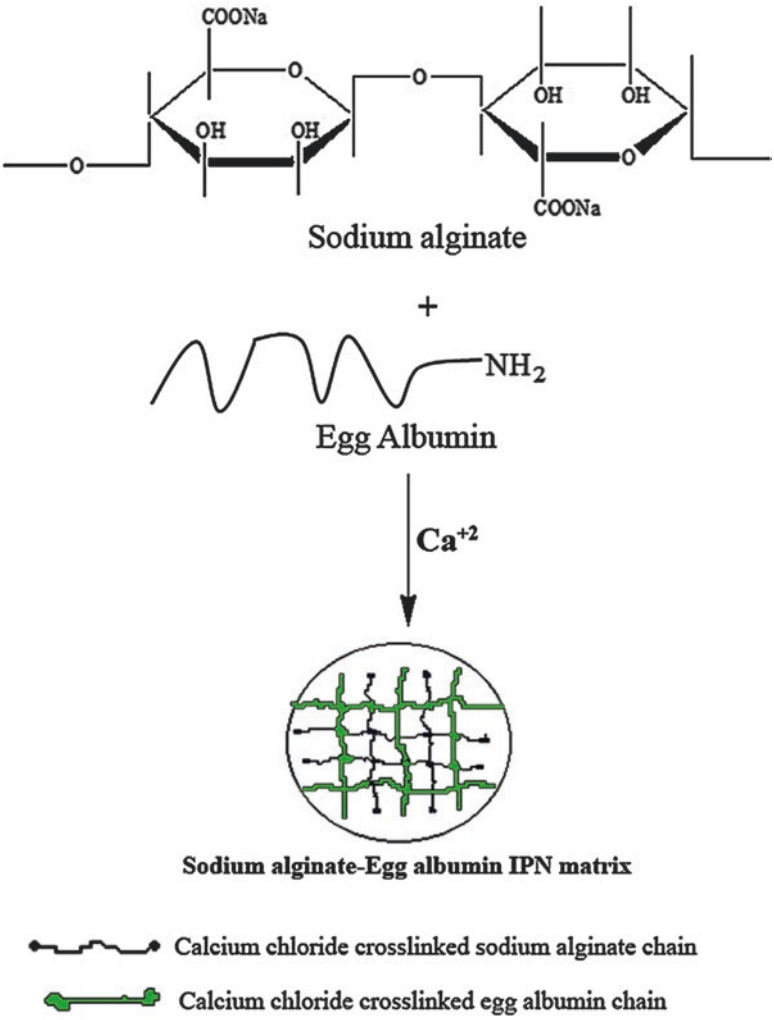


Fig. 1.4 Schematic representation of IPN matrix formation of sodium alginate-egg albumin. (Reprinted from Vipul et al. 2015; Copyright 2015, with permission from Elsevier)

(Kumari and Kundu 2007). A sedating antihistamine, triprolidine hydrochloride monohydrate, was encapsulated in IPN microspheres of sodium alginate and carboxymethyl cellulose prepared by W/O emulsification cross-linking method. A narrow particle size distribution was observed by particle size analysis result. The swelling study revealed that a higher concentration of sodium alginate resulted a high percentage of water uptake. The IPN microspheres can be used for gastroretentive delivery of triprolidine hydrochloride monohydrate up to 12 h due to the lower density of it (Ramakrishna et al. 2013).

1.2.10 Delivery of Protein Drug

An oral insulin delivery system was developed through semi-IPN microparticles made of alginate and poly(methacrylic acid). Insulin was loaded into the microparticle core by diffusion filling technology. The insulin-loaded microparticles were tested for in vitro dissolution in different buffer solution. At pH 1.2 almost 30% of loaded insulin was released from the microparticle matrix within 2 h. At pH 7.4, for poly(methacrylic acid)-alginate microparticles, burst release was observed for insulin within 60 min (Sajeesh and Sharma 2004).

1.2.11 Delivery of Prodrugs

Emulsification cross-linking method was used for delivering a prodrug capecitabine. Semi-IPN hydrogel microsphere was prepared by using chitosan and poly(ethylene oxide) grafted polyacrylamide. Grafting was done by free radical reaction with a redox initiator (ceric ammonium nitrate). Capecitabine goes for thymidine phosphorylase to convert into fluorouracil in the target tissue. Studies like FTIR, DSC and XRD confirmed the formation of IPN and chemical stability of capecitabine. Simulated gastric buffer solution (pH 1.2) was used as dissolution buffer for first 2 h, and then it was changed to simulated intestinal buffer (pH 7.4). It was observed that the drug release was continued up to 10 h from the microspheric core (Agnihotri and Aminabhavi 2006).

1.2.12 Delivery of Antituberculosis Drugs

Manjeshwar et al. have investigated glutaraldehyde cross-linked IPN microspheres of hydroxyl ethyl cellulose and chitosan to obtain controlled release of an antituberculosis agent isoniazid. The uniform distribution of isoniazid was confirmed by XRD study. The DSC and thermogravimetric analysis confirmed the thermal stabilities of the IPN matrix. The in vitro drug release study showed dependence on polymeric ratio of IPN matrix (Angadi et al. 2010).

1.2.13 Delivery of Steroids

IPN hydrogel microbeads encapsulated with glucocorticoid corticosteroid betamethasone acetate were developed by using carrageenan and sodium alginate. Being a hydrophilic drug, betamethasone acetate was loaded in IPN matrix during hydrogel network formation. The drug entrapment efficiency was found to vary with change in temperature and pH. The in vitro release from IPN matrix showed controlled release of drug (Mohamadnia et al. 2007).

1.2.14 Delivery of Antimalarial Drugs

An antimalarial drug pyronaridine was loaded into gelatin-lignosulfonic acid IPN microspheres prepared by desolvation method. The crystallinity of pyronaridine in polymer matrix was studied by XRD. The SEM image confirmed smooth surfaces of microspheric surfaces. Drug release pattern in pH 1.2 and pH 7.4 showed that the developed microspheres were pH sensitive. The dissolution study showed that the release of the drug was dependent on cross-linking density and polymeric composition and it was controlled up to 10 h (Chen and Zheng 1992; Sekhar et al. 2014).

1.2.15 Delivery of Drugs in Combination

PVA and methyl cellulose-based controlled release IPN microsphere was developed for delivering antihypertensive drug losartan potassium and an antiplatelet drug clopidogrel bisulphate used for preventing heart attack and strokes. The IPN microspheres were developed by emulsification cross-linking technology. The swelling study was performed at pH 7.4 buffer solution whereas in vitro drug release was performed at simulated gastric buffer solution and simulated intestinal buffer solution to understand the exact release profile. It was also observed that the cross-linking density of the matrix was affected by the amount of glutaraldehyde and the concentration of methyl cellulose (Sullad et al. 2014).

1.2.16 Delivery of Drugs to Lungs

IPN microspheres were prepared by PVA and acrylamide grafted pullulan to incorporate pirfenidone used to treat idiopathic pulmonary fibrosis. W/O emulsification cross-linking technology was used to develop the microspheres. Enzymatic degradation was studied in vitro and the result showed after 24 h 34.30% degradation. Biocompatibility study was performed in vitro which showed unaltered cell morphology which indicated good biocompatibility. The in vivo pharmacokinetic test showed extended T_{max} and higher AUC (Soni et al. 2018a, b). Soni et al. have investigated a pH-dependent system of pirfenidone-loaded IPN microsphere. Previous study showed the need of pH-dependent matrix to control the drug release in both the stomach and intestine, so that it meets the criteria of loading dose and maintenance dose (Jana et al. 2016). Poly(vinyl alcohol) and carboxymethylated pullulan were used in different ratio to study the swelling property. In vitro enzymatic degradation study was performed by using pullulanase. Acute oral toxicity reports suggested normal haematological and serum biochemical level. The histopathological report of the heart, kidney, liver and stomach did not show any significant difference. The drug release from the microsphere matrix was found to follow non-Fickian trends (Soni et al. 2018a, b).

1.2.17 Delivery of Drugs to Treat Claudication

A controlled release natural polymer-based microparticulate delivery system was developed for time-dependent target-specific delivery of buflomedil hydrochloride. Locust bean gum and PVA were used along with glutaraldehyde to develop IPN microspheres by emulsification cross-linking technique. Buflomedil hydrochloride is used to treat claudication (Kaity et al. 2013). The microspheres exhibited controlled release of buflomedil hydrochloride from without any incompatibility. The biodegradability and toxicity study results assured the safety of the delivery system in vivo. The T_{max} value of the developed microspheres was found similar to oral suspension of buflomedil hydrochloride (Kaity and Ghosh 2015).

1.3 Biomedical Application

1.3.1 Tissue Regeneration

IPN can be effectively applied to act as a functional substitution of damaged tissues or in case of impaired organ function. The science of tissue engineering is predominantly dependent on scaffold that must be biocompatible and biodegradable with good mechanical stability. The scaffold allows cell attachment and cellular growth in the impaired tissue. Many synthetic and natural polymers have been widely used in tissue engineering for bones, skin, cartilage, ligaments, etc. (George et al. 2008; Jain et al. 2011).

Mahou et al. have pioneered a tissue engineering method from collagen-alginate-based IPN hydrogel microspheres. The inadequate vascularization can inhibit the process of tissue engineering. The collagen-alginate IPN hydrogel matrix was developed and evaluated for various parameters required in tissue engineering. The hydrogel network was made by combining collagen fibrillogenesis with ionic gelation of alginate. The resulted hydrogel matrix showed higher resistance compared to collagen when subjected to enzymatic degradation without altering the property of embedded mesenchymal stromal cells. The microspheres were developed by a homocentric air flow method and a further coating of collagen was provided to facilitate the attachment of endothelial cells of umbilical vein collected from human body. The developed microspheres were subcutaneously implanted in SCID/bg mice. After 7 days, it was observed that the microspheres embedded with mesenchymal stromal cells and coated with endothelial cells of human umbilical vein have generated more blood vessels than microspheres without embedded mesenchymal stromal cells. Perfusion study was conducted and the results confirmed a connection of blood vessels with host vasculature. After 3 weeks of implant fewer vessels were found and a controlled leakage of blood was detected. The study findings showed promising application of IPN microspheres in the field of tissue engineering (Mahou et al. 2018).

1.3.2 IVD Degeneration Treatment

Surgical procedure is not always preferred due to their complexity and restricted post-surgery motility for the treatment of intervertebral disc (IVD) degeneration which is a major cause of lower back pain. Loose network-like structure of collagen fibres and increased water content of nucleus pulposus (NP) encourage the application IPN hydrogel for regeneration of NP. The hydrogel could be inserted into NP in liquid state followed by polymerization to generate sufficient mechanical strength.

A novel semi-IPN microsphere of gelatin was made of hyaluronic acid and collagen. Low molecular weight hyaluronic acid was used to develop the microspheres which were investigated as scaffold for tissue engineering of intervertebral disc degeneration. The semi-IPN hydrogel network was formed by boosting collagen fibrillogenesis in the intimate presence of low molecular weight hyaluronic acid. The IPN exhibited a gel-like nature and the viscosity was found to decrease with an increase in shear rate. The injectability of the resultant semi-IPN was confirmed by suitable tests and was found non-inflammatory and non-cytotoxic in nature to support tissue growth. The results showed that the composite hydrogel supports chondrogenic differentiation for mesenchymal stem cells and nasal chondrocytes both in vitro and in vivo. The hydrogel-based microspheres can act as a suitable vehicle for delivering growth factor (Tsaryk et al. 2015).

1.3.3 Heart Valve Regeneration

Commonly developed heart implants show many undesirable features such as uncontrollable degradation rate and mechanical incompatibility. Two extensively found biopolymers present in heart valve are hyaluronic acid and collagen. A tailored IPN matrix of these two biopolymers could be a useful alternative to the existing treatment approaches. Study results recommended that unmodified hyaluronic acid should not be used for heart tissue regeneration. The conducted animal study on tissue engineered heart valve has been unsuccessful due to insufficient mechanical stability. The study finding suggests that chemically cross-linked full IPN can assure a degradation rate synchronizable with the tissue regeneration rate (Jin et al. 2010; Nazir 2016). An investigation by Kane et al. has shown modified freeze-drying method for improving the mechanical property of hyaluronic acid-collagen scaffolds. Compression moulding was used for attaching collagen fibrils, paraffin microspheres and hyaluronic acid. Afterwards the paraffin microspheres came out of the matrix resulting a chemically cross-linked IPN (Kane, et al. 2015).

1.3.4 Cartilage Tissue Engineering

Formation of IPN with three components has been reported to have increased proliferation of cells, significant gene expression and reduced cytotoxicity compared to bi-polymeric IPN hydrogel (Dinescu et al. 2015). Recent advancements such as

formation of IPN microspheres, spinning and 3D printing show possibility of manipulating hydrogels into 3D scaffolds. The microspheres can generate a required microenvironment supporting cartilage regeneration and recovery (Vega et al. 2017).

Chang et al. have developed TGF-β1-loaded sustained release chitosan microspheres by emulsification cross-linking technology. ATDC-5 was additionally incorporated into chitosan microspheres along with TGF-β1. The microspheres were incubated with 107 U/L lysozyme, to achieve degradation rate of 51.0% ± 1.8% after 4 weeks. To understand the TGF-β1 effect different scaffolds were used to estimate ATDC-5 growth by fluorescence staining method and MTT assay. The study results suggested that TGF-β1 could effectively promote ATDC-5 growth. A quick release of TGF-β1 was observed in first day and afterwards a gradual decreased release to attain plateau after 5 days (Chang et al. 2017).

1.3.5 Ophthalmic Implant

Inverted colloidal crystals were developed from IPN hydrogel of poly(acrylic acid) and poly(ethylene glycol). For achieving long-term retention of the artificial cornea, a sponge-like porous interlinked scaffold at peripheral region is important. For preparing scaffolds of inverted colloidal crystals with predetermined pore size and channel dimensions, microsphere templates were applied. Sequentially polymerized IPN hydrogels with more than 80% water content were used. Moulded annular scaffolds were used for developing artificial cornea implant which consist a clear optic vision with porous periphery (Parke-Houben et al. 2015).

1.4 Conclusion

Over the past few years the development of novel IPN system has become a rapidly growing area. In this entry the development of various microparticulate systems and their applications in the field of controlled release medication was discussed. It is also showcased that IPN microparticulate system can be a major resource of drug delivery system in a controlled manner to specific target site. The biomedical application of IPN microparticles is another flourishing area which has a lot to offer through tissue engineering. This promising area needs a lot of future investigation on IPN microparticles for its successful administration in therapeutic or biomedical field.

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2 Interpenetrating Polymer Network (IPN) Nanoparticles for Drug Delivery Applications

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Abstract

Recent studies emphasize the interpenetrating polymer network (IPN) as a combination of two or more polymers in the form of network where covalent bond is unlikely to be formed when at least one of the polymers is synthesized or cross-linked in the presence of the other during formation of IPN. One of the most IPN hydrogel applications is the formation of stimuli-sensitive delivery system. Researchers have used them alone or in combination with synthetic polymers to

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fabricate IPN hydrogels with desired properties. Those polymers include polysaccharides such as chitosan, cellulose, dextran and xyloglucan, and proteins such as gelatin as well as synthetic polymers such as poloxamer. The IPN nanoparticle-based hydrogels have the ability to form aqueous solutions with high colloidal stability in vivo and entrap of macromolecules, such as proteins and peptides, target, and control the drug release, feasibility of surface modifications by various types of site-specific ligands in order to improve targeted delivery in the body in addition to feasibility for administration through different pathways, such as oral, parenteral, nasal, pulmonary, and ocular routes.

Keywords
Interpenetrating polymeric network · Drug delivery · Nanoparticles · Microparticles · Hydrogels

2.1 Introduction

In the recent decades of industry, polymers are extensively used as biomaterials due to their unique properties such as good biocompatibility, easy design and preparation, a variety of structures, and interesting bio-mimetic character. Polymers have a significant effect in drug delivery since they deliver the drugs efficiently to their desired site of action and enhance solubility, permeation, and bioavailability of some hydrophobic drugs (Kawashima 2001; Soppimath et al. 2001) as well as control the release of the drugs at the target site (Allemann et al. 1993). Natural semi-synthetic and synthetic polymers are used as drug carriers from the nanoscale to microscale range (Bennet and Kim 2014).

Mixing of polymers and producing many new types of polymer mixture with sophisticated uses has become very common since the industrial era has invaded our time. In drug delivery systems, the use of more than one polymer was found to have a great impact on practical and academic level for controlling the release of many drugs, owing to the modification ease in the system which is provided by combining more than a polymer regarding physical and chemical properties. This field got its intensive development during the recent years and the literature on this topic is really extensive.

No doubt that the first interpenetrating polymer network (IPN) was invented by Aylsworth, since the idea itself existed from the last decade, where Miller gave the term IPN in his study (Lohani et al. 2014). Some studies emphasize the IPN as a combination of two or more polymers in the form of network (Singh et al. 2012), where covalent bond is unlikely to be formed when at least one of the polymers is synthesized or cross-linked in the presence of the other during formation of IPN (Qadri et al. 2015). The network is linked together in chain or series that cannot be separated unless chemical bonds are broken unlike polymer complex and graft copolymer (Work et al. 2004). Not only they differ from other polymer combination in that but also IPN swells without dissolving in the solvent and prevents creep and flow action (Kudela 1987). A new advanced polymeric system form must be

produced from the combination of the polymers with new physicochemical properties (Kim et al. 2004). There are specific criteria for choosing the IPN-forming polymers for production of intact network involving the presence of one polymer during synthesis or cross-linking of the other polymers without dramatically phase separation; both polymers should show same absorption, distribution, metabolism, and excretion routes in our bodies (Singh et al. 2012).

2.2 Types of IPN According to Chemical Bonding

2.2.1 IPN Arranged According to Chemical Bonding

Hydrogels are simple example for permanent covalent cross-linking network structure with irreversible chemical links which allows drug release by diffusion and permits the absorption of drugs without dissolutions.

Covalent semi-IPN: A single polymer network is formed by cross-linking of two separate polymer systems, for example, the synthesis of water-soluble polysaccharide-based semi-IPN cryogel hemostatic dressing via Schiff's base cross-linking between the polyaldehyde groups of oxidized dextran and thiolated chitosan in the presence of locust bean gum (LBG) known for its hydrophilicity, leading to increase of hydrophilicity and swelling ability of IPN. That improved the mechanical strength and fast fluid responsiveness aiding blood clotting, without acidic medium found in the conventional chitosan dressing (Meena et al. 2018). The synthesis and characterization of a natural polymeric system consist of the interpenetrating polymer network (IPN) comprising curcumin as a bioactive. Biopolymers and bioactives such as chitosan, hypromellose, citric acid, genipin, and curcumin were used to develop an effective, biodegradable, and biocompatible film used in wound healing (Mayet et al. 2014).

Non-covalent semi-IPN: It is formed when just one polymer is cross-linked, for example, cross-linked chitosan composites functionalized with silver and gold nanoparticles for antimicrobial applications. Chitosan was cross-linked in the presence of siloxane composite and metal NPs were introduced (Ryan et al. 2017).

Non-covalent Full IPN: It involves the cross-linking of two separate polymers independently.

2.2.2 According to Arrangement Sequence

1. **Sequential IPN**: the second polymer starts polymerizing instantly after the complete polymerization of the first polymer network.
2. **Novel IPN polymer**: interlocking of two or more polymer networks on a molecular scale but not covalently bonded to each other and can only be separated by chemical bond breaking – cannot be separated unless chemical bonds are broken.

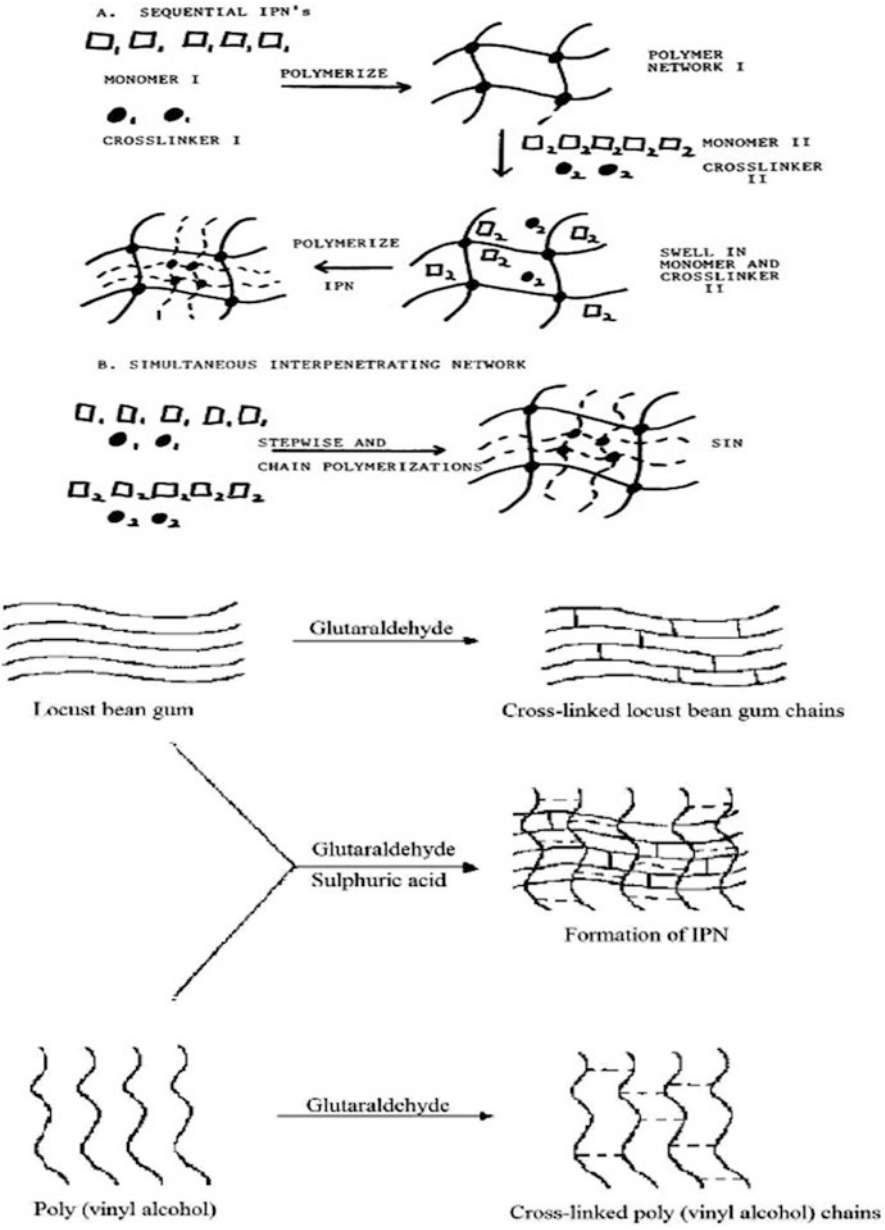

Fig. 2.1 Demonstrating types of IPN

3. **Simultaneously IPN**: in situ polymerization of both polymers at the same time, such as synthesis of pH-, thermo-, and salt-sensitive modified poly(aspartic acid)/poly(vinyl alcohol) IPN hydrogel release, by a simple one-step method in aqueous system using poly(aspartic acid) grafting 3-aminopropyltriethoxysilane (KH-550) and poly(vinyl alcohol) (PVA) and drug release-controlling ability (Lu et al. 2015). Various types of IPN are shown in Fig. 2.1.

2.3 IPN Properties

1. Increased elasticity and mechanical property were accompanied by a gel composed of two interpenetrating networks by cross-linking a polymer (or polyelectrolyte) into a pre-existing highly cross-linked network of a polymer (or polyelectrolyte) of another polymer type. That was emphasized by the stress-strain behavior with comparative study of elastic moduli and breaking points, where elastic moduli and tensile strength modification can be done through changing molecular weight (Yin et al. 2008; Bhattarai et al. 2005).
2. PEG as the first network and poly(acrylic acid) as the second network forming IPN hydrogels of oxygen permeability of 95.9 ± 28.5 (Wang et al. 2010).
3. Equilibrium water content – IPN can swell in solvent without dissolving. The water content of hydrogels was evaluated in terms of the swollen weight to dry weight ratio. The dry hydrogel was weighed and then immersed in water as well as phosphate-buffered saline. At regular intervals the swollen gel was lifted, patted dried, and weighed until the equilibrium was attained.
4. High thermostability increasing the phase stability of the final product thus overcoming thermodynamic incompatibility by interlacing the networks together (Yan et al. 2009).
5. Good dielectric properties.

2.4 Some Features of IPN in Drug Delivery Systems

IPNs have a synergistic effect as they consist of different types of polymers forming networks together with one rubbery phase and one glassy phase, where phases remain separated even when subjected to stress. Many different types of IPN systems are formed as a result of diversity in the number and types of cross-links (Singh et al. 2012). IPNs have a significant adhesive property as they can be swelled in the medium without dissolving the solvent (Suresh et al. 2011). IPNs showed a great impact in developing the controlled release system for delivering the drug for the required period of time with low fluctuation (Wang et al. 2007; Rokhade et al. 2007).

2.5 Drawbacks of IPNs

The problem is encountered with the release of the drug that sometimes becomes hard as a result of strong interpenetration of the polymers. That was demonstrated more in non-covalent system as well as the covalent system due to the lack of an effective interface (Qadri et al. 2015; Suresh et al. 2011).

2.6 Methods of Preparation of IPNs

As shown in Fig. 2.2, simultaneous and sequential synthetic methods are the most common methods for IPN preparation. On the other hand, in situ technique was implemented where the networks can be formed at the same time which was found to be the most convenient way (Vancaeyzeele et al. 2005).

1. **Simultaneous synthetic method:** where polymer network is formed in situ by mixing the monomers together by different reaction routes.
2. **Sequential synthetic method:** Different network reactions are controlled sequentially by adding different monomers. Mostly commercial materials are prepared by sequential IPNs, due to their flexibility and easy-to-process ability. When IPNs are used for coating purpose, they cannot be prepared by the sequential or simultaneous interpenetrating polymerization because of the presence of volatile monomer. For this purpose, they can be prepared from preforming prepolymers which contain complementary functional groups that increase their miscibility (Anzlovar and Zigon 2005). In IPNs, cross-linking of mutual chain entanglement produces finer dispersion of one polymer into the other (Merlin and Sivasankar 2009).

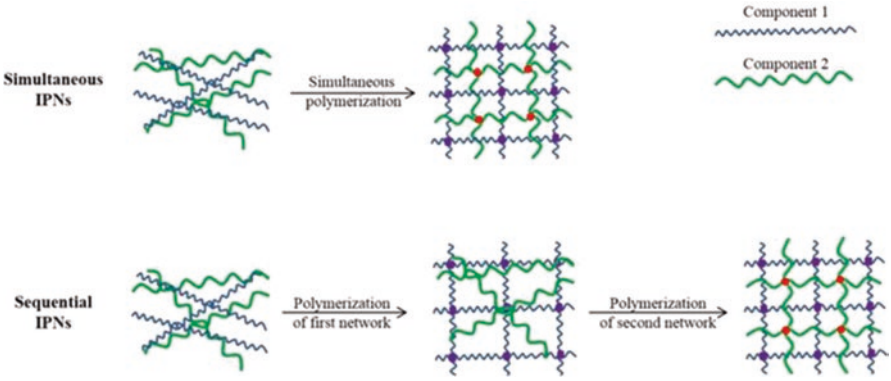

Fig. 2.2 Illustrating different methods of IPN preparation

2.7 Applications of IPN

IPN-based drug delivery system: involves many applications used to improve the delivery of active constituents such as hydrogel films, tablets, capsules, microsphere, sheets, sponges, matrix, transdermal patches, nanoparticles, and others (Hou and Siow 2001).

Hydrogel: It's prepared from combination of different polymers chemically cross-linked with the ability to retain water in its structure as a result of presence of hydrophilic function of polymers. Hydrogels are three-dimensional polymeric network as well (Bhardwaj et al. 2012; Peppas et al. 2000; Zhao et al. 2006). Moreover, in situ forming hydrogels can be a method for developing new therapeutics and diagnostics. A polymer solution can turn into gel immediately, after photopolymerization (Burkoth and Anseth 2000; Burdick et al. 2003), chemical cross-linking (Ossipov and Hilborn 2006), ionic cross-linking (Kuo and Ma 2001), or responding to an environmental stimulus as temperature, pH, or ionic strength of the media (Peppas et al. 2000).

2.8 General Properties of Hydrogels

1. Increase the mechanical strength of the natural polymers.
2. Easy manufacturing.
3. Resilient and stable (Burkoth and Anseth 2000).
4. The hydrogel is cell compatible.

2.9 Thermosensitive Hydrogels

Temperature here is the only stimulus that affects hydrogel gelation without other factors such as chemical or environmental and can be thus produced upon injection to the body, when temperature is increased from room temperature to physiological. Some hydrogels separate from solution and solidify above a known temperature. This is known as the lower critical solution temperature (LCST). Below the LCST, the polymers are soluble. Above the LCST, they exhibit hydrophobic and insoluble features, leading to gel formation. On the contrary, hydrogels are produced when cooling polymer solution exhibits an upper critical solution temperature (UCST) (Li et al. 2002). The sol-gel transition of thermosensitive hydrogels can be shown by numerous techniques such as spectroscopy, differential scanning calorimetry (DSC) (Li et al. 2002), and rheology (Yin et al. 2006).

In Fig. 2.3, temperature can cause changes in the solubility of polymers, as a result in changing of hydrophilicity of the polymer chain, producing gels in aqueous solution. This can be explained by further investigation into the three types of interactions that happen when polymer dissolved in water, whether it happens between polymer molecules, between polymer and water, and between water molecules. For polymers

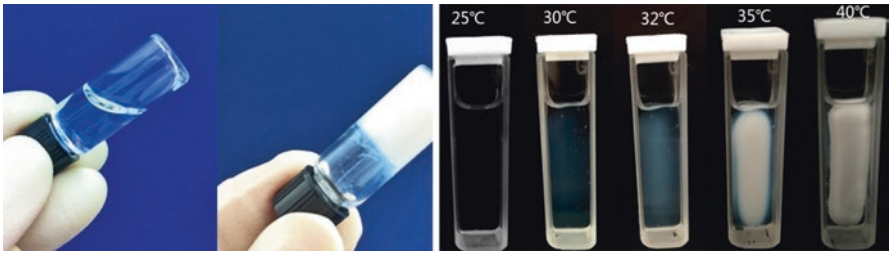

Fig. 2.3 Showing gel formation of thermosensitive polymer upon heating

having an LCST, temperature affects negatively the system which makes water-polymer reaction unfavorable while allowing the other two types of interactions. This negative free energy (ΔG) is attributed to the higher entropy term (ΔS) as a result in increase of enthalpy term (ΔH) in the thermodynamic relation $\Delta G = \Delta H - T\Delta S$. The entropy increases due to water–water associations which are the governing interactions in the system. This phenomenon is known as hydrophobic effect (Schild 1992).

2.10 IPN Thermosensitive Hydrogel-Based Polymers

2.10.1 1-Natural Polymers

Many natural polymers have been shown to exhibit gelation upon temperature change. Researchers have used them alone or in combination with synthetic polymers to fabricate thermally responsive hydrogels with desired properties.

2.11 Polysaccharides

2.11.1 Cellulose Derivatives

Cellulose is a natural polysaccharide which is insoluble in water. Removal of the hydroxyl groups on cellulose and addition of hydrophobic units as methyl or hydroxypropyl groups results in decreasing the water solubility of cellulose in water-soluble (Schild 1992). Methylcellulose (MC) is a cellulose derivative that has been widely used in many biomedical applications. It forms gels in water at temperatures in the range of 60–80 °C turning into a solution upon cooling (Takahashi et al. 2001). Liu et al. (2004) have impeded methylcellulose with the synthetic *N*-isopropylacrylamide (NiPAAm), uniting thermogelling properties of both materials. It was possible to prepare fast reversibly thermogelling hydrogels by adjusting the ratios of the two components. It was reported that a low percentage of methylcellulose decreases the LCST as compared to pNiPAAm, but with a high MC ratio the LCST increases. It was also proved that combining MC with NiPAAm polymers improves the mechanical strength of the hydrogel.

2.11.2 Chitosan

It is a deacylation product of chitin, which can be found in the outer skeleton of shrimp and insects; many approaches had developed thermosensitive chitosan-polyol salt hydrogels. More recently, several reports have incorporated poly(ethylene glycol) (PEG) into chitosan and without need of any cross-linker to produce thermoreversible hydrogel (Chenite et al. 2000).

The incorporation of PEG into chitosan has improved its solubility in water and provided a platform for controlled release delivery of the drugs. The gelation was shown mostly in physiological pH values (Supper et al. 2014). An initial burst release was shown followed by a steady release from the hydrogel for about 3 days upon using albumin as a model protein. It required dissolving the gel into the media for complete release of albumin. When cross-linking the PEG-grafted chitosan with genipin in situ, it showed quasi-linear drug release for up to 40 days; however, thermo-reversibility was lost at 37 °C.

2.11.3 Dextran

Besides being biodegradable, a modification was given to the structure of dextran to provide it with thermoresponsive properties, where dextran reacted with maleic anhydride (MA) to form the Dex-MA polysaccharide by photocross-linking it with NiPAAm. The formed hydrogel was partially biodegradable and had a higher LCST due to the hydrophilic and biodegradable nature of Dex-MA. Additionally, the carboxylic end groups of Dex-MA provide the hydrogel with pH sensitivity property (Cho et al. 2009). Other studies reported dextran macromer containing oligolactate and 2-hydroxyethyl methacrylate units (Dex-lactate-HEMA), which has hydrolytically degradable blocks, was copolymerized with NiPAAm. This hydrogel showed an LCST close to that of pNiPAAm. The hydrogels had disintegrated within 2 weeks at temperature below LCST. The degradation was much slower due to increased hydrophobic effects at 37 °C. Interestingly, when the hydrogel was tested for drug delivery, it was shown that a low molecular weight drug (methylene blue) was released slower at 25 °C than at 37 °C, on contrast to the high molecular weight substance (bovine serum albumin, BSA). It was concluded a number of factors such as the temperature, the swelling and degradation characteristics of the hydrogel, as well as the interactions of the drug and the hydrogel macromolecules affect the drug release profile.

2.11.4 Xyloglucan

Xyloglucan is a biocompatible polysaccharide and was found to have thermally responsive behavior when more than 35% of its galactose residues are removed (Hastings et al. 2012). Xyloglucan gels are found to be a promising drug delivery vehicle for many applications (Kim et al. 2014), but there are not enough proven

data on rheological and morphological properties of these hydrogels. Some have examined the gelation characteristics of xyloglucan hydrogels and also their morphology when put under physiological conditions (Chien et al. 2012). The gelation process seemed to be affected by the presence of ions in PBS in comparison to deionized water. As to the optimum concentration, it was found that 3% (wt.) xyloglucan in aqueous media possess an elastic modulus that is significantly higher than other natural or synthetic hydrogels. Moreover, this concentration yielded a gel that could be freeze-dried and examined with scanning electron microscopy. The images appear macroporous, interconnected, and three-dimensional.

2.12 Proteins

2.12.1 Gelatin

Gelatin is a biocompatible, biodegradable polymer with thermoreversible properties. At temperatures below 25 °C, an aqueous gelatin solution becomes gel as a result of the formation of triple helices and a rigid three-dimensional network. When raising the temperature up to 30 °C, liquification of gel takes place (Csóka et al. 2007). Researchers have modified gelatin easily due to presence of amino group in its structure, as it is required to have the opposite thermal behavior for biomedical applications, where gelation occurs at temperature nearly to body temperature (Luca et al. 2011). For most compositions of gelatin and mPEG-DLLA, the hydrogel was shown to flow at 37 °C and gel at room temperature; however a 100 mg/mL gelatin solution underwent fast gelation at 37 °C when combined with 30% wt. mPEG-DLLA. Different hydrogel compositions were also examined for drug release kinetics with gentamycin sulfate as the model drug. At room temperature, 5 days or longer was necessary for 50% drug release, and the release lasted up to 40 days. At 37 °C, gelatin-mPEG-DLLA showed an even slower release profile; however after 1 week, the release was no longer detectable due to degradation of the hydrogel matrices (Cui et al. 2011).

2.13 PEO-/PPO-Based Systems

Triblock copolymers poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO), also called as Pluronics® or Poloxamers, are thermoreversible synthetic polymers. This reversible gelation can happen at physiological temperature and pH by modifying the composition, the molecular weight, and the concentration (Billiet et al. 2014). The amphiphilic nature of these polymers is due to the hydrophilic ethylene oxide and the hydrophobic propylene oxide. The gelation mechanism of PEO/PPO block copolymers in aqueous solution occurs by the changes in micellar characteristics depending on concentration and temperature. Amphiphilic block copolymer molecules can self-assemble into micelles in aqueous solutions. Above a certain concentration, termed as the critical micelle concentration (CMC), the polymer molecules, which were previously in solution, aggregate

and form micelles. Also, the micelle formation has strong temperature dependence. Below a certain temperature, termed as the critical micelle temperature (CMT), both ethylene and propylene oxide blocks are hydrated, and poly(propylene oxide) is relatively soluble in water. With temperature increase, poly(propylene oxide) chains become less soluble, resulting in micelle formation (Kabanov et al. 2002). Over the last years, these copolymers have been widely used in applications such as drug and gene delivery (Kabanov et al. 2002).

2.14 Smart Drug Delivery Systems

One of the most important IPN hydrogel applications is the formation of stimuli-sensitive delivery systems known as smart drug delivery systems (SDDS) which.

2.15 Environmentally Sensitive Hydrogels

It is formed from hydrophilic, stimuli-responsive polymer networks that can change the volume in response to an external signal, for example, change in temperature or chemical environment. They are used in many biomedical applications (Kaufman et al. 2018). Hydrogels of calcium alginate and dextran hydroxyethylmethacrylate were prepared in situ and evaluated for protein release and also for the behavior of embedded cells. It was noticed that after an initial burst release, bovine serum albumin was gradually released from the IPN hydrogels for up to 15 days. Encapsulation of expanded chondrocytes in the IPNs showed that cells remained viable and were able to remodel.

The preparation of IPN hydrogels is based on polyvinyl alcohol (PVA) networking with polyacrylic acid (PAA) in situ with no cross-linker addition using benzoyl peroxide as initiator and sodium chloride (NaCl) as additive. The response of the hydrogels with and without NaCl was observed by studying their swelling behavior, biodegradability, and thermal stability. Scanning electron microscopic study showed that the pores of the prepared IPN were widely open in presence of NaCl, therefore making the hydrogel macroporous. (PVA-co-PAA)/NaCl was found to be more biodegradable than without NaCl. The IPN hydrogel showed a higher swelling ability in intestinal pH than that of gastric medium, and the presence of NaCl in the IPN showed a significant increase in the swelling properties in both media.

2.16 Injectable In Situ Forming Hydrogels

It's used in delivering protein and tissue engineering (Ray et al. 2011). Eltjani-Eltahir Hago et al. have produced interpenetrating polymer network PVA/GE hydrogels by a combination of enzymatic and physical methods, using freezing-thawing process and in situ with synthesis of gelatin/mTG in PVA solution. Scanning electronic microscope (SEM) is used to determine the morphology and crystalline structures of interpenetrating polymer network PVA/GE.

2.17 Soft Hydrogels

Soft hydrogels interpenetrating silicone polymer networks were developed by Steffensen et al. drug-releasing medical devices. IPN materials with PHEMA content in the range of 13–38% (w/w) were synthesized by using carbon dioxide-based solvent mixtures under high pressure. These IPNs were characterized according to microstructure and ability of the hydrogel to form a surface-connected hydrophilic carrier network inside the silicone. A critical limit for hydrogel connectivity was found both *through* simulation and by visualization of water uptake in approximately 25% (w/w) PHEMA, indicating that entrapment of gel occurs at low gel concentrations. The optimized IPN material was loaded with the antibiotic ciprofloxacin, and the resulting drug release was shown to inhibit bacterial growth when placed on agar, thus demonstrating the potential of this IPN material for future prospective applications in drug-releasing medical devices (Steffensen et al. 2016). Some of IPN hydrogel applications are shown in Table 2.1.

2.18 Nanocomposite Polymer Hydrogels

The need for new and better soft materials and also exploring for new knowledge and fundamental understanding showed a wide development in nanocomposite gels. It is a variety of complex gel structures with different chemical, physical, and biological characteristics that have been formulated at nanoscale. In general, nanocomposite polymer hydrogels exhibited a definition of cross-linked polymer networks swollen within water in the presence of nanoparticles or other nanostructures. The polymer is cross-linked to form a network through chemical or physical interactions. Covalent bonds make the chemical cross-linking permanent. The physical interactions are non-covalent in nature and are due to hydrogen bonding and hydrophobic and ionic interactions.

The incorporation of nanoparticles has added unique physical characteristics to polymer hydrogels, for example, responsiveness to mechanical, optical, thermal, barrier, sound, magnetic, electric stimulation, etc. These unique properties provide new applications in the electronics, optics, sensors, actuators, and micro-fluidics sectors, as well as catalysis, separation devices, drug delivery, and many other biotechnological areas. Various nanocomposite hydrogels are shown below:

Polymer-/silicate-based nanocomposite gels
Poly(ethylene oxide)–silicate nanocomposites
Poly(acryl amide)– and poly(vinyl alcohol)–silicate nanocomposites
Polymer–metal nanoparticle hydrogels
Polymer–magnetic nanoparticle hydrogels

Applications of nanocomposite hydrogels for tissue engineering usually depend on the combination of functional properties that are engineered into the nanocomposite hydrogels to make these materials more versatile. Creative approaches incorporate

Table 2.1 List of some prepared polymers through IPN-based hydrogel

Polymer	Nanoparticles	Method of preparation	Drug	Type of nanocomposite	Key outcome	References
Carboxymethyl cellulose–hydroxyethyl	Na-montmorillonite (NaMMT)	Sequential solution blending technique	Cisplatin		Increased cross-link density of hydrogel improved the elastic modulus (up to 99%) and improved the drug retention time (up to 72 h at both pH 7.4 and 4.0)	Kouser et al. (2018)
Cellulose-acrylonitrile-linseed oil polyol (CHAP)					The release rate of cisplatin in nanocomposite hydrogel films was found to be higher (83 and 69%) at both pH 4.0 and 7.4. The release rate of cisplatin in nanocomposite hydrogel films was pH-responsive and increased with decrease of pH	
Polyvinyl alcohol (PVA)	Chitosan	Sequential	Tetracycline	Nanocomposite hydrogels based on PVA	Increasing antibacterial activity against gram-negative and gram-positive bacteria and cell growth in the presence of tetracycline	Parsa et al. (2019)
Alginate Acrylamide Montmorillonite	Ca^{2+}	In situ polymerization			Shows remarkable reversible pH-dependent swelling/deswelling behaviors	Su and Chen (2018)
Carboxymethyl chitosan	Fe_3O_4 $MnFe_2O_4$	In situ polymerization	Curcumin	Polymer–metal nanoparticle hydrogels	Sustained release of curcumin	Naderi and Azizian (2018)

(continued)

Table 2.1 (continued)

Polymer	Nanoparticles	Method of preparation	Drug	Type of nanocomposite	Key outcome	References
Chitosan	Graphene oxide–Ag		Doxorubicin		Higher sustained and controlled drug release profile was enhanced by increasing the GO–Ag nanohybrid particle content	Rasoulzadehzali and Namazi (2018)
N-Isopropylacrylamide + Laponite (XLG)/ carboxymethyl chitosan (CMCTs)	Genipin	Facile, one-pot free radical polymerization	Aspirin		The hydrogels showed an appropriate controlled release property of aspirin by tuning their inner cross-link density	Chen et al. (2018)
N,*N*'-methylenebisacrylamide	Clay	In situ polymerization	–	–	Increased swelling degrees, slowed de-swelling process, and enhanced mechanical properties depending on the clay type	Ianchis et al. (2017)
Chitosan	Calcium phosphate	In situ polymerization	BSA	–	Controlled release	Salama (2018)

biomolecules into magnetic nanoparticle (5–10 nm) gels for chemotherapeutic loading, and for tumor-associated bimolecular binding, nanocomposite polymer hydrogels with bentonite have shown promising potential in drug delivery applications. Thermal stability of IPN was affected by copolymerization, due to increasing porosity of the IPN. The prepared nontoxic, hydrophilic IPN hydrogel system holds good for further drug delivery studies in connection to its super swelling and biodegradability (Ray et al. 2008; Thakur et al. 2014; Xiao et al. 2011; Honmute et al. 2012).

2.19 Properties of Nanoparticle Hydrogels

(1) The ability to form aqueous solutions with high colloidal stability in vivo and entrap macro-molecules, such as proteins and peptides.
(2) Targeting and controlling the release of the drug. High drug loading without chemical reactions.
(3) Feasibility of surface modifications by various types of site-specific ligands in order to improve targeted delivery in the body.
(4) The excellent choice for internalization by the cells like dendritic cells, through phagocytosis.
(5) Feasibility for administration through different pathways, such as oral, parenteral, nasal, pulmonary, and ocular (Baklaushev et al. 2015; Gonçalves et al. 2010; Debauche 2011). A wide range of natural or synthetic polymers may be used for the preparation of nanogels. Among these polymers, polysaccharides are the mainly used ones (Boaro et al. 2010) (Fig. 2.4 and Table 2.2).

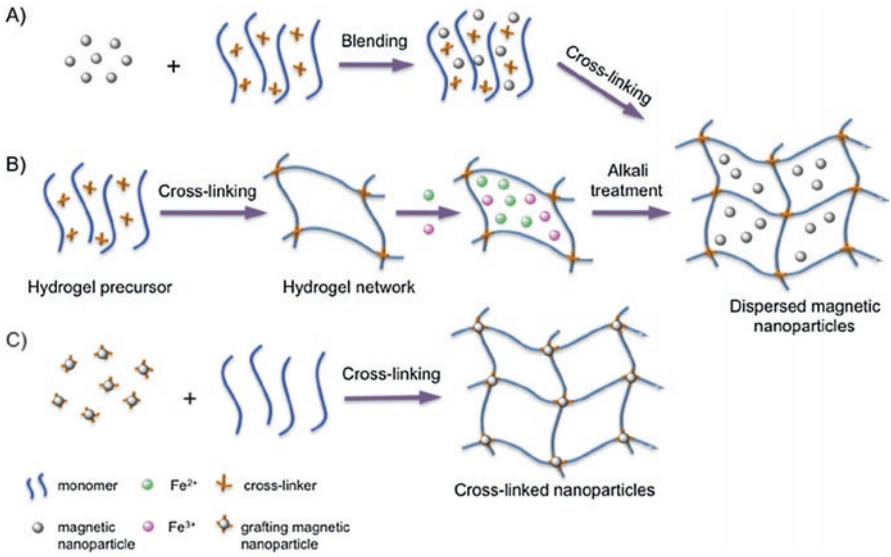

Fig. 2.4 Various methods of preparation of hydrogels

Table 2.2 List of some prepared polymers through IPN-based hydrogel

Polymers	Method	Drug	Type of IPN	Key outcome	References
PAM-SA +cellulose nanofibers	Covalent cross-linking	–	Semi-IPN	Cellulose nanofibers with SA-PAM improved adsorption capacity and mechanical strength	Yue et al. (2019)
Alginate + F-gelatin	Physical and chemical cross-linking	Bioactive materials	Semi-IPN	Lowering swelling and degradation rate	Zhang et al. (2018)
				Increases cell adhesive ability	
Collagen + alginate (b-CAFs)		–	Semi- IPN	Physical entrapment effect on the b-CAFs limiting its metastasis and phenotype in a manner that effectively muted its pro-tumorigenic activity	Cao et al. (2018)
Monomer	Response surface methodology-central composite design (RSM-CCD)	Dyes	Semi-IPN nanocomposite hydrogel	Enhancement of the % dye removal (96.82% EY and 98.73% EBT) along with fluorescent behavior	Sharma et al. (2019)
Chitosan + 2-acrylamido-2-methylpropane sulfonic acid (AMPS) + acrylic acid (AA)		–	Semi-IPN pH-sensitive hydrogels	Improving of drug loading, drug release, and swelling with increasing the concentration of chitosan and AA, on contrary to AMPS	Ullah et al. (2018)
Cellulose (BC) + chitosan (CS)	(In situ) simultaneous with glutaraldehyde cross-linker	–	(Semi-IPN) hydrogels	BC-CS hydrogels exhibited higher thermal stability than pure BC film or CS hydrogel alone. Hydrogels with 20% BC to CS reduced the viable bacterial count by ~88%	Wahid et al. (2019)
Carboxymethyl guar gum and gelatin	Sequential method	Ciprofloxacin	(Semi-IPN) hydrogels	Sustained-release profile of ciprofloxacin	Ghosh et al. (2018)

Polymers	Method	Drug	Type of IPN	Key outcome	References
Polysaccharide and poly(methacrylic acid)	In situ Michael-type reaction combined with covalent cross-linking with N,N-methylenebisacrylamide (MBA)	–	(Semi-IPN) hydrogels	The hydrogels showed reversible ductility up to 70% in response to cyclic loading-unloading cycle which is an obvious phenomenon of rubber-like	Fares et al. (2018)
				Controlled drug release can be applied by affecting the pH	
Carrageenan + alginate	Green method in situ free radical gelation into semi-IPN hydrogel matrix	–	(Semi-IPN) hydrogels	Hydrogels showed dual stimuli-responsiveness, that is, environmental pH and external electrical stimulation	Ganguly et al. (2018)
Hyaluronic acid + poly (N-isopropylacrylamide)		Luteolin	Double cross-linked (IPN) hydrogels	An IPN hydrogel with 3% cross-linker amount had the most adhesive and stable cross-linked network	Kim et al. (2018)
				Delivering luteolin successfully as a result of dual pH sensitivity	
Sugarcane bagasse + cellulose (SBC), carboxymethylcellulose (CMC) and poly(N-isopropylacrylamide)	In-situ polymerization with N,N'-methylene-bis(acrylamide) as a cross-linker	Bovine serum albumin (BSA)	pH and thermoresponsive (IPN) hydrogels	The in situ polymerization produced thermoresponsive IPN hydrogels	Pan et al. (2018)
Aminated silver nanoparticles + gelatin +carboxylated cellulose nanofibers (CNF)	In situ (simultaneous) method	–	Non-covalent full IPN hydrogel	Stronger mechanical, self-recovery, antibacterial properties, satisfactory hemostatic performance, and appropriate balance of fluids on the wound bed (2093.9 /m^2 per day)	Liu et al. (2018)

(continued)

Table 2.2 (continued)

Polymers	Method	Drug	Type of IPN	Key outcome	References
Salecan (N-(3-dimethylaminopropyl) acrylamide-co-acrylamide)	Free radical polymerization	Amoxicillin	Non-covalent full IPN hydrogel	Amoxicillin showed better encapsulation into the developed hydrogels and released in a Salecan dose-dependent and pH-sensitive environment	Qi et al. (2017)
Acrylic acid (AA) + 2-acrylamido2-methylpropane sulfonic acid+starch	Sequential Michael-type addition	Memantine		Degradation of the hydrogel around 60% of its primary mass has suggested this as a promising pH-tunable, biodegradable candidate for control drug delivery vehicle	Ganguly et al. (2017)
Hydroxypropyl-β-cyclodextrin	Simultaneously with an emulsification/solvent evaporation process	–	–	Provided a potential ability for nanogels to incorporate a molecule that can form inclusion complexes	Moya-Ortega et al. (2012)
γ-cyclodextrin				Capability for sustained release	
Methacrylic acid + polyethylene glycol	–	Atorvastatin, theophylline	–	Showed higher efficacy in controlling the drug release	Qadri et al. (2015)
PEO+gellan gum	–	Sulpiride		Increasing the intestinal absorption of sulpiride to a greater extent than the marketed product in vivo	Hoosain et al. (2017)

Films: IPN-based films are used as piezodialysis membrane which are non-mosaic membrane. The most predominant application of IPN delivery system is the uralkyd/poly(glycidyl methacrylate)-based film which illustrated a better mechanical and tensile strength (Hoosain et al. 2017; Kim et al. 2007). Biodegradable collagen films or matrices have served as scaffolds for the survival of transfected fibroblasts (Rosenthal and Köhler 1997). IPN-based films which are prepared by the mixture of collagen and polyvinyl alcohol, cross-linked with glutaraldehyde vapor, show depot formulation for recombinant human growth hormones (Zakharchenko et al. 2010). In many animal models, after implantation of transfected cells, a long-term expression of the foreign gene has not been achieved (Zakharchenko et al. 2010). Suh et al. studied the graft copolymerization of type I atelocollagen onto the surface of polyurethane (PU) films treated with ozone (Park et al. 2000). It has been observed that they could enhance an attachment and proliferation of fibroblasts and growth of cells. An interesting use of thermoresponsive polymer films was shown by Zakharchenko et al. who prepared a belayed of PVCL on top of pNiPAAm with encapsulated magnetic nanoparticles (Ward and Georgiou 2011). At temperatures greater than the lower critical solution temperature (LCST), the films were flat and allowed for adsorption of nanoparticles, cells, or drugs onto the surface, upon cooling the films rolled up entrapping the absorbed particles which could then be released by heating again. This is a novel approach for the encapsulation and release of nanoparticles and cells with the addition of the magnetic particles allowing manipulation of the films by an external field (Zakharchenko et al. 2010) (Table 2.3).

Microspheres: Microspheres are novel IPN-based drug delivery system. They are free-flowing powder, which are small spherical particles made up of natural or synthetic polymers with particle size ranging from 1 to 1000 um in diameter (Lohani and Chaudhary 2012). Microspheres consist of core at which drug is entrapped inside and an outer layer of polymer is the coating material (Swapna et al. 2013). IPN microspheres are employed in drug controlled release and targeting application as they encapsulate a wide range of drugs; increased bioavailability, biocompatibility, and patient compliance; and sustained-release characteristics (Bhattacharya et al. 2013).

The hydrogel microspheres were formulated of polyvinyl alcohol and guar gum for controlling the release of nifedipine by emulsion cross-linking method for improving the treatment of severe hypertension (Soppimath et al. 2000). A microsphere interpenetrating polymer network of sodium alginate and polyvinyl alcohol was formulated by the emulsion cross-linking method in which glutaraldehyde is used as a cross-linker. This IPN was used for the controlled release of diclofenac sodium (Banerjee et al. 2010a). Interpenetrating polymer network microspheres were also used in prolonging the delivery of anti-cancer drug (Somya et al. 2015). The idea of developing mucoadhesive microspheres is that the formulation will remain on the surface for localized delivery of the drug, and the release of the drug will be close to the site of action with more improvement in the bioavailability (Alexander et al. 2011). IPN microspheres are formulated

Table 2.3 List of some prepared polymers through IPN-based films

Polymer	Method of preparation	Drug	Type of IPN	Key outcome	References
Chitosan + xanthan gum	Simultaneously	Amoxicillin	Hydrogel film	Indicated higher drug release in SGF than in SIF of amoxicillin	Thakur et al. (2014)
Sod. alginate + gelatin	Simultaneously	Azure B	IPN film	Showing controlled release of azure B, in opposite to non-IPN formula	Qadri et al. (2015)
Polyvinyl alcohol + polyacrylic acid	Simultaneously	Crystal violet	IPN film	Enhancement of swelling properties leading to higher control of release of substances	Yue et al. (2008)
Poly(acrylic acid-*co*-acrylamide) + *O*-carboxymethyl chitosan	–	Insulin	Hydrogel film	Enhanced loading capacity for insulin, and more than 90% of the insulin was released within 1 h	Yin et al. (2007)
Polyaniline + polyvinyl alcohol	–	Ammonium persulfate	Thin film		Honmute et al. (2012)

with xanthan gum and polyvinyl alcohol by emulsion cross-linked method to deliver the anti-inflammatory drug (Jain et al. 2011). Glutaraldehyde was used as a cross-linking agent. Theophylline, an anti-asthmatic drug, was successfully entrapped into it by changing the ratio of dextran-g-acrylamide and glutaraldehyde. The % encapsulation efficiency is in between 50 and 70. In vitro release studies of theophylline from these IPN at pH 1.2 and 7.4 dissolution media showed that slow release was elevated up to 18 h at 37 °C (Al-Kahtani and Sherigara 2009). Some of the IPN-based microspheres with their applications are shown in Table 2.4.

Tablets: An extended release IPN tablet can be formed from chitosan/Carbopol inter-polymer complex. IPN-based tablets are solid in nature and have great potential for anti-hypertensive action by blending with hydrophilic inter-polymer complexes or a hydrophobic waxy polymer (Ganguly et al. 2017). Kulkarni et al. formulated IPN tablets of sodium alginate and carrageenan for controlling the release of propranolol HCl. The pure drug demonstrated a rapid and complete

Table 2.4 Some examples of IPN microsphere preparation

Polymer	Method	Drug	Type of IPN	Key outcome	References
Sodium alginate + polyvinyl alcohol	Water-in-oil emulsion cross-linking	Diclofenac sodium	IPN microspheres	Drug entrapment efficiency (72%) was obtained depending upon concentration of cross-linking density	Banerjee et al. (2010b)
Gellan gum + Poly(N-isopropylacrylamide)	–	Atenolol	Semi-IPN microspheres	Release of atenolol was extended up to 12 h	Mundargi et al. (2010)
Sodium alginate + poly(vinyl alcohol)	Oil (W/O) emulsification method	Naproxen	IPN microspheres		Solak (2011)
Xanthan gum + superabsorbent polymers + poly(vinyl alcohol)	–	Ciprofloxacin HCl	IPN hydrogel microspheres	Sustained release of ciprofloxacin	Bhattacharya et al. (2013)
Chitosan + hydroxyethyl cellulose	–	Isoniazid	IPN blend microspheres	Improved the encapsulation efficiency from 50 to 66%	Angadi et al. (2010)
Hydroxypropyl-methylcellulose + poly(vinyl alcohol)	–	Ciprofloxacin	IPN microspheres	Show a sustained and controlled release of ciprofloxacin hydrochloride from the IPN microspheres up to 10 h	YerriSwamy et al. (2011)
Acryl amide grafted carboxymethylcellulose + sodium alginate	–	Triprolidine hydrochloride Monohydrate	IPN microspheres		Banerjee et al. (2012)
Gelatin + sodium carboxymethyl		Ketorolac	Semi-IPN Microspheres		Qadri et al. (2015)
Cellulose		Tromethamine			
Pullulan+ poly(vinyl alcohol)	Water-in-oil emulsion cross-linking method	Pirfenidone	Semi-IPN Microspheres	Controlled delivery of pirfenidone	Soni and Ghosh (2017)

(continued)

Table 2.4 (continued)

Polymer	Method	Drug	Type of IPN	Key outcome	References
Sodium alginate + guar gum	Water-in-oil emulsion cross-linking method	Zidovudine	hydrogel microspheres	Demonstrated 68% encapsulation efficiency	Eswaramma and Rao (2017)
				Release time enhancement up to 34 h in pH 7.4 at 37 °C	
Low-methoxyl (LM) pectinate +sterculia gum (SG)	Simultaneous ionotropic gelation	Ziprasidone HCl	IPN microspheres	Enhanced encapsulation of ziprasidone HCl (87.98 ± 1.15%)	Bera et al. (2015)
				Good mucoadhesivity with gastric mucosa	
Acrylamide-grafted chitosan + methylcellulose	Water-in-oil emulsion-cross-linking method	Ibuprofen	IPN microspheres	Release of IBU from microspheres decreased when the amount of CS-g-PAAm in the polymer matrix and amount of cross-linker added were increased, while it increased with the increase of the IBU/polymer ratio	Bulut (2016)

dissolution within 60 min, contrary to IPN tablets that showed slower and prolonged drug release over 18 h (Kulkarni et al. 2011; Rao et al. 2006). Some of the IPN-based tablets with their applications are shown in Table 2.5.

Sponges: IPN-based sponges are also used as drug delivery system. They were mainly used in wound dressings and hemostyptics and also very helpful in the treatment of severe burns (Chvapil 1982). The advantages are:

a) Their capacity to easily take up large quantities of tissue exudates and provides smooth adherence to the wet wound bed with preservation of moist climate.
b) Its protection against mechanical harm and secondary bacterial infection.

Capsules: IPN-based capsules are widely used for delivery of drug. IPN capsules are also used as drug delivery systems for sustained release of drug. Interpenetrating polymer network (IPN) hydrogel capsules consisting of polyacrylamide and polyvinyl alcohol are used for sustained drug release (Bon et al. 2007). Some of the IPN-based capsules with their applications are shown in Table 2.6.

Table 2.5 Some examples of IPN tablet preparation

Polymer	Method of preparation	Drug	Key outcome	References
Chitosan + tamarind seed polysaccharide	Simultaneous blending	Aceclofenac	Showing sustained release of aceclofenac over 8 h	Jana et al. (2014)
			Enhanced encapsulation efficiency up to 91.97 ± 1.30%	
Polyacrylamide grafted-sodium alginate + sodium alginate	Simultaneous	Diltiazem HCl	Sustained release of diltiazem HCl	Mandal et al. (2010)
	Wet granulation method			
Tamarind seed polysaccharide+ sodium alginate	–	Propranolol HCl	Prolonged drug release from tablets up to 21 h	Kulkarni et al. (2013)
			Increasing cross-linking time slows the release rate	
Sodium alginate + carrageenan	Wet granulation/ covalent cross-linking method	Propranolol HCl		Kulkarni et al. (2011)

Table 2.6 Examples showing some IPN capsule preparation

Polymer	Method of preparation	Drug	Key outcome	References
Poly(vinyl alcohol) + polyacrylamide	Bulk polymerization	Crystal violet + bromothymol blue	Higher drug release for semi-IPN than full-IPN.	Ramaraj and Radhakrishnan (1994)
Poly(dimethyl siloxane) + poly(2-hydroxyethyl methacrylate)	Monomer immersion method	–	Enhanced hydrophilicity Reduced protein adsorption for long-term use	Tang et al. (2011)
Poly(N-isopropylacrylamide) (PNIPAM) + calcium alginate	Concentric two-fluid nozzles (monomer immersion method)	Suspension of cocoa particle	Producing a thermosensitive IPN core–shell structure	Ochi et al. (2014)

2.20 Conclusion

Mixing of polymers and producing many new types of polymer mixture with sophisticated uses has become very common since the industrial era has invaded our time. IPNs have a synergistic effect as they consist of different types of polymers forming network together with one rubbery phase and one glassy phase, where phases remain separated even when subjected to stress. Many different types of IPN systems are formed as a result of diversity in the number and types of cross-links. IPNs have a significant adhesive property as it can be swelled in the medium without dissolving the solvent. IPNs showed a great impact in developing the controlled release system for delivering the drug for the required period of time with low fluctuation. There are specific criteria for choosing the IPN-forming polymers for production of intact network involving the presence of one polymer during synthesize or cross-linking of the other polymers without dramatic phase separation; both polymers should show same absorption, distribution, metabolism, and excretion routes in our bodies.

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Fabrication Technology of Chitosan-Based IPN: Drug Delivery Application

3

Ali Rastegari, Fatemeh Mottaghitalab, and Mehdi Farokhi

Abstract

Chitosan as a biocompatible and biodegradable polymer is a good candidate for preparation of interpenetrating polymer network (IPN) hydrogel with amino and hydroxyl groups. Chitosan-based IPNs can release their active ingredient in response to environmental stimuli like pH and temperature. This chapter describes the preparation of chitosan-based IPNs according to chemical or physical interactions to make semi- or full-IPN hydrogels. Furthermore, drug delivery applications of chitosan-based IPN hydrogel are discussed based on the delivery route.

Keywords

Chitosan · Interpenetrating polymer networks · Hydrogel · Drug delivery

3.1 Introduction

Interpenetrating polymer networks (IPNs) are the entangled polymers formed by interlacing the secondary polymers within the crosslinked networks. Single-network hydrogels have weak mechanical properties and also slow response to swelling and

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deswelling. The IPN hydrogels have advantages compared with single hydrogels and exhibited enhancement in the mechanical strength and swelling/deswelling response properties. Chitosan-based IPNs are three-dimensional and hydrophilic structures that retain large amounts of water and are useful as drug carriers. In general, hydrogels are categorized into single and multicomponent networks. The single-network hydrogels face weak mechanical properties that limit their biomedical applications. To overcome this limitation, multicomponent networks like IPN hydrogels have been developed through chemical or physical reactions like molecular entanglements and/or ionic bonds, hydrogen bonds, and hydrophobic interactions (Hoffman 2012; Peppas et al. 2000). Based on these types of physical interactions, they could be disintegrated in response to changes in environmental conditions such as ionic strength, pH, and temperature. Indeed, the volume and shape of IPN hydrogels are changed in response to environmental changes such as temperature and pH (Bajpai et al. 2008; Peak et al. 2013; Peng et al. 2008; Richter et al. 2008). The main advantage of IPN hydrogel over single-network hydrogels is good mechanical strength and swelling/deswelling responses.

IPN hydrogels are classified based on types of blending and/or method of preparation. There are two categories in the context of polymer blends including mechanical blends (no chemical bonds between the polymers) and graft copolymers (containing primary bonds between the polymeric components) (Zoratto and Matricardi 2018). Furthermore, preparation methods of IPN hydrogels are classified into simultaneous and sequential methods. The precursors of two networks are mixed for preparation of IPN hydrogels based on simultaneous strategy. In this method, both networks are synthesized at the same time by independent and non-interfering chemical ways (Fig. 3.1a) (Myung et al. 2008; Mark 2013; Wang and Liu 2013). However, for sequential method, the first network polymer is initially prepared and then swelled into a solution containing the monomer, initiator, and activator of other polymeric networks, with or without a crosslinker (Fig. 3.1b). The full-IPN hydrogel is formed when a crosslinker is present in the reaction to crosslink both components existed in the solution. But in the semi-IPN hydrogel, only one component is crosslinked and the other is not crosslinked and is just used as a liner polymer physically entrapped in a matrix of crosslinked polymer (Myung et al. 2008; Sperling 1991; Chivukula et al. 2006; Hoare and Kohane 2008; Dragan et al. 2012a, b; Yin et al. 2007) (Fig. 3.1c).

Chitosan (CS)-based IPN hydrogel is composed of a cationic polysaccharide b-(1–4)-2-amino-2-deoxy-D-glucopyranose and b-(1–4)-2-acetamido-2-deoxy-D-glucopyranose units and other polymeric components (Dragan 2014). CS-based IPN could undergo various modifications due to the presence of many amino and hydroxyl groups in their structure. Specifically, the presence of amino groups is essential for pH-dependent responsive materials (Hamman 2010). CS-based IPN hydrogels are categorized into semi- or full-IPN hydrogels and could be used as control drug delivery systems. They could encapsulate the drug molecules as control drug delivery system for releasing therapeutic agents at controlled rate for long period of time and also could release the encapsulated drugs in responsive to pH and temperature alteration (Hamman 2010; Berger et al. 2004).

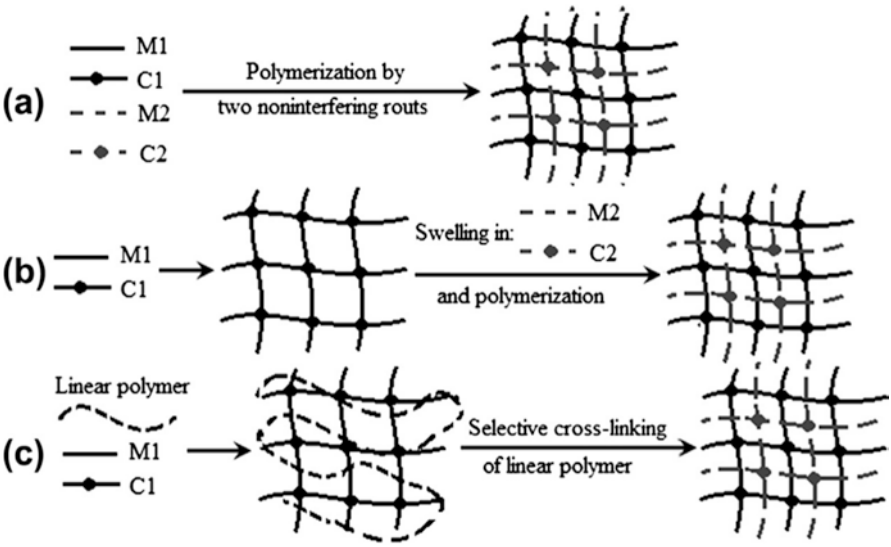

Fig. 3.1 Schematic representation of IPN hydrogel preparation. (**a**) Simultaneous strategy, (**b**) sequential strategy, and (**c**) selective crosslinking of a linear polymer entrapped in IPN. (Reprinted with permission from Dragan (2014))

In this chapter, we firstly discuss the most important preparation strategies of semi- and full-IPN hydrogels. After that, the importance of CS-based responsive carriers is described in detail. Finally, an overview of the applications of CS-based IPNs in drug delivery is explained.

3.2 Chitosan-Based Semi- and Full-IPN Hydrogels

CS-based IPN can be categorized in two main categories including semi- and full-IPN hydrogels. They have been widely used for drug delivery applications especially as a responsive and control released drug delivery system (Hamman 2010; Berger et al. 2004). Preparation of CS-based semi- or full-IPN hydrogels requires CS polymer, a crosslinker, and additional linear or branched polymeric components.

The intermolecular hydrogen bonds play an important role for preparation of CS-based semi-IPN hydrogel like intermolecular hydrogen bonds between CS with other polymers and polyether-based polymers, silk, polyethylene oxide (PEO), and polyethylene glycol (PEG) (Yao et al. 1993; Guan et al. 1996a; Wang et al. 2000; Guan et al. 1996b; Yao et al. 1998; Lee et al. 2000; Risbud et al. 2000). In the preparation process of IPN hydrogel, the added polymer can be crosslinked to have full-IPN hydrogel. The UV irradiation or a second crosslinker such as methylene bis-acrylamide could be added to the reaction for second crosslinking process;

however, adding the second crosslinker could decrease the biocompatibility of IPN hydrogels (Lee et al. 2000).

Drug release and mechanical strength of IPNs strongly depend on the density of crosslinking. This parameter is mainly influenced by the concentration of crosslinkers, molecular weight of CS, deacetylation degree (DD) of CS, and temperature of reactions (Mi et al. 2000; Knaul et al. 1999).

Numerous investigations have been performed to prepare CS-based semi- and full-IPN hydrogel. Ahmed et al. synthesized semi-IPN hydrogel composed of crosslinked CS and hydroxyethyl cellulose grafted acrylamide for increasing the loading efficiency of diclofenac sodium. In this study, glutaraldehyde (GA) was used as a crosslinker which increased the encapsulation efficiency of the drug to 83%. The release of diclofenac sodium was dependent on pH, the content of entrapped polymer, and degree of crosslinking. The results have shown that the percent equilibrium water uptake of IPN was related to the pH of the media. In acidic condition (pH of 1.2), the equilibrium swelling of IPNs was higher than in the media with pH of 7.4. This behavior might be attributed to the protonation of amine groups of CS polymer that lead to the repulsion of the polymeric chains and also the dissociation of secondary interactions by swelling. Furthermore, they observed that the equilibrium water uptake of IPN hydrogel was also related to the hydrophilic and hydrophobic nature of IPN hydrogels. The equilibrium water uptake was significantly increased by enhancing the hydrophilic parts of the IPN hydrogels (from 188% to 239%). Also increasing the amount of GA in the matrices decreased the equilibrium water uptake (245% to 150%) at pH 1.2, while at pH 7.4, it decreased from 295% to 170%. The reduction of water uptake could be related to the formation of a rigid network structure by using high amounts of the crosslinking agent. Moreover, the value of diffusion coefficient (D) of the prepared IPN hydrogels was dependent on the extent of crosslinking agents in the polymeric matrix. Indeed, the D value decreased with enhancement of GA content, as a crosslinker agent. The free volume of the polymeric matrix of IPN hydrogel was decreased by increasing the amount of crosslinker. Therefore, it could hinder the diffusion of small molecules through the IPN matrix. The researcher mentioned that the D value was higher at pH 7.4 in comparison to the acidic medium. Indeed, more solvent molecules could be transported through the hydrogel matrix in the basic media (Ahmed et al. 2009). GA is usually used for CS crosslinking because it facilitates the Schiff base between $–NH_2$ groups of CS and aldehyde groups of GA. However, it is very toxic with limited use for drug delivery applications. Alternatively, genipin can be used as a natural crosslinker for preparing CS-based IPN hydrogels with no cytotoxic effects (Khurma and Nand 2008; Cui et al. 2014; Muzzarelli 2009; Yuan et al. 2007). Cui et al. developed CS/gelatin full-IPN hydrogel by using genipin. The obtained results exhibited that CS/gelatin IPN hydrogel had maximum adsorption capacity for removing the acid orange II as an anionic dye from the medium when the genipin content was 0.25 mmol/L. Furthermore, the full-IPN hydrogel displayed pH-sensitive behavior and rapid response in adsorption and desorption with pH alterations. The adsorption abilities of chitosan and gelatin have shown to be related to the amino groups (NH_2) on chitosan and gelatin molecules. They could

be transformed into the primary amino groups (NH^{3+}) in the acidic solution by making electrostatic interactions with sulfo (SO^{3-}) groups of acid orange II. Their results have shown that the adsorption ability of chitosan and gelatin was attributed to the number of free amino groups; low crosslinking with genipin enhanced the number of free amino groups. Moreover, the porous structure of the IPN hydrogel influenced the adsorption properties. They found that with an increase in the genipin content, the morphology of the pores on the surface was changed from open to close and the pore size was also decreased, gradually (Cui et al. 2015).

The semi-IPN hydrogels could be used as ion responsive systems. In this manner, the semi-IPN hydrogel is prepared in the presence of CS polymer and a polyelectrolyte (Wang et al. 1998; Chen et al. 2005; Kim et al. 2005; Povea et al. 2011; Lee et al. 1999). Accordingly, Wang et al. fabricated a semi-IPN composed of crosslinked chitosan (cr-CS) and poly(acrylic acid) (PAA) by making electrostatic interaction between NH_3 groups of CS and COO^- groups of PAA. The swelling ratio of CS/PAA IPN hydrogel was in the maximum capacity at acidic region (lower than pH = 2). The swelling of prepared semi-IPN in the acidic region can be related to the dissociation of ionic crosslinks. The swelling degree of the prepared semi-IPN increased with increasing the ionic valence of solution. The maximum swelling degree was observed in the solution of trivalent salts (Al^{3+}) and the minimum swelling degree exhibited in the solution of monovalent salts (K^+, Na^+). This behavior could be attributed to the different dissociation degree of electrostatic interaction when the prepared semi-IPN hydrogel swelled in various valent salt solutions (Wang et al. 1998).

Crosslinking polymerization of nonionic monomers in the presence of CS is also used for preparing semi-IPN hydrogels. The most applicable monomers are acrylamide (AAm), N-isopropylacrylamide (NIPAAm), N,N-dimethylacrylamide, and 2-hydroxyethyl methacrylate (HEMA). This type of IPN hydrogels is applicable to control release systems. Dragan et al. prepared a semi-IPN composed of CS and PAAm and epichlorohydrin (ECH) for selective crosslinking of CS. The water transport of these IPNs was based on Fickian diffusion (15, 17). Recently, a semi-IPN hydrogel based on nanocellulose, CS, and GA was developed for drug delivery and tissue engineering. Firstly, the cellulose nanocrystals (CNCs) were extracted from microcrystalline cellulose by sulfuric acid hydrolysis. Then the nanocrystals were homogenized through ultrasonication and CS was added and crosslinked with GA to form Schiff base linkages. SEM analyses showed that CNCs were uniformly distributed within the CS polymeric matrix. Adding CNCs (up to 2.5%) to CS hydrogels improved the maximum compression of the hydrogel to form semi-IPN (Sampath et al. 2017).

3.3 Chitosan-Based IPN Microspheres

Microspheres are particles with size 0.1 to 100 μm that could be used for drug delivery applications. Polymeric microsphere composed of a biodegradable polymeric matrix that can encapsulate many types of drugs and proteins with ability to

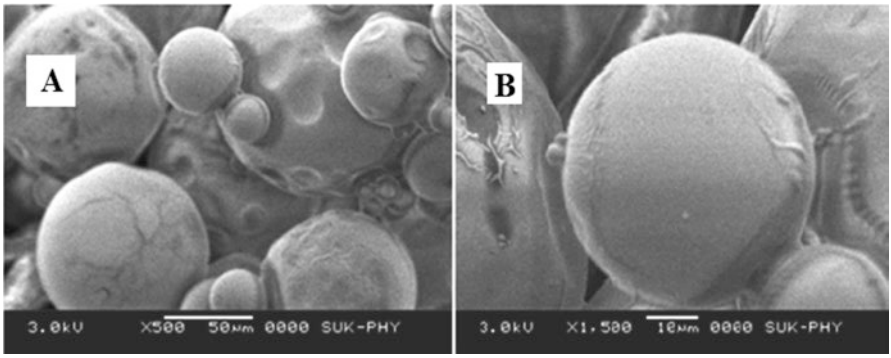

Fig. 3.2 Scanning electron micrographs of (**a**) group of IPN microspheres and (**b**) single IPN microspheres comprising CS and hydroxyethyl cellulose. (Reprinted with permission from Angadi et al. (2011))

control the release rate of them. CS-based IPN microspheres are useful for drug delivery applications. An IPN microsphere comprising CS and hydroxyethyl cellulose crosslinked with GA was prepared for loading isoniazid (Fig. 3.2) (Angadi et al. 2011). The IPN microspheres are more swollen in acidic pH solution (pH = 1.2) since CS and gel have positive charges in acidic pH and they repel each other. The prepared IPN microsphere controlled the release of isoniazid in a sustained manner up to 30 h. Formulations with low amount of GA (5 ml) have shown higher release than high amount of GA (7.5 ml) that could be related to crosslinking density of formulations.

In another study, CS/polyvinylpyrrolidone (PVP) semi-IPN microspheres were developed by water/oil emulsification method for delivering 5-fluorouracil (5-FU). At first, CS was crosslinked with GA and then PVP polymer was entrapped in the matrix of CS. By enhancing the concentration of PVP in the structure of microsphere and changing the ratio of CS/PVP from 1:0.33 to 1:1, the release rate of 5-FU was increased from 39 μg to 79 μg that related to enhancing of porosity with increasing of PVP in the formulations. In the regard of tensile properties, the mean ultimate tensile strength (UTS) value of CS/PVP (1:1), CS/PVP (1:0.5), and CS/PVP (1:0.33) was 66.40 MPa, 108.50 MPa, and 118.20 MPa, respectively. According to the results, the UTS value increased when the amount of PVP decreases in the formulation. This behavior could be attributed to disturbing of CS crystallinity by adding PVP and thus caused to decreasing the UTS values (Ozerkan et al. 2013). CS-based semi-IPN microspheres are also used in blend with other polymers such as PEO-g-acrylamide, acrylamide-dextran, and methylcellulose (Agnihotri and Aminabhavi 2006; Rokhade et al. 2007, 2009). For example, CS-poly(ethylene oxide-*g*-acrylamide) semi-IPN microspheres were prepared by emulsion crosslinking using GA to make a sustained release system for capecitabine. Encapsulation efficiency of capecitabine was 87% and in vitro release of the drug was sustained during 10 h. The release rate of the drug was dependent on the amount of GA for crosslinking. The release kinetics was slower for formulations with higher amounts of GA in the

matrix. The higher crosslinking decreased the free volume of the matrix. Higher crosslinking also reduced the rate of swelling and drug release from the matrix. Furthermore, they performed dynamic swelling experiments. The obtained results have shown that the swelling capacity of IPN hydrogels was decreased when using higher amounts of GA. This could be related to formation of a highly crosslinked rigid network. However, the swelling capacity of the prepared IPN hydrogels was decreased by enhancing the amount of graft copolymer into the polymeric matrix. The main reason for decreasing the swelling capacity seems to be related to the formation of a rigid semi-IPN structure. In this condition, the amount of chitosan into the polymeric network was decreased and consequently reduced the swelling ration of IPN hydrogel. The polyelectrolyte nature of chitosan induces high osmotic pressure and therefore a high swelling occurs due to enhancing the translational entropy of counterions (Agnihotri and Aminabhavi 2006).

3.4 Smart Chitosan Carriers

3.4.1 pH-Responsive Systems

Ionizable polymers with pKa value in the range of 3–10 could be used for preparation of pH-responsive drug delivery systems. There are three polymeric categories as pH-responsive polymers including polyacids (containing acidic groups like carboxylic acid), polybases (containing basic groups like amines), and natural polymers (CS, dextran, and hyaluronic acid). The pH value of human organs is different and it will be changed in disease conditions like infections, inflammations, ischemia, and cancers (Gerweck and Seetharaman 1996; Ganta et al. 2008). Thus, pH-sensitive polymers are capable of respond to variations in pH in different organs.

The primary NH_2 groups, OH groups, and other functional groups in the structure of CS are responsible for pH value detection (Carreira et al. 2010). The primary amines of CS accept the protons at low pH and become positive at pH below their pK_b and therefore could be used as a pH-responsive controlled release system (Zhai et al. 2012). Indeed, the repulsion between ion species induces the swelling of CS IPN in low pH. The conformational transition of hydrogels from collapsed to swollen states mainly occurs at ionization state as a consequence of osmotic pressure exerted by the mobile counterions neutralizing the ionic charges (Philippova et al. 1997).

The CS-based IPN hydrogels prepared from polyelectrolytes containing carboxylic groups enhanced the mechanical properties of hydrogels due to ionic interactions between -NH_3 groups of CS and -COO groups of anionic polyelectrolyte. The ionic interaction also decreases the swelling degree of hydrogel by enhancing the crosslinking density of hydrogel. For example, CS/poly(methacrylic acid) (PMAA) semi-IPN hydrogel had the highest swelling capacity at pH = 2.3; by increasing the pH to 4.5, the degree of swelling was decreased gradually due to formation of more ionic bonds between $–NH_{3+}$ in CS and $–COO^-$ in PMAA that enhanced the crosslinking density of the hydrogel (Chen et al. 2005; Milosavljević et al. 2011; Risbud et al. 2001).

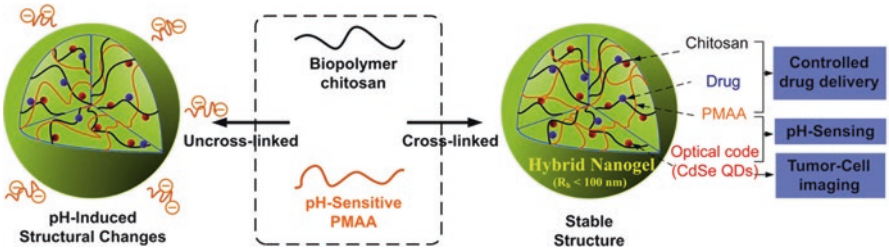

Fig. 3.3 Schematic representation of the concept for designing multifunctional CS/PMAA/CdSe semi-IPN hydrogel and its potential extending applications in biomedical field. (Reprinted with permission from Wu et al. (2010))

In one study, cadmium selenide (CdSe) quantum dots (QDs) were immobilized on CS-poly(methacrylic acid) (CS-PMAA) semi-IPN hydrogel for optical cell imaging and controlled drug delivery (Fig. 3.3). QDs and temozolomide were entrapped into the pH-sensitive IPN hydrogel. The results showed the covalent crosslinking of PMAA with CS made high colloidal and structural stability and also good responsiveness to pH variation. The drug release was examined in buffer solutions with different pH values (5.03, 6.17, 6.67, and 7.38) at 37 °C. The results showed that enhancement of pH induced a gradual dissociation of the -COOH groups to form $–COO^-$ groups, which caused to breaking the PMAA-TMZ hydrogen bond complexes and therefore increased drug mobility (Wu et al. 2010).

3.4.2 Temperature-Responsive Systems

The gelling and swelling properties of temperature-responsive IPN hydrogels can be activated by changing the temperature. These polymeric systems have temperature-dependent phase transitions at either an upper critical solution temperature (UCST) or at lower critical solution temperature (LCST) (Gandhi et al. 2015). In this context, the CS polymer could be copolymerized with one or more temperature-responsive polymers including methylcellulose, PVA, PEO, PAA, PNVP, N-vinyl caprolactam (NVCL), and N-substituted polyacrylamides to form IPN hydrogels (Jain et al. 2013). IPN hydrogels containing these polymers are characterized with temperature-dependent sol-gel transition (Tgel) which is related to LCST polymer and also temperature-dependent gel-sol transition (Tp) which is related to UCST polymer. The conformation of these polymers is changed following the change in solubility due to interactions between solvent and polymeric chain. For example, the thermosensitive poly(NIPAM-co-NVP)/CS semi-IPN hydrogel was prepared for increasing the loading capacity of naproxen as an anionic drug. The CS semi-IPN networks improved the swelling behavior and provided high affinity for naproxen. The drug release was suppressed at acidic pH (2.2–5.0) which was increased at pH 7.4 due to deprotonation of amino groups in CS. The semi-IPN hydrogels showed a continuous release of drug without burst release with enhancing the temperature above LCST. Increasing the temperature above LCST

resulted in PNIPAM shrinkage and restraining the release of drug (Jagur-Grodzinski 2010; Ghaz-Jahanian et al. 2015; Chenite et al. 2000).

In one study, an injectable and thermo-reversible CS-PEG copolymeric IPN hydrogel was prepared for controlling the release of bovine protein as a model drug. The copolymer was an injectable liquid at low temperature and transformed into a semisolid hydrogel in body temperature when 40 wt. % of PEG was covalently grafted to CS chains. Indeed, CS-PEG IPN hydrogel was viscous liquid below the transition temperature that could be injectable through a 20-gauge needle. When the solutions were heated to the body temperature, the prepared IPN hydrogel was transformed into gels. An initial burst release of bovine protein was observed in the first 5 h and after that a steady linear release was achieved for a period of ~70 h. They also investigated the effect of hydrogel environment on the structure of encapsulated protein particularly protein denaturation, aggregation, hydrolysis, and reaction with the crosslinking agents. It was found that all the performed conditions decreased the activity of loaded protein in the hydrogels. In all the experiments, the release of bovine protein from the hydrogels, whether crosslinked with genipin or not, was evaluated during 3 days which was consequently compared with an original bovine protein in solution. The obtained results showed that most of the released protein from the IPN hydrogels, whether crosslinked with genipin or not, was monomer and therefore demonstrated that the majority of bovine protein retained their integrity and structure (Bhattarai et al. 2005). Zhou and coworkers also developed a thermo-responsive IPN hydrogel with CS and αβ-glycerophosphate (αβ-GP). They investigated the effect of degree of deacetylation (DDA) and molecular weight of CS polymer on release of Adriamycin and 6-mercaptopurine. They showed that the thermosensitive properties, appearance, and structure of IPN hydrogel were affected by concentration, molecular weight, and DDA of CS polymer. CS polymer with molecular weight of 1360 kDa and DD 75.4% with concentration 2% was the optimal condition for preparation of CS-αβ-GP thermosensitive hydrogel. It was shown that increasing the molecular weight of CS decreased the release rate of Adriamycin and 6-mercaptopurine. About 60–70% of Adriamycin (hydrophilic drug) was released during 24 h which was slower than 6-mercaptopurine (hydrophobic drug) (Zhou et al. 2008).

3.4.3 pH- and Temperature-Responsive Systems

Recently, several pH- and temperature-responsive systems have been developed by using poly(isopropyl acrylamide-*co*-acrylic acid) (PNIAA) as pH- and/or temperature-responsive material (Guo and Gao 2007; Chen et al. 2007). CS-based dual pH- and temperature-responsive semi-IPN polyampholyte (polymeric systems composed of monomers of varying charge) hydrogel systems were prepared for developing pH/temperature oral drug delivery systems. The semi-IPN hydrogel was synthesized using carboxymethyl CS and PNIPAM using *N*,*N*-methylenebisacrylamide as a crosslinking reagent. Coenzyme A was loaded on semi-IPN hydrogels which exhibited 22.60% cumulative release in pH 2.1 and 89.10% in pH 7.4 at 37 °C within 24 h. Additionally, the release rate was higher

at 37 °C compared with 25 °C in pH 7.4 (Guo and Gao 2007). Some studies have shown CS/PVA and PVA/CS/PAA are useful for formation of dual pH- and temperature-responsive IPN hydrogel (Zhang et al. 2007; El-Sherbiny et al. 2006; Abdelaal et al. 2007). For example, a full-IPN hydrogel composed of PVA/CS/PAA exhibited fast swelling properties within 5 h. The swelling ratio of IPN hydrogels was significantly increased by changing the pH value from 2 to 10 (Zhang et al. 2007).

In a study, CS-PEG-PNIPAAm (or poly(N-isopropylacrylamide) (CPN) hydrogels were prepared as temperature- and pH-sensitive systems for biomedical applications. The designed hydrogels were prepared by physical crosslinking CS/PEG/PNIPAAm by dissolution/evaporation method. CPN hydrogel had an LCST around 32 °C because of using PNIPAAm. The maximum swelling was obtained at pH 3 which decreased by increasing the pH. The high swelling in acidic condition was related to the protonation of amino groups of CS (Sun et al. 2008). Swelling degree of hydrogel was increased slightly by increasing the molecular weight of PEG. However, increasing the concentration of PEG and NIPAAm had a negative effect on swelling degree of IPN hydrogel at room temperature.

Recently, a triple pH-, temperature-, and salinity-responsive semi-IPN hydrogel (PCAMO) was prepared for controlled release of bovine serum albumin (BSA) and 5-FU. The IPN hydrogel was prepared based on concurrent free radical polymerization of acrylic acid (AA), oligo(ethylene glycol) methacrylate (OEGMA), and 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) combined with CS polymer (Fig. 3.4). The mechanical and swelling properties of hydrogel were affected by the content of AA and MEO_2MA. The hydrogel with short hydrophobic side chains formed a more compact structure that hinders the mobility of the hydrogel chains and water absorbency. Indeed, the swelling ratio gradually increased with increasing hydrophilic monomers of AA and MEO_2MA that could be related to the hydrogel affinity for water. The amount of drug release was relatively low in acidic condition (pH 1.2) but it was high in neutral condition (pH 7.4). Furthermore, the drug release rate was slower at 37 °C compared with 25 °C. The obtained results confirmed the potential of smart semi-IPN hydrogel for using as a site-specific oral drug delivery system in the intestine and colon (Che et al. 2018).

Sokker and coworkers developed a temperature- and pH-responsive hydrogels consisting of CS grafted PAA, poly(hydroxypropyl methacrylate) (PHPMA), PVA, and gelatin. The IPN hydrogel was synthesized by gamma irradiation technique with degree of gelation over 90%. The degree of gelation was increased as the content of PVA, PAA, and CS was increased or in the absence of PHPMA. Also with enhancement of gamma irradiation dose, the degree of gelation was slightly changed. High degree of gelation was related to the chemistry of CS. The CS polymers have large quantities of amino groups in its structure and therefore a polyelectrolyte complex will form with PAA, PVA, and gelatin. Furthermore, increasing the irradiation dose formed high degree of breaking and crosslinking of the double bonds located in the vinyl monomers. Increasing the pH improved the swelling degree of IPN; the highest swelling of IPN hydrogel was observed at pH 9. The prepared IPN hydrogels with different network structures and composition ratios

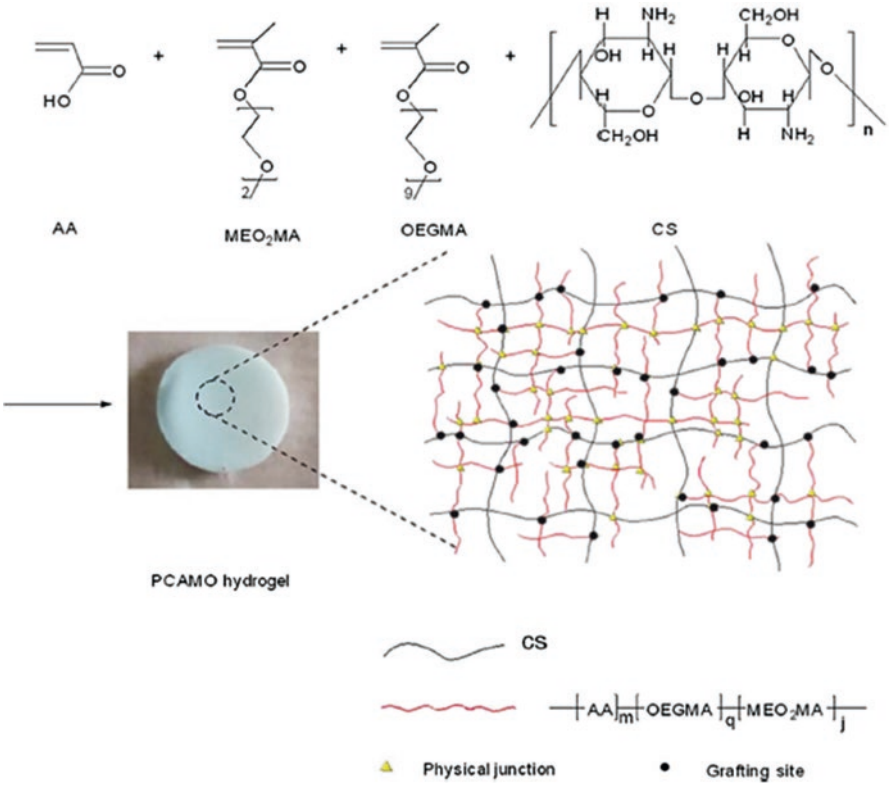

Fig. 3.4 Schematic preparation of PCAMO hydrogel. (Reprinted with permission from Che et al. (2018))

showed different swelling percentages. The results showed that the swelling percent was low when high amount of hydrophobic polymer (PHPMA) was used in the structure of hydrogel. In this condition, the hydrophobic segments of the chain tend to aggregate and therefore low swelling (%) in the presence of PHPMA Cs 1 and Cs 2 was achieved. This is due to its hydrophobic nature, where most of the hydrophobic segments of the chain tend to be aggregated, and therefore the water formed hydrogen bonds with the polar groups that were located on the surroundings and decreased the swelling ratio of the hydrogel. However, the swelling degree was increased when the amount of CS, PAA, and PVA was enhanced in the structure of hydrogel. Results showed that the release of oxytetracycline was increased within time and increasing the pH (maximum after 48 h at pH 9). The drug release rate from the IPN hydrogel was increased with enhancing the percentage of drug loading but the percentage of drug loading was not significant among different formulations except in the presence of PHPMA (as a hydrophobic segment) where the drug release was reached to the maximum rate. Also, the higher percentage of drug loading forced the drug particles on the surface of the polymeric matrix of IPN hydrogel to touch each other. In this condition, the large cluster of drug particles was extended

from the surface of matrix into the core of matrix. Upon dissolution of the drug molecules from the polymeric matrix, connected pore spaces were created and therefore the percentage of drug release was increased. The drug release also was affected by pH of the medium in which the release rate of the drug was drastically enhanced by changing the pH from 2 to 9. This finding could be related to the hydrolysis of ester linkage, which connects hydrogel networks and therefore opened the channels into the IPN hydrogel matrix. This condition caused the drug molecules to freely locate in the inner core of the hydrogels and release to the surroundings. Interestingly, the radiation dose of gamma also influences the release rate of the drug loaded into the polymeric matrix of IPN hydrogel. Indeed, high radiation dose during the process of IPN hydrogel preparation increased the crosslinking into the IPN hydrogel network that enhanced the rate of the drug release. Thus, the surface of the polymeric hydrogels became soft and smooth and the surface showed a lot of pores. These types of inner cavities and pores will be more pronounced with increasing crosslinking (Sokker et al. 2009).

3.4.4 pH and Ionic-Responsive Systems

Blending CS polymer with polyacids produce pH- and ionic-responsive systems. These types of IPN hydrogels are susceptible to dissociations by any change in pH and ionic strengths of the environment (Chen and Cheng 2008). CS derivations like carboxymethyl CS have numerous -COO and -NH_2 groups that produce excellent pH and ionic responsiveness capability. Zhai et al. developed pH and ionic strength-responsive polyampholyte carboxymethyl CS-based IPN hydrogels through blending carboxymethyl CS (CM-CS) and gelatin by GA. The hydrogel showed good responsive to pH and ionic strength since to have -COO and -NH2 groups. It seems that the most important factor to regulate the swelling behavior of the hydrogel was related to redistribution of mobile ions and related osmotic pressure difference (Zhai et al. 2012).

3.5 Drug Delivery Routes of CS-Based IPN Hydrogels

Drugs are incorporated into CS-based IPN hydrogels by two approaches. The first way relies on mixing polymeric IPN hydrogel with the drug molecule and an initiator in the presence or absence of a crosslinking agent (incorporation method). In the second approach, the hydrogel is firstly formed and allowed to swell in drug solution to induce drug absorption on the surface of the hydrogel (incubation method). The incorporation method faces major limitations because of the changes in the properties of the drugs during the polymerization process and difficulties for purifications process. The incorporation method is appropriate for water-soluble drugs. The loading in this approach relies on swelling degree of hydrogel in water (Kawashima et al. 1985). However, the water-insoluble drugs could be loaded on CS-based IPN hydrogel by multiple emulsion technique. For this, the drug

molecules are firstly dissolved in the suitable solvent and then emulsified in CS solution to form emulsion (oil in water emulsion). The obtained tiny drops become hydrogel and hardened with using a proper crosslinking agent (Rokhade et al. 2006). CS-based IPN hydrogels are applied via oral, colon, transdermal, ocular, and nasal drug delivery systems. In the following sections, we will discuss the most relevant drug delivery systems based on CS IPN hydrogel in detail.

3.5.1 Oral and Colon Drug Delivery

Oral drug delivery is a versatile, cost-effective method with high patient compliance for drug administration. In oral drug delivery approach, a drug should overcome several barriers before reaching to the systemic blood circulation. In the gastrointestinal tract, the drug molecules (peptides or nucleic acids) could be degraded by digestive enzymes. However, the short residence time of drug in the intestine restricts the use of oral routes (Crini and Badot 2008; Sun et al. 2011).

CS polymer has mucoadhesive properties and therefore could be used as a proper drug carrier in oral drug delivery approaches. Its mucoadhesive properties can be related to the ionic interactions between the cationic amino groups of chitosan and negative functional groups of sialic acid of the mucus in the gastrointestinal tract. CS-based IPN hydrogels are useful for improving the drug bioavailability and its delivery to the stomach, small intestine, and colon. In general, pH-sensitive IPN hydrogels are mainly used for preparing oral drug delivery systems because they mediate the release of drug in different pH values of the stomach and intestine. Moreover, the low swelling capacity of polycationic IPN hydrogels at neutral pH avoids the release of drug in the mouth. Studies have shown that polyionic IPN hydrogel could serve as suitable candidates for amoxicillin and clarithromycin site-specific delivery into the stomach (Chang et al. 2009; Gisbert et al. 2006). In a study, pH-sensitive CS-based IPN hydrogel composed of CS and poly(g-glutamic acid) was loaded with amoxicillin for treating *Helicobacter pylori* (*H. pylori*) infection in the peptic ulcer disease. The results of confocal microscopy demonstrated that the developed IPN hydrogel could permeate the cell-cell junction and released the drug in the site of bacterial infection which is located in the intercellular spaces. Indeed, the hydrogel firstly adhered to the gastric mucosal surface in the acidic environment (pH = 6) and then swelled and released the drug at the higher pH (pH = 7) of the gastric mucosal surface that is the site of *H. pylori* infection on the gastric (Chang et al. 2009).

Patel et al. fabricated a semi-IPN hydrogel composed of crosslinked CS and PEO that exhibited more swelling in the stomach compared with the intestine. The obtained results seem to be related to cationic properties of CS in gastric fluid with acidic pH. The IPN hydrogel exhibited good functionality for delivery of amoxicillin and metronidazole (Patel and Amiji 1996). Wang et al. fabricated a pH-sensitive hydrogel composed of chitosan-g-poly(acrylic acid)/attapulgite/sodium alginate (CS-g-PAA/APT/SA) loaded with diclofenac sodium. The hydrogel had good pH sensitivity. Indeed, the swelling ratio of hydrogel significantly increased to 42.5

when the pH enhanced from 5.0 to 8.0. This behavior seems to be related to -COO groups that converted to –COOH groups in acidic condition and form hydrogen bonds which is responsible for the small swelling ratio. In the basic regions, most of –COOH groups changed to –COO groups and therefore the hydrogen bonds dissociated and enhanced the swelling ratio. The cumulative release ratio of diclofenac sodium from the IPN hydrogel was 3.76% in pH 2.1 and 100% in pH 6.8 within 24 h. Additionally, the cumulative release of diclofenac sodium reached 100% in pH 7.4 within 2 h. However, when 10 wt% APT was added to hydrogel matrix, the release time prolonged to 12 h. This may be attributed to the absorbance of drug on APT that need to migrate out of the hydrogel through a longer path (Wang et al. 2009).

Many efforts have been made for delivering insulin by CS-based IPN hydrogels. In this regard, Yin and coworkers developed a superporous IPN hydrogel containing poly(acrylic acid-co-acrylamide)/O-carboxymethyl CS for oral delivery of insulin. The designed system exhibited good enzymatic inhibition capacities which notably enhanced the paracellular permeability of insulin in Caco-2 cell monolayers and in rat intestine model by 4.9 and 4.2 folds, respectively. The results of oral delivery of insulin in rat intestine showed good retentive properties for more than 8 h. In their study, the release rate of insulin was increased when the pH was enhanced from 1.0 to 6.2. In acidic pH, the carboxylic groups of the polymeric chains were protonated which leads to shrinkage of the IPN hydrogel. So, hydrogen bonds were formed between insulin and polymeric chains which restricted the release of insulin release. However, when pH elevated the carboxylic groups in the polymeric chains, they were partly ionized and significantly swelled the IPN hydrogel and insulin was easily released from the IPN hydrogels. The obtained results demonstrated that the ionic strength influenced the release of insulin from IPN hydrogel. In this context, 60% and 99% of the insulin was released within 2 h under the ionic strength of 1 and 0.1 M, respectively. However, no insulin was released when the ionic strength was lower than 0.01 M. Indeed, under the ionic strength of 0.1 M, the IPN hydrogel was swelled and rapidly released the insulin. However, when the ionic strength was lower than 0.01 M, the insulin was not dissolved and therefore it was not released (Yin et al. 2008).

A pH-sensitive IPN hydrogel composed of N,O-carboxymethyl CS (NOCC) and alginate crosslinked with genipin was also developed for site-specific protein delivery into the intestine (Chen et al. 2004). In this study, BSA was added to the dissolved NOCC/alginate solution with continuous stirring. The release of BSA was relatively low at pH 1.2 but it significantly increased at pH 7.4. This release pattern might be related to formation of hydrogen bonds between carboxymethyl CS and alginate at pH 1.2 which restricted the swelling capacity. Moreover, the electrostatic repulsion between the ionized acid groups at pH 7.4 increased the swelling capacity of IPN hydrogels (Chen et al. 2004).

Recently, colon-specific drug delivery systems have received good attention for the systemic delivery of protein and peptide drugs. This route of drug delivery is suitable for treatment of chronic diseases like irritable bowel syndrome, inflammatory bowel disease, and ulcerative colitis. For example, IPN hydrogel of alginate-chitosan (ALG-CS) was prepared based on Ca^{2+} or dual crosslinking with various

proportions of alginate and CS. The IPN hydrogel was used for colon-specific delivery of BSA. The researchers investigated the sustained release profiles of single and dual crosslinked IPN hydrogels in simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and simulated colonic fluid (SCF). In SGF, Ca^{2+} single crosslinked showed fast release and the cumulative drug release percentage was about 80% during 4 h. However, dual crosslinking limited the drug release to 3% within 8 h. Conversely, in SIF and SCF, the Ca^{2+} single crosslinked beads were disrupted earlier; but the dual crosslinked hydrogels exhibited a higher cumulative release. This study showed that dual crosslinked hydrogel has good potential for small intestine or colon site-specific drug delivery (Xu et al. 2007).

Lai and Shum fabricated a compatible IPN hydrogel with gastrointestinal site composed of CS polymer. The CS polymer was copolymerized with hypromellose via 1,1'carbonyldiimidazole to form a water-soluble and nontoxic copolymer (hypromellose-graft-CS). The complex copolymer with carboxymethylcellulose (CMC) generated an IPN hydrogel with high pH buffering capacity to provide a pH-stable environment for drug delivery. Furthermore, the IPN hydrogel showed a drug encapsulation efficiency over 90% for mometasone, tetracycline, and metronidazole. The results indicated that CS-based IPN hydrogel has a good potential for localized drug delivery to the intestinal environment (Lai and Shum 2015).

3.5.2 Transdermal Drug Delivery

Transdermal drug delivery is a favorite route for delivery of therapeutic agents because it avoids the first pass metabolism effect and facilitates the delivery of macromolecules and drugs at lower dose during a larger period of time. CS-based IPN hydrogel membranes have shown a good capability for delivering drug molecules through skin (Viyoch et al. 2005). IPN hydrogel patch composed of crosslinked CS-starch was developed for controlled release of natural alpha-hydroxy acid (AHA). CS with molecular weight 100 kDa was blended with corn, tapioca, or rice starch in various ratios and then crosslinked with GA. The following formulations provided flexible and elastic patches with good bioadhesive properties (formulation of CS/corn starch with ratio 4.5/0.5 by using GA 0.02% w/w or 0.04% w/w, formulation of CS/tapioca starch with ratio 4.5/0.5 with GA 0.04% w/w or 0.05% w/w, formulation of CS/rice starch with ratio 4.5/0.5 with GA 0.04% w/w, and formulation of CS/rice starch with ratio 4.0/1.0 with GA 0.03% w/w). Also, the drug release pattern from the hydrogel patches was based on Higuchi's model and was proportional to a square root of time (Viyoch et al. 2005).

CS-based IPN hydrogel could be also used for drug delivery of lipophilic and hydrophilic drugs. Thacharodi and Rao developed an IPN hydrogel composed of CS and collagen for forming membrane permeation-controlled transdermal patches for delivery of nifedipine and propranolol hydrochloride. Nifedipine and propranolol were released about 9.5 mg and 5 mg, respectively, from the patches through the full-thickness skin in a near-zero order fashion during 24 h. They showed that the IPN hydrogel membrane could be useful for delivery of both lipophilic and hydrophilic

drugs (Thacharodi and Rao 1996). Zhou et al. also prepared a semi-IPN hydrogel by UV irradiation of water-soluble N-carboxymethyl chitosan (CECS) and 2-hydroxyethyl methacrylate (HEMA) in the presence of D-2959, as a photo-initiator. SEM results showed that semi-IPN hydrogels displayed porous surface. The swelling degree was improved by increasing the CECS content and the results showed that semi-IPN hydrogels reached equilibrium within 25 h. Increasing the amount of CECS improved swelling ratios. This phenomenon could be related to the formation of high amount of loosely crosslinked networks of polymers by enhancing the amount of CECS. Indeed, the crosslink density of poly(HEMA) was decreased by increasing the amount of CECS and thus the molecular entanglement was weakened between CECS and poly(HEMA) which improved its water absorbing ability. This study demonstrated the effectiveness of CECS/poly(HEMA) hydrogels as a transdermal drug delivery matrix or wound dressing materials (Zhou et al. 2009).

Recently, a pH-responsive hydrogel based on carboxymethyl cellulose (CMC)/2-hydroxyethyl acrylate was prepared for transdermal delivery of naringenin (NRG) for treatment of atopic dermatitis. The hydrogel was developed by radical polymerization. The results showed that the swelling ratio at pH 7.5 and 8.5 was greater than at pH 5.5. These results were related to the ionization of the carboxyl group of CMC. Indeed, at pH 5.5, the carboxyl groups were protonated and strong hydrogen bonds between them caused dominant polymer-polymer interactions over polymer-water interactions. However, at pH 7.5 and 8.5, the carboxyl group ionized and the electrostatic repulsion between them extended the network further and increased the swelling ratio of the hydrogel. The drug release from prepared hydrogel was examined at pH 5.5 (the normal skin pH), 7.5 (the acne skin pH), and 8.5 (the atopic skin pH). According to the results, the cumulative release of NRG at pH 5.5, 7.5, and 8.5 after 24 h was 42, 70, and 73%, respectively (Fig. 3.5). The behavior of drug release rate at different pH suggested that pH could influence drug release in hydrogel system and the NRG release will be easier in the basic skin like atopic skin (Park et al. 2018).

3.5.3 Ocular Drug Delivery

Preparation of ocular drug delivery systems is a major challenge for drug delivery applications due to the unique anatomy and physiology of the eyes. The eyes have static barriers including cornea, sclera, and retina that contain blood aqueous and blood-retinal barriers. The eyes also have dynamic barriers including choroidal and conjunctival blood flow, lymphatic clearance, and tear dilution. Furthermore, the efflux pumps of conjunction make a major challenge for ocular drug delivery especially to the posterior segments (Gaudana et al. 2010). The primary requirement for ocular delivery is bioadhesive properties that increase the contact time with the cornea. The conventional eye drops had not good retention time into the eyes. Studies have shown that administration of ophthalmic drugs in the form of IPN hydrogels could increase the contact time of the drug molecules with the cornea and therefore increase the ocular bioavailability of drug. Hosny developed an in situ thermosensitive prolonged release liposomal hydrogel based on CS and

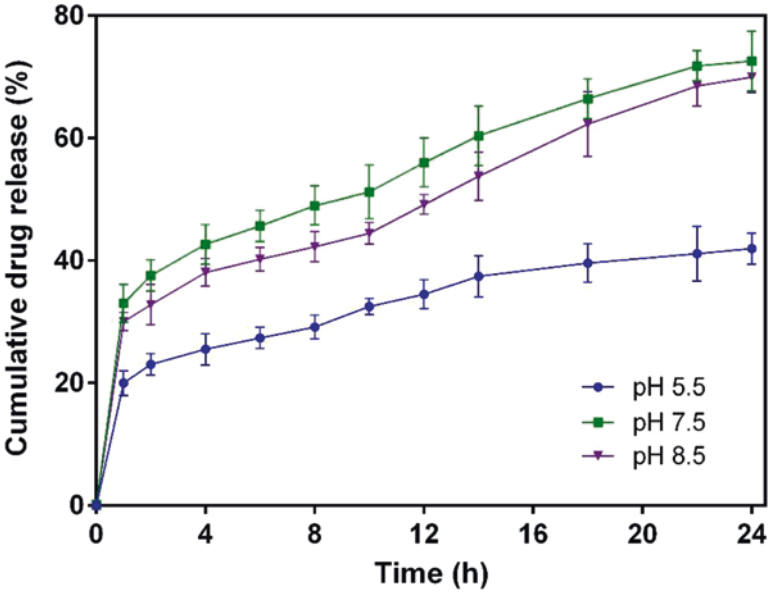

Fig. 3.5 In vitro drug release profiles of carboxymethyl cellulose/2-hydroxyethyl acrylate hydrogels with different pH levels. Values are presented as mean ± SD (n = 3). (Reprinted permission from Park et al. (2018))

β-glycerophosphate to deliver ofloxacin into the eyes. The main problems of using ofloxacin are the frequent administration (every 4 h or even every 1 h to treat severe eye infection) and also formation of white crystalline deposit on the cornea due to very low solubility of drugs at pH of corneal fluid. The gelation time of hydrogel was decreased by adding liposomes into the IPN hydrogel and the prepared system enhanced the transcorneal permeation sevenfold more than the aqueous solution. This could be related to mucoadhesive and cationic properties of prepared hydrogel that make a long contact time to corneal membrane. The obtained results suggested that the in situ thermosensitive IPN hydrogel can improve the ocular bioavailability, minimize the need for frequent administration, and decrease the ocular side effect of ofloxacin (Hosny 2009).

A copolymer composed of poly(*N*-isopropylacrylamide)-chitosan (PNIPAAm–CS) loaded by timolol maleate has been fabricated as a thermosensitive IPN hydrogel for ocular drug delivery. The synthesized copolymer had a LCST of 32 °C, which was close to the surface temperature of the eye. The C_{max} (maximum blood concentration of drug) of timolol maleate was 11.2 μg/mL in a rabbit model. The obtained C_{max} was twofold higher than of the conventional eye drop. Interestingly, the fabricated CS-based IPN hydrogel had a stronger capacity to reduce the intraocular pressure (IOP) than conventional eye drop during 12 h (Cao et al. 2007).

In another study, CS nanoparticles or acrylic acid-functionalized CS with NIPAM or HEMA were prepared for ocular drug delivery. NIPAM-based IPN hydrogel

containing a low amount of CS showed the highest adhesion to mucosal surfaces. The degradation of nanoparticles by lysozyme was dependent on CS content. Both formulations exhibited good loading capacities (up to 45%) for the broad-spectrum antibiotics and anticholinergic drugs. The release profile of drugs showed that the release rate of pilocarpine from HEMA- or NIPAM-based hydrogels was decreased by reducing the CS/AA content. The atropine release from HEMA-based IPN hydrogels exhibited a slower rate than NIPAM-based IPN hydrogels. Atropine, as a water-soluble drug, showed a fast release rate due to collapsing polymer structure under the experimental conditions (37 °C) (Barbu et al. 2009).

3.5.4 Nasal Drug Delivery

The mucosal membrane of nasal made a useful site for drug delivery. Nasal drug delivery can decrease the first pass metabolism effect and also have high patient compliance (Nazar et al. 2011). However, intranasal drug delivery faces some challenges for delivering therapeutic agents and drug molecules into the blood circulation. The challenges included mucosal membrane barrier for absorption of macromolecules, enzymatic degradation of proteins and peptides that influence their bioavailability, and nasal clearance based on mucus turnover time that is limited to 15 to 20 min (Nazar et al. 2011). Studies have shown that CS-based IPN hydrogels could improve the nasal absorption of drugs by opening the tight junctions and facilitating the paracellular transport of large molecules across the mucosal surface. IPN hydrogel composed of N-trimethyl CS chloride polymers with three different average molecular weights co-formulated with PEG and glycerophosphate for developing a thermosensitive nasal drug delivery system. The IPN hydrogels with a low degree of quaternization and high or medium molecular weight showed relatively short sol-gel transition times at 37 °C. Moreover, this type of hydrogel had a good capacity for holding water with improved mucoadhesiveness. The hydrogel composed of N-trimethyl CS with medium molecular weight and low degree of quaternization showed most promising rheological and mucoadhesive behavior. The point of sol-gel transition of hydrogels occurred at 32.5 °C within 7 min (Nazar et al. 2011).

Agrawal et al. prepared a thermosensitive in situ IPN hydrogel system based on CS and PVA for insulin nasal delivery. The IPN hydrogel was obtained by mixing CS and PVA polymer which was liquid at room temperature. Thermal transition from solution to gel with high viscosity occurred after incubation at 37 °C for approximately 12 min. The release of insulin from gel network exhibited a good potential for maintaining the blood glucose level for 6 h (Agarwal et al. 2010). Furthermore, a thermosensitive CS-based IPN hydrogel was prepared by simple mixing of N-[(2-hydroxy-3-trimethylammonium)propyl] CS chloride (HTCC) and PEG fabricated with small amount of alpha-beta-glycerophosphate (alpha-beta-GP). The prepared IPN hydrogel underwent thermal transition from solution to non-flowing hydrogel in

several minutes. It was shown that the developed IPN hydrogel can be dropped or sprayed easily into nasal cavity and spread on the nasal mucosa in a solution state. The solution transformed into viscous hydrogel at the body temperature after administration of hydrogel into nasal cavity. This thermal transition decreased the nasal mucociliary clearance and released the drug more slowly (Wu et al. 2007).

3.6 Conclusion

This chapter discussed the CS-based IPNs and their potential applications for drug delivery. CS polymer with possibilities for several modifications creates a window for having novel biomaterials with useful properties and functions for drug delivery applications, especially as IPN hydrogels. These hydrogels could be categorized into two models of semi- and full-IPN hydrogels. They have a great ability to respond to external stimuli like temperature and pH that make them smart materials for biomedical applications. The most important issue about CS-based IPN hydrogels is their ability to control the releases of therapeutic agent in a sustained manner. It can be concluded that CS-based IPN shaves a great potential for drug delivery and it is expected to become a promising matrix for drug delivery in the near future.

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4 Alginate-Based Interpenetrating Network Carriers for Biomedical Applications

Subhraseema Das and Usharani Subuddhi

Abstract

With advances in modern medicine, there has been a constant need to develop a single material that caters to all demands such as high tensile strength, biocompatibility and biodegradability. The development of an interpenetrating polymer network (IPN) is both an outstanding innovation and contribution that has led to massive technological advances across a wide spectrum of applications in medicine. IPNs comprising natural and synthetic polymers are typically endowed with improved properties compared to monolithic materials and offer superior properties. Most importantly, synergism of properties has also been observed in most of the systems. This chapter discusses the potential of alginate-based IPN carriers for biomedical applications.

Alginate is a naturally occurring anionic polysaccharide widely employed in a broad spectrum of biomedical applications. The ability to assemble alginate with a diversity of polymers and to fabricate IPNs makes it a promising choice for various applications in biomedicine. This chapter discusses at length the various inherent properties of alginate that make it suitable as a biomaterial. The state-of-art applications of alginate IPNs in drug delivery, wound healing and tissue engineering have also been elaborated. The prospective of alginate in delivery of small molecule drugs as well as protein drugs has been presented. This chapter further focuses on the potential of alginate IPNs in wound dressings and regenerative medicine.

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Keywords
Alginate IPNs · Drug delivery · Protein delivery · Wound dressing · Tissue engineering

4.1 Introduction

Utilization of naturally occurring polysaccharides towards the design of biomaterials for biomedical applications has blossomed into a promising approach owing to their biocompatibility and biodegradability. The diversity in the properties of these natural polysaccharides could be well ascribed to the presence of a large number of reactive groups, wide range of molecular weight and varying chemical composition (Prabaharan 2011). Due to the presence of various functional groups in their backbone, these polysaccharides are greatly suitable for chemical and biochemical modifications resulting in a wide range of derivatives (Prabaharan and Jayakumar 2009). In particular, their abundant resources in nature and low processing cost have fuelled up their utilities in pharmaceutical industries. In view of these magnificent attributes, alginate, a naturally occurring anionic polysaccharide, has carved a niche for itself for myriad biomedical applications. Alginate has been extensively investigated in biomedical utilities due to its natural abundance, relative low cost, biocompatibility and ease of fabrication of formulations (Gombotz and Wee 1998).

4.2 Chemistry of Alginate

4.2.1 Sources and Extraction

Alginate is a naturally occurring anionic polysaccharide primarily extracted from the species of brown seaweed viz. *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum* and *Macrocystis pyrifera* (Smidsrod and Skjak-Braek 1990). In all these algae, alginate constitutes almost 40% of dry weight (Sutherland 1991). Alginate is extracted from the dried algae after treatment with dilute mineral acid (mostly HCl) (Rinaudo 2008). Free alginic acid, thus obtained, is then converted to a salt, mostly the sodium salt, i.e. sodium alginate, which finds prominence in biomedical applications (Tonnesen and Karlsen 2002). Alginate also exists as mixed salts of various cations found in seawater such as Mg^{2+}, Ca^{2+}, Ba^{2+} and Na^{+}. To extract the alginate, the cations are exchanged for H^{+}. The alginate is then converted to its soluble sodium salt by addition of sodium carbonate while maintaining the pH below 10 (Sutherland 1991).

Bacterial alginates have also been isolated from *Azotobacter vinelandii* and several species of *Pseudomonas* (Smidsrod and Skjak-Braek 1990). The pathway for alginate biosynthesis mostly comprises of four decisive steps: (i) synthesis of precursor substrate, (ii) polymerization and cytoplasmic membrane transfer, (iii) periplasmic transfer and modification and, lastly, (iv) export through the outer membrane

(Lee and Mooney 2012; Remminghorst and Rehm 2006). Further regulation and tailored development features in bacterial alginate biosynthesis could provide a new direction towards alginate production and its wide applications in biomedicine.

4.2.2 Chemical Structure

Alginates are a family of linear block copolymers comprising of 1,4'-linked β-D-mannuronic and α-L-guluronic acid residues (George and Abraham 2006). The composition and properties of alginates are strongly dependent on the ratio of guluronate (G) to mannuronate (M) residues. Depending on the source of extraction, the contents of M and G vary and to date, more than 200 different alginates have been manufactured (Thakur et al. 2018). The blocks might be arranged with consecutive G residues, M residues and alternating G and M residues. Because of the difference in the mode of linkage between the G residues and M residues, the geometries of the G-block regions, M-block regions and the alternating regions are significantly different (Fig. 4.1a) (from Lee and Mooney 2012). The G-blocks are considered to be buckled shaped whereas M-blocks are often referred to as extended ribbons. The

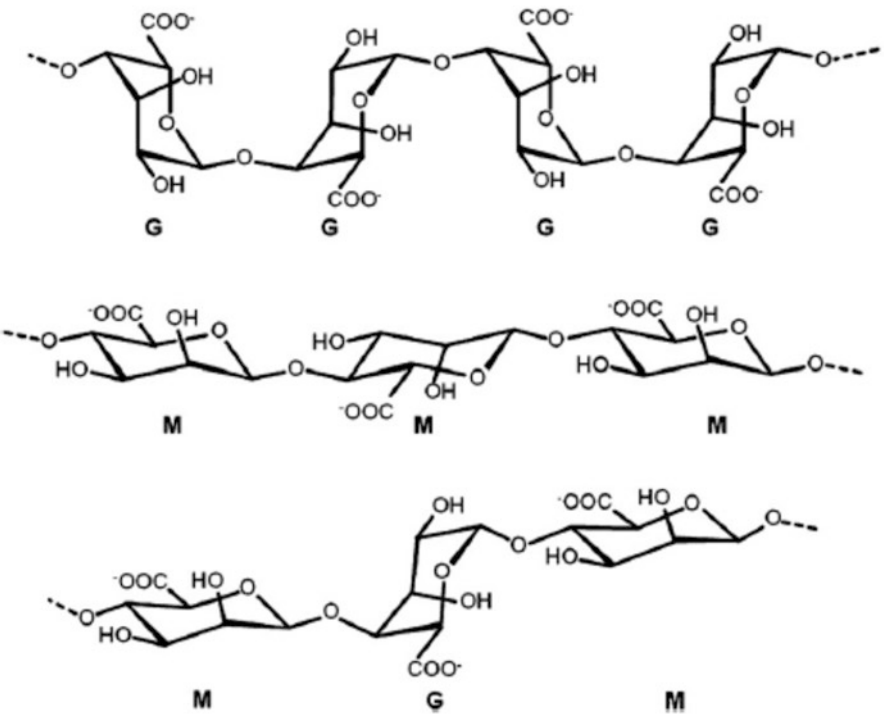

Fig. 4.1a Chemical structures of G-block, M-block and alternating block in alginate. (Lee and Mooney 2012)

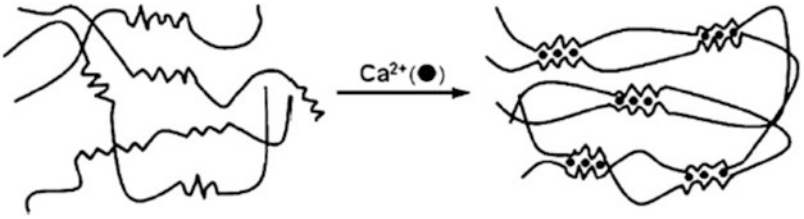

Fig. 4.1b Alginate hydrogels prepared by ionic crosslinking (egg-box model). (Lee and Mooney 2012)

specific arrangement of G residues results in diamond-shaped holes of suitable dimensions that are ideal for accommodating divalent cations such as Ca^{+2} to form stacked egg-box-like structures of the G residues resulting in gelation (Fig. 4.1b) (from Lee and Mooney 2012) (George and Abraham 2006). Thus, the composition (M/G ratio), the sequence and the G-block length are crucial factors that determine the physical properties of the alginate and its resultant formulations (George and Abraham 2006).

4.2.3 Molecular Weight and Viscosity

The molecular weights (MW) of commercially available Na-alginates mostly range between 32,000 and 400,000 g/mol (Rinaudo 1992). The intrinsic viscosity ($[\eta]$) of alginate solution depends on its MW. According to Mark-Houwink relationship ($[\eta] = KM_v^a$) the parameters are $K = 2 \times 10^{-3}$ and $a = 0.97$ for Na-alginate in 0.1 M NaCl and at 25 °C, where M_v is the viscosity average molecular weight (Rinaudo 1992). The viscosity of alginate solutions is also sensitive to the pH and it increases as the pH is lowered. A maximum viscosity is observed around pH 3–3.5 wherein the carboxylate groups in the polysaccharide become protonated and take part in hydrogen bonding (Berth 1992). It has also been demonstrated that alginate solutions with high MW become greatly viscous which is undesirable for further processing (LeRoux et al. 1999). Thus, judicious manipulation of MW can tailor the solution viscosity and render alginates with desired properties. Ideally, by employing a combination of high and low MW polymers, the viscosity of the resulting alginate gels could be adjusted (Kong et al. 2002).

4.3 Properties of Alginate Making It Suitable for Biomedical Applications

4.3.1 Gelation

The gel formation capacity or gelation property of alginate is of great importance in widespread applications of this polysaccharide in food and pharmaceutical industries. The ability of alginate to form two types of gels (i) pH dependent (acid gel)

and (ii) ionotropic gel confers unique advantages over neutral polysaccharides. Hydration of alginic acid, especially the high MW variants, leads to formation of high viscosity 'acid gels' due to strong intermolecular hydrogen bonding. These gels can entrap large amount of labile water molecules and are of great importance in many applications such as cell immobilization and encapsulation (Nahar et al. 2017). Alginate can form ionotropic gels with a variety of metal ions under mild conditions. Monovalent cations generally form soluble salt with alginate whereas divalent and multivalent cations often result in gelation. Ionic crosslinking mostly involves divalent cations such as Ca^{2+}, Ba^{2+} and Sr^{2+}. The ionotropic gelation of alginate is mostly due to the ion-induced association of G-block regions in the polymer. Various functional properties such as porosity, swelling behaviour, stability, mechanical strength, biodegradability, immunological characteristics and biocompatibility of these gels are found to be greatly influenced by the chemical structure, MW, gel formation kinetics of the polysaccharide and the cation. Alginate gels can be prepared in various shapes and sizes among which alginate beads are largely explored.

Alginate beads have been prepared by extruding a solution of sodium alginate containing the desired protein or drug, as droplets, into divalent crosslinking solutions of Ca^{2+}, Ba^{2+} and Sr^{2+} (Sutherland 1991). The viscosity of alginate solution and the diameter of the extruder are paramount in influencing the shapes and sizes of the resulting beads. Recent studies point to alginate gelation by covalent crosslinking (mostly esterification, carbodiimide, methacrylation, click chemistry) or by photocrosslinking (Bidarra et al. 2014).

4.3.2 pH Sensitivity

Alginate is a well-known pH-responsive polymer due to its ionic nature. At low pH, (gastric environment) it shrinks into a porous insoluble matrix usually referred to as alginic acid skin but as soon as it experiences higher pH in the intestinal tract, the alginic acid skin converts to a soluble layer (Chen et al. 2004). The strong pH-dependent responsivity of alginate could be accredited to the acidic β-D-mannuronic and α-L-guluronic acid structural units on its backbone that get protonated in acidic environment (pH 1.2 of gastric fluid). In alkaline media (pH 7.4 of intestinal fluid) the repulsion between the deprotonated COO^- groups renders the alginate matrix a greater swellability (Kajjari et al. 2012). This pH-sensitive behaviour has been categorically exploited to customize drug release kinetics from alginate matrices. The various biomedical applications pertaining to pH sensitivity of alginate have been discussed in the later sections.

4.3.3 Mechanical Properties

Materials intended as extracellular matrices (ECMs) often require specific and reproducible elastic moduli that can be modulated in a controlled manner (Schneider et al. 2006). The mechanical properties of a polymer such as stiffness can mostly be

manipulated by physical factors like crosslinker type, crosslinking density, MWs and also by chemical modification of the polymer (Peppas 2004). Generally, in the case of high MW alginate, an increase in stiffness is observed upon increasing the polymer concentration. This, however, leads to a greatly viscous polymer solution that makes further processing difficult (Kong et al. 2003; Augst et al. 2006). To circumvent this hindrance, specifically formulated combination of high and low MW alginates is usually employed. In these formulations, the low MW chains reduce the physical interactions in solution while the high MW chains maintain the long-range interactions in the gel (Augst et al. 2006). Thus, the synergistic effects of these two factors tend to minimize the alginate gel brittleness.

Reinforcement of cationic polymers such polyethylene imine into alginate is also an effectual strategy to improve its mechanical strength (Kong and Mooney 2003). Gelling condition such as gelation time and type of crosslinker are pivotal that dramatically influence the swelling and mechanical properties of the alginate gel.

4.3.4 Bioadhesiveness

Studies have revealed that polymers possessing a charge density serve as good mucoadhesive agents (Chickering and Mathiowitz 1995; Chang et al. 1985; George and Abraham 2006). More so, anionic polymers are more efficient bioadhesive candidates than their polycationic and nonionic counterparts. Literature reports have illustrated alginate with excellent bioadhesion characteristics (El-Kamel et al. 2002; Pinkas et al. 2017; Singh et al. 2017; Shtenberg et al. 2017; Hong et al. 2018;). Moreover, due to adherence of alginate to mucosal tissues, protein transit time is delayed and thus the drug is localized to the absorptive surfaces, thereby improving the drug bioavailability and efficacy (Szekalska et al. 2016). In a recent development, an oral mucoadhesive drug delivery system constituting alginate and liposomes were fabricated by Shtenberg and co-workers that displayed retention of around 80% of the biomaterial in the tongue tissues (Shtenberg et al. 2018).

4.3.5 Biodegradation

Degradation of a biomaterial is often a key issue in tissue replacement and drug release. Ionically crosslinked alginates mostly dissolve in neutral pH by losing their divalent crosslinking cation (Augst et al. 2006). Gamma irradiation-assisted alginate degradation has also been reported at the optimum dose 100 kGy (Lee et al. 2003). Enzymic degradation of alginates is a well-known phenomenon (Gacesa 1992). Schaumann and Weide (1990) have explicitly detailed on alginase-mediated structural and molecular degradation of Na-alginate by marine fungi. The review article by Wong and co-authors (2000) particularly emphasizes on the enzyme characteristics of alginate lyase to engineer and fine-tune the degradation of alginate-based biomaterials. Recently, controlled degradation of alginate has also been achieved by crosslinked enzyme aggregates of alginate lyase (Kunjukunju et al.

2018). The study revealed that controlled degradation of alginate was obtained over a period of 28 days. These findings altogether suggest that it is possible to tailor the degradation kinetics of alginate and its derivatives with proper use of conditions.

4.3.6 Biocompatibility

The biocompatibility of alginate has been studied extensively by various authors in vitro as well as in vivo. However there still remains some debate about the varying levels of impurity particularly for commercially available alginates. Since crude alginates often contain contaminants such as proteins or endotoxins, it is desirable to properly purify the alginates to minimize the risk of an immune response. Tam et al. (2011) have studied the factors influencing the alginate gel biocompatibility and inferred that the mannuronate/guluronate content is the key factor. Orive and co-authors (2005) have presented a battery of in vitro techniques to access the biocompatibility of alginates with different compositions and purities. Their findings established that the differences in protein and polyphenol content amount to purified and non-purified alginates.

4.4 Limitations of Pure Alginate

The foremost hindrance associated with pure alginate is its poor mechanical strength which usually warrants reinforcement (Li et al. 2005; Zineh et al. 2018). The application of alginate is further limited by its high moisture sensitivity (Jost and Reinelt 2018). Composite materials usually exhibit better tensile strength in comparison to monolithic materials and thus, alginate has been assimilated with a diversity of polymers to engineer composites of biomedical interest.

4.5 Modifications of Alginate

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 myriad biomedical applications (Dragan 2014). 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.

IPNs are ‘alloys’ of crosslinked polymers, at least one of them being synthesized and/or crosslinked within the immediate presence of the other, without any covalent bonds between them, which cannot be separated unless chemical bonds are broken (Dragan 2014). IPNs can be basically classified as simultaneous IPN and sequential IPN based on the preparation method. When the precursors of both networks are mixed and the two networks are synthesized at the same time, simultaneous IPN

results. A sequential IPN is typically performed by swelling of a single-network hydrogel into a solution containing the mixture of monomer, initiator and activator, with or without a crosslinker. In the presence of a crosslinker, a full IPN results while the absence of a crosslinker forms a semi-IPN (Matricardi et al. 2013; Dragan 2014; Lohani et al. 2014).

The high rates of success of IPNs and/or semi-IPNs used for biomedical and pharmaceutical applications could be attributed to the combination of favourable properties of each constituent polymer. Formation of IPNs leads to new systems with improved properties, which quite often are substantially different from those of the individual polymers. Most importantly, synergism of properties has also been observed in most of the IPNs (Matricardi et al. 2013). The combination and synergism of properties can be judiciously exploited to modify the characteristics of the resulting biomaterial to cater to specific needs, particularly in the biomedical and pharmaceutical fields. Development of IPNs has led to an assortment of architectures such as hydrogels, microspheres, microgels, nanoparticles, tablets, capsules and scaffolds, among others, to accomplish the biomedical objective (Lohani et al. 2014).

4.6 IPNs of Alginate

Alginates have been assembled with a diversity of polymers for the fabrications of IPNs and/or semi-IPNs intended for biomedical applications in the form of films, microspheres, microgels, nanoparticles or depot matrices. They have also been designed as scaffolds in tissue engineering for bone, cartilage and soft tissue generation. Because of the high versatility and tailorable properties, IPNs of alginate are promising candidates in pharmacy. Alginates have often been taken in combination with synthetic polymers or with naturally occurring biopolymers for the generation of IPNs. Depending on the nature and the intrinsic properties of components, the resulting IPNs can be fine-tuned. Moreover, the range of reachable properties can be broadened substantially.

4.6.1 IPNs with Synthetic Polymers

A combination of synthetic polymers with alginate further widens the window of pharmaceutical and biomedical utilities. Synthetic polymers such as polyvinyl alcohol (PVA), polyethylene glycol (PEG), polyacrylic acid (PAA), polyacrylamide (PAAm), poly(N-isopropylacrylamide) (PNIPAAm), polycaprolactone (PCL), polylactic acid (PLA), polyvinyl pyrrolidone (PVP), etc., have been taken in conjugation with alginate to form IPNs.

Table 4.1 reviews the IPNs of alginate with various synthetic polymers and their potential applications in biomedicine.

Table 4.1 IPNs of alginate with synthetic polymers and their biomedical applications

Polymer	IPN component	Formulation	Biomedical applications	References
PVA	PVA-g-PAA	Semi-IPN hydrogels	Controlled release of loxoprofen sodium	Khalid et al. (2018)
	PVA-graphene nanosheet	Fibres	Scaffolds for engineering neural construct	Golafshan et al. (2017)
	PVA	Hydrogels	Controlled release of tramadol	Anwar et al. (2017)
	PVA	Hydrogel	Controlled delivery of carbidopa	Tareq (2016)
	PVA	Semi-IPN and IPN hydrogels	Cardiac tissue engineering	Thankam et al. (2013)
	PVA	Hydrogel	Controlled release of prazosin HCl	Kulkarni et al. (2010)
PNIPAAm	PNIPAAm	Hydrogels	Wound dressing	Li et al. (2018)
	PNIPAAm/acryloyl phenylalanine	Hydrogels	Controlled release of imatinib mesylate	Jalababu et al. (2018)
	PNIPAAm	Semi-IPN microspheres	Controlled delivery of 5-fluorouracil	Reddy et al. (2008)
	PNIPAAm	Semi-IPN beads	Sustained release of indomethacin	Shi et al. (2006)
PAA	PAA	Hydrogel	Controlled release of diclofenac potassium to the colon	Jalil et al. (2016)
	Starch-g-PAA	Hydrogel	Controlled release of diclofenac sodium in pH 7.4	Chang (2015)
	PAA	Microgels	Controlled release of Ibuprofen	Babu et al. (2006)
PAAm	PAAm-graphene oxide-gelatin	Hydrogels	Scaffolds for nerve tissue engineering application	Zhao et al. (2018)
	PAAm	Hydrogels	Cartilage replacement	Arjmandi et al. (2018a, b)
	PAAm-montmorillonite platelets	Hydrogels	Aquatic load-bearing materials for artificial tissues, actuators, agriculture, etc.	Su and Chen (2018)
	PAAm-gum ghatti	Microbeads	Ketoprofen delivery	Boppana et al. (2015)
	PAAm	Semi-IPN hydrogels	Acetaminophen release	Samanta and Ray (2014)
	PAAm	Hydrogels	Biomaterials	Darnell et al. (2013)

(continued)

Table 4.1 (continued)

Polymer	IPN component	Formulation	Biomedical applications	References
PEG	PNIPAAm-PEG-co-poly(ε-aprolactone)	Hydrogel	Oral delivery of BSA	Zhao et al. (2010)
	PEG acrylate	Hydrogel	Encapsulation of islets of Langerhans	Desai et al. (2000)
	PEG	Hydrogel	Drug-eluting stents	Livnat et al. (2005)
	PEG	Fibres	Controlled delivery of salicylic acid	Wang et al. (2007)
	Chitosan-PEG	Microcapsule	Controlled delivery of hirudin in pH 7.4	Chandy et al. (1998)
PVP	PVP	Beads	Controlled delivery of ibuprofen	Yigitoglu et al. (2014)
Pluronic	PF68	Nanoparticles	Sustained release of zidovudine in pH 7.4	Joshy et al. (2017)
	Au nanoparticle-loaded F127	Microparticles	Controlled release of FITC-dextran	Park et al. (2017)
	Chitosan-Pluronic	Nanoparticles	Sustained delivery of meloxicam	Fattahpour et al. (2015)
	Chitosan-Pluronic	Nanoparticles	Enhanced biological activity of nisin	Bernela et al. (2014)
PCL	PCL	Scaffolds	Tissue regeneration	Hu and Ting (2019)
	PCL nanofibers	Scaffolds	In situ transfection	Hu et al. (2018a)
	PCL	Scaffolds	Improved cellular responses	Kim and Kim (2014)
PLA	Ciprofloxacin-loaded poly(lactic-co-glycolic acid)	Microparticles	Wound dressings	Liu et al. (2018a, b)
	Doxorubicin-loaded poly(lactic-co-glycolic acid)	Hydrogels	Antitumor applications	Chai et al. (2017)
	PLA nanofibers	Hydrogels	Bioink for 3D bioprinting of constructs	Narayanan et al. (2016)

4.6.2 IPN with Biopolymers

Biopolymers such as chitosan, guar gum, cellulose, pectin, soya protein and others have been integrated with alginate and explored for manifold biomedical applications. Table 4.2 enlists the recent advances in the IPNs of alginates with various biopolymers and their diverse pharmaceutical applications.

Table 4.2 IPNs of alginate with biopolymers and their biomedical applications

Biopolymer	IPN component	Formulation	Biomedical applications	References
Chitosan and its derivatives	Chitosan	Hydrogel scaffolds	Tissue engineering	Kolanthai et al. (2018) and Naghizadeh et al. (2018)
	Chitosan/β--cyclodextrin	Polyelectrolyte complexes	Piroxicam release	Hardy et al. (2018)
	Chitosan/gelatin	Gel	Sustained release of ranitidine	Belhadji et al. (2018)
	Chitosan	Hydrogels	Controlled delivery of deferoxamine	Rassu et al. (2016)
	Chitosan	Hydrogel	Cell growth applications	Baysal et al. (2013)
	Chitosan	IPN scaffolds	Cartilage tissue engineering	Tigli and Gumusderelioglu (2009)
	Chitosan-g-(PAA/attapulgite/Na-alginate)	Hydrogel beads	Controlled delivery of diclofenac sodium to intestine	Wang et al. (2009)
	Chitosan-coated alginate beads containing PNIPAAm	Beads	pH-/temperature-sensitive controlled delivery system for indomethacin	Shi et al. (2008)
	Chitosan	Fibrous scaffolds	Intervertebral disc tissue engineering	Shao and Hunter (2007)
	Carboxymethyl chitosan	Beads	Intestinal delivery of BSA	Lin et al. (2005)
	Chitosan	IPN scaffolds	Cartilage tissue engineering	Li and Zhang (2005)
	Chitosan	IPN scaffolds	Bone tissue engineering	Li et al. (2005)
Guar gum and its derivatives	Guar gum	Hydrogel	Controlled delivery of BSA	George and Abraham (2007)
	Carboxymethyl cellulose/guar gum	Fibres	Antibacterial activity	Riaz et al. (2018)
	Guar gum succinate	Beads	Colon-specific delivery of ibuprofen	Seeli et al. (2016)
	Guar gum-garose	Hydrogels	Entrapment of ginger peroxidase	Ali and Husain (2018)
	Carboxymethyl guar gum	Beads	Oral delivery of drugs along GIT	Bajpai et al. (2006)

(continued)

Table 4.2 (continued)

Biopolymer	IPN component	Formulation	Biomedical applications	References
Cellulose and its derivatives	Polypyrrole-TEMPO-oxidized microfibrillated cellulose	Hydrogels	Excellent swelling and biocompatibility	Lin et al. (2019)
	Rifampicin-loaded cellulose nanocrystal hybrids	Nanoparticles	Potential for treatment against *M. tuberculosis*	Thomas et al. (2018)
	Bacterial cellulose	Nanofibers	Drug delivery carriers	Shi et al. (2014)
Gelatin and its derivatives	Gelatin-agar	Scaffolds	Improved growth of microbial cells	Aljohani et al. (2018)
	Gelatin	Hydrogels	Oral delivery of hydrophobic drugs	Bhutani et al. (2016)
	Gelatin	Nanogels	Controlled delivery of curcumin to cancer cells	Sarika et al. (2016)
	Methacrylamide-modified gelatin	Hydrogels	Suitable cell carriers	Graulus et al. (2015)
Pectin	Cefazolin-loaded pectin	Nanoparticles	Wound dressing agents	Shahzad et al. (2019)
	Pectin	Hydrogels	Optimized delivery of anthocyanins	Guo et al. (2018)
	Pectin	Microgels	Controlled release of antacids	Chen et al. (2018a)
	Pectin	Hydrogels	Treatment of gastro-oesophageal reflux disorders	Hanif and Abbas (2018)
Xanthan gum	Xanthan gum	Hydrogels	Inhibition of *H. pylori* infection	Vega-Sagardia et al. (2018)
	Xanthan gum reinforced with cellulose nanocrystals and halloysite nanotubes	Scaffolds	Bone tissue engineering	Kumar et al. (2017)
	Xanthan gum-β-cyclodextrin	Hydrogels	Cholesterol reducing potential	Fareez et al. (2017)

(continued)

Table 4.2 (continued)

Biopolymer	IPN component	Formulation	Biomedical applications	References
Carrageenan	Carrageenan-gelatin	Hydrogels	Tissue engineering	Vignesh et al. (2018)
	Carrageenan-carboxymethyl cellulose-grapefruit seed extract	Hydrogels	Antibacterial applications	Shankar and Rhim (2018)
	κ-Carrageenan	Hydrogel beads	Controlled delivery of oral insulin	Lim et al. (2017)
	κ-Carrageenan	Microparticle	Mucosal delivery of ketoprofen and quercetin	Goncalves et al. (2016)
Locust bean gum	Locust bean gum	Microbeads	Controlled delivery of capecitabine for treatment of colon cancer	Upadhyay et al. (2018)
	Locust bean gum	Microspheres	Sustained release of aceclofenac	Jana et al. (2015)
	Locust bean gum	Microcapsule	Controlled release of *Lactobacillus rhamnosus* probiotic cells	Cheow et al. (2014)
Soy protein isolates	Soy protein isolates	Beads	Thyme oil encapsulation for intestinal delivery	Volic et al. (2018)
	Soy protein isolates	Scaffolds	Tissue engineering	Nooeaid et al. (2017)
	Soy protein isolates	Hydrogels	Tissue regeneration and wound healing materials	Tansaz et al. (2017)
Acacia gum	Acacia gum	Beads	Oral delivery of diclofenac sodium to intestine	Benfattoum et al. (2018)
	ZnO nanoparticle-loaded acacia gum	Hydrogels	Antibacterial and wound healing effects	Raguvaran et al. (2017)

4.7 Biomedical Applications of IPN of Alginate

Owing to its biocompatible, non-immunogenic, non-toxic, mucoadhesive and biodegradable features, applications of alginate are manifold in biomedical field. Alginate biocompatibility has been affirmed in vivo after ocular (Lin et al. 2004), nasal (Sarei et al. 2013), topical (Coskun et al. 2014), vascular (Rottensteiner et al. 2014; Amirian et al. 2017) and oral (Nunamaker et al. 2007; Thomas et al. 2018; Yang et al. 2018a, b) administration. Alginate has been greatly studied for pharmaceutical applications like targeted drug delivery, protein delivery, tissue engineering and wound healing purposes. The following sections explore the biomedical wonders of alginates in greater details.

4.7.1 Pharmaceutical Applications

Oral formulations are currently the most frequent and preferred mode for pharmaceutical applications (Nahar et al. 2017). The design of oral dosage forms usually follows any one of these two principles: (i) the entire drug dose is in the same physical unit or (ii) the dose is encapsulated in an assembly of small subunits (Nahar et al. 2017). In the latter case, the subunits are further compressed into tablets which, thereby, experience a 'barrier' that is pivotal to provide a controlled release profile. In particular, hydrocolloids like alginates undergo an immediate hydration to create a high viscosity layer around themselves which act as diffusion barrier, thereby decreasing the migration of small chemical drugs, thus sustaining the delivery. Additionally, the intrinsic pH responsivity of alginates makes them wonderful agents for colon-specific drug delivery. Alginates are widely used as delivery vehicles due to their ability to encapsulate and release a wide range of drugs in a gentle and biocompatible manner.

4.7.1.1 Delivery of Small Chemical Drugs

Alginate hydrogels may be broadly useful for the sustained and localized delivery of traditional small chemical drugs. Conventional approaches to drug delivery (oral mostly) usually lead to burst release of drugs and poor targeting to the site of interest causing side effects and inefficacy. The release kinetics of low MW drugs from alginate gels can be controlled by regulating drug-alginate interactions. When there are no chemical interactions between the drug and the polymer, the release depends largely on the charge polarization of the molecule; i.e. hydrophilic molecules may diffuse very quickly while hydrophobic drugs diffuse slowly through the gel pores (Augst et al. 2006).

pH-Sensitive Alginate Hydrogels in Drug Delivery

A plethora of alginate-based IPN formulations have been developed towards the controlled delivery of drug molecules to the target. pH-sensitive composite hydrogel beads of Ca-alginate and agar have been developed towards controlled drug

delivery (Yin et al. 2018). Biodegradable and biocompatible semi-IPN hydrogels of Na-alginate and poly(methacrylic acid) have been utilized for controlled delivery of theophylline to colon (Ganguly et al. 2018). Upadhay and co-workers (2018) have optimized capecitabine-loaded IPN beads of locust bean gum and Na-alginate by ionotropic gelation method and investigated their controlled drug delivery features. In vitro drug release studies indicated sustained delivery for 12 h. More so, cytotoxicity assay against HT-29 cells revealed significant reduction in cell growth, validating their worth towards the treatment of colon cancer. IPN hybrid hydrogels comprising Ca-alginate and peptide Fmoc-tyrosine have been constructed with an aim to achieve a controlled release drug profile for small molecules (Chen et al. 2018b). The IPN hydrogels exhibited enhanced storage moduli and fracture energy in comparison to Ca-alginate and revealed minimal drug release at acidic pH and sustained release at intestinal pH. Basu et al. (2017) have developed silver nanocomposite semi-IPN hydrogels of Na-alginate and acrylamide and explored their drug delivery features. The results demonstrated sustained release of ciprofloxacin for colonic delivery. Anwar and co-authors (2017) constructed Na-alginate and PVA hydrogels by free radical polymerization using 2-ccylamido-2-methylpropane-sulfonic acid. The hydrogels exhibited prolonged delivery of tramadol HCl with improved entrapment efficiency.

Samanta and Ray (2014) synthesized various grades of Na-alginate-acrylamide IPN hydrogels using N,N'-methylenebisacrylamide as crosslinker and studied the release of acetaminophen. It was observed that the IPN with 6 wt% Na-alginate content was the best of the lot in terms of optimum swelling, drug entrapment and controlled release drug profile at physiological pH. Na-alginate has also been assimilated with carrageenan to formulate pH-responsive IPN hydrogel beads (Mohamadnia et al. 2007). The beads displayed sustained release profile for betamethasone acetate at pH 7.4. Literature reports pertaining to carrageenan and alginate-based IPN suggest their utility as colon-targeted controlled drug delivery systems (Mohamadnia et al. 2008; Kulkarni et al. 2011; Kulkarni et al. 2012; Mahdavinia et al. 2014).

In situ forming IPN hydrogels of Ca-alginate and hydroxyethyl-methacrylate-derivatized dextran have been prepared by Pescosolido and co-workers (2011) and bovine serum albumin (BSA) was loaded as a model drug to evaluate the drug delivery efficacy of the hydrogels. Surprisingly the drug release spanned approximately for 15 days. Treenate and Monvisade (2017) investigated the release profile of paracetamol from hydroxyethylacryl chitosan and Na-alginate IPN hydrogels crosslinked by ionic crosslinkers such as Ca^{2+}, Zn^{2+} and Cu^{2+}. The comprehensive results of the study demonstrated their potential in the applications of site-specific oral drug delivery to the intestine and colon. Na-alginate-carboxymethyl cellulose hydrogel beads have been prepared in ferric chloride solution that showed excellent pH responsivity and controlled delivery features for metformin in pH 7.4 (Swamy and Yun 2015). IPN hydrogel membranes of Na-alginate and PVA prepared by Kulkarni et al. (2010) by solvent casting method were explored for prolonged transdermal delivery of antihypertensive drug prazosin hydrochloride.

Temperature-Sensitive Alginate Hydrogels in Drug Delivery

Thermo-sensitive smart polymers are one of the most common classes of smart polymers studied in drug delivery research. Two major classifications of thermo-responsive IPNs can be categorized as:

1. IPNs that exhibit an upper critical solution temperature (UCST) that show a transition from gel to sol state above this temperature
2. IPNs that exhibit a lower critical solution temperature (LCST) that show a transition from gel to sol state below this temperature

Temperature-sensitive smart IPN hydrogels of alginate have often been prepared by taking in combination with one of the most commonly used thermo-responsive polymers, poly(N-isopropylacrylamide) (PNIPAAm) that has its phase transition near body temperature (~33 °C). The use of PNIPAAm in interpenetrating architectures has been particularly investigated for tailoring the release of drugs. Integrating alginates with PNIPAAm result in a special class of dual stimuli-sensitive composites with responsivity to both pH and temperature.

Doxorubicin-loaded alginate-g-PNIPAAm IPN hydrogels demonstrated a sustained release profile of drug in physiological pH and were found to be effective in cancer therapy (Liu et al. 2017). Alginate-g-PNIPAAm injectable temperature-sensitive hydrogels with potential for localized and sustained delivery of stem cells and bioactive molecules have been successful and are ready to serve their purpose (Pentlavalli et al. 2017). Temperature-sensitive alginate beads synthesized via microwave-assisted graft copolymerization were found to prolong the delivery rate of indomethacin (Isiklan and Kucukbalci 2016). Muniz and co-workers studied the effect of temperature on the mechanical properties and permeability of PNIPAAm-Ca-alginate semi-IPNs and IPNs (Guilherme et al. 2002; de Moura et al. 2005; Guilherme et al. 2005). The authors evaluated the IPN hydrogel strength and observed a synergism of the mechanical performances of the polymers both below and above the LCST. The effect was more pronounced above LCST where the uniaxial compressive modulus of the hydrogel was much higher than individual components. More so, sustained release of drug was observed above LCST, which is attributed to the tighter structure of the hydrogel matrices with smaller pore sizes.

Ju and co-workers (2002) prepared semi-IPN hydrogels of alginate and amine-terminated PNIPAAm using calcium chloride as crosslinker. Also, they reported that semi-IPN hydrogel exhibited a remarkable sensitivity to temperature, pH and ionic strength of swelling. Li et al. (2018) employed Ca-alginate-PNIPAAm IPN onto cotton fibre surfaces using 1,2,3,4-butanetetracarboxylic acid crosslinking. The cotton fibre-supported hydrogels exhibited stiffer mechanical properties and controlled drug release characteristics at 37 °C. The in vitro drug release of anticancer drug 5-fluorouracil from semi-IPN networks of Na- alginate-PNIPAAm microspheres was reported by Reddy and co-workers (2008). The drug release at 25 °C and 37 °C confirmed about the thermo-responsive nature of the microspheres.

pH/Temperature Dual-Sensitive Alginate Hydrogels in Drug Delivery

Several studies have also focused on the development of pH and temperature dual stimuli-sensitive smart alginate hydrogels. The properties of alginate-PNIPAAm semi-IPNs or IPNs were found to be strongly affected not only by pH but also by temperature. Shi et al. (2006) studied the effect of pH and temperature on the release of indomethacin from semi-IPN beads of Ca-alginate and PNIPAAm. At pH 2.1 and 37 °C, the drug release was slow and less than 10% of drug was released in 7 h while the release completed in 3 h at pH 7.4. Temperature-dependant drug release was also observed from the same gel, leading to a faster release at 37 °C than 25 °C. An overview of the properties and performances of alginate-based smart IPNs for drug delivery applications has been elucidated in the review article by Matricardi and co-authors (2013).

Alginate Microparticles in Drug Delivery

Alginate-based microparticles have been fabricated by various groups and are found to be quite efficacious towards oral targeted drug delivery. The microparticles have been formulated mostly by the conventional shredding method in a commercial food processor or by a water/oil (w/o) emulsion and external gelation method. Chitosan-reinforced alginate microparticles for sustained release of antineoplastic drugs have been successfully synthesized and drug delivery potential has been explored (Yu et al. 2008). The same authors have also developed composite microparticle drug delivery systems composed of alginate, chitosan and pectin for site-specific delivery via oral route (Yu et al. 2009). Moebus et al. (2009) illustrated the efficacy of alginate-poloxamer microparticles for controlled drug delivery to mucosal tissues. The recently published review article by Aguero and co-authors (2017) summarizes the utility of alginate microparticles as oral colonic drug delivery devices with greater precision.

Alginate Nanoparticles in Drug Delivery

The development of nanoparticles has proven to be a boon to the pharmaceutical sector. The idea of IPN nanoparticles as drug delivery agents may be utilized to modify or control the drug distribution at the tissue, cellular or subcellular levels (Lohani et al. 2014). Chitosan-alginate nanoparticles as delivery systems for ε-polylysine were designed and a sustained release profile was achieved (Liu et al. 2018a, b, c). In another report of the above nanoparticulate systems by Motwani and co-workers (2008), prolonged ocular delivery of the ophthalmic antibiotic gatifloxacin was revealed for a period of 24 h. Hybrid nanoparticles of alginate and stearic acid-polyethylene glycol were loaded with an antiviral drug, zidovudine (Joshy et al. 2017). The optimized formulations were stable up to a period of six months and represented potential carrier for the drug by enhancing its efficiency.

Alginate Microgels in Drug Delivery

Microgels offer unique advantages for polymeric drug delivery systems in terms of high degree of control over properties such as stability for prolonged circulation in

blood stream, control over particle size and biodegradability for sustained release of drugs. The potential of alginate microgels as sustained drug delivery agents is well-established. Chen and co-authors (2018a) have demonstrated the controlled release of an antacid (magnesium hydroxide) from alginate-pectin microgels. Microgels of varying dimensions were formed using either a handheld syringe or a vibrating nozzle encapsulation device with different nozzle sizes. The slowest antacid releasing features was observed from the microgel containing 80% alginate and 20% pectin. The authors inferred that the role of alginate was crucial for the design of microgels for the release of antacids in stomach. In another study by Wang and Newby (2018), coating of poly(allylamine) and poly(styrene sulfonate) was deposited layer by layer on alginate microgels and evaluated for sustained release of two hydrophilic drugs, sodium benzoate and zosteric acid. The results revealed that a prolonged release of the drugs could be achieved from the microgels spanning from hours to at least three days. Na-alginate microgel spheres encapsulated with tea polyphenol were designed for the treatment of bone infection (Chen et al. 2018d). The drug loading efficiency was reported to be 92.96% and the polyphenol release was therapeutic. Apart from exhibiting excellent antibacterial activity against *S. aureus*, the microgels promoted proliferation and differentiation of osteoblasts. Microgel suspensions of alginate have been developed for controlled release of vascular endothelial growth factor (VEGF)-encoding lentivectors (Madrigal et al. 2018). Babu and co-workers (2005) have reported IPN microgels of Na-alginate-acrylic acid prepared by w/o emulsion technique for the controlled release of ibuprofen. The developed systems showed pH responsivity and prolonged the drug delivery at physiological pH.

4.7.1.2 Protein Delivery

The various inimitable features of alginate have enabled it to be used as a matrix for encapsulation and delivery for a variety of protein drugs. These features include (Gombotz and Wee 2006):

1. An almost inert aqueous environment within the matrix
2. An encapsulation process devoid of any organic solvents that proceeds at room temperature
3. A high gel porosity which allows for high diffusion rates for macromolecules
4. Ease of controlling the porosity with simple procedures
5. Biodegradation of the system under normal physiological conditions

Proteins encapsulated in alginate matrices are essentially released by two mechanisms: (i) diffusion of the protein through the pores of the polymer network and (ii) degradation of the polymeric network (George and Abraham 2006). In order to achieve a sustained release profile for protein drugs, the matrix degradation may not be the suitable method since it usually results in the rapid release of the protein. Therefore, for protein delivery, it is apt that the matrix remains intact and the protein diffuses out through the pores. Numerous reports have been published on the encapsulation and release of proteins from alginate matrices.

In a recently published article by Lima et al. (2018), pH-responsive alginate hydrogels have been evaluated for oral delivery of bovine serum albumin (BSA) in acidic and alkaline environments. The hydrogels were found to be compatible with living cells and higher BSA release was observed at pH 7.4, thereby validating them to be ideal for oral protein delivery. In another study utilizing Na-alginate-glycerol dressings for the delivery of therapeutic proteins to wounds, the authors found that the protein release was sustained for more than 72 h (Momoh et al. 2015). The films also showed ideal moisture content that is required for protein conformation and exhibited a good balance of flexibility and toughness.

Rahmani and Sheardown (2018) have explored the potential of protein-alginate complexes as pH-/ion-sensitive carriers for protein delivery. The authors used cytochrome C, lysozyme, myoglobin, chymotrypsin and BSA as model proteins for preparing the complexes. They observed that the proteins could be complexed with alginate in the absence of a cation and the complexes displayed decreased release rates. Furthermore, the protein release was facilitated by environmental triggers such as pH and ionic strength. Reports pertaining to protein encapsulation in alginate hydrogel beads also point to the utility of alginate for controlled protein delivery. Whey protein was encapsulated in Ca-alginate beads using an extrusion device (Zhang et al. 2016). The results suggested that hydrogel beads were suitable for encapsulation and the pH-triggered release of proteins was monitored. L-arginine-g-alginate beads were synthesized by Eldin et al. (2015) to be utilized as carriers for BSA. The grafting of alginate improved its release profile. The preliminary results clearly suggested that the Arg-g-alginate hydrogel may be a potential candidate for polymeric carrier for oral delivery of protein. IPN hydrogel beads composed of Na-alginate-carboxymethyl chitosan have shown great potential towards the oral delivery of BSA (Hu et al. 2016).

Injectable pH-/thermo-responsive hydrogels of poly(ethylene glycol) methacrylate, N-isopropylacrylamide and methacrylated alginate were prepared by Zhao and co-workers (2014). BSA as a model protein drug was encapsulated in situ in the hydrogel. BSA release results indicated that these hydrogels, as carriers, have great potential for long-term localized protein release.

A study by Pescosolido et al. (2011) demonstrated the potential of in situ forming IPN hydrogels based on a physical network of Ca-alginate, interpenetrated with a chemical one based on hydroxyethyl-methacrylate-derivatized dextran (dex-HEMA). BSA was gradually released from the IPNs over approximately 15 days. In situ semi-IPN hydrogels of Ca-alginate and dextran methacrylate (Dex-MA) were obtained by a dispersion of Dex-MA chains into a Ca-alginate hydrogel (Matricardi et al. 2008). The release of protein from these hydrogels validated their efficacy as sustained protein delivery systems.

4.7.2 Wound Dressing

An ideal dressing should always protect the wound from bacterial infection as well as promote healing. Hydrogels have received widespread applications as wound

healing agents because they provide a moist environment, accelerate wound healing capacity, allow gaseous exchange and protect against bacterial infection. Moreover, they are non-toxic and have suitable mechanical and water vapour retention capabilities that can facilitate the healing process. Alginate-based formulations have been used in an array of wound healing applications. Bakhshayesh et al. (2018) reviewed that alginate-based wound dressing formulations such as sponges, hydrogels and electrospun mats are promising candidates for wound healing and have numerous advantages in terms of haemostatic capability and gel-forming ability upon absorption of wound exudates.

The antibacterial and wound healing properties of Na-alginate-PAAm hydrogels have been reported by Zhou et al. (2018). The influence of different divalent ion crosslinking (Cu, Zn, Sr and Ca) on the efficacy of the hydrogels has been explored. In vitro and in vivo study results showed that Zn-crosslinked hydrogel has a spectrum of advantages such as antibacterial activities, cell viability, better mechanical strength and higher ability of wound closure. Studies comprising chitosan-alginate-collagen have also proven them to be quite effective wound dressing materials (Xie et al. 2018). In another study, ZnO nanoparticle-loaded Na-alginate-gum acacia hydrogels displayed excellent wound healing effect on fibroblast cells (Raguvaran et al. 2017).

Summa et al. (2018) studied the in vitro biocompatibility and the efficiency of Na-alginate and povidone iodine (PVPI) polymeric composite in a mouse model for wound healing features. The hydrogel exhibited outstanding wound healing properties of alginates with the bactericidal and fungicidal properties of PVPI, providing a controlled release of antiseptic; thus, it is a good candidate for reduction in inflammatory response both in human foreskin fibroblasts and in rodents after wound induction. The synthesis of ciprofloxacin-loaded electrospun hydrophobic poly(lactic-co-glycolic acid) (PLGA) fibrous mats modified by Na-alginate microparticles was reported by Liu et al. (2018b). The results revealed that alginate improved the wettability and water absorption capacity and improved the release rate of ciprofloxacin from the PLGA fibrous mats and reduced the stiffness of the mats for better protection of the injured area. Furthermore, the burst release of ciprofloxacin which resulted from the addition of alginate could offer an improved antibacterial effect to the PLGA mats.

Alginate crosslinked by calcium gluconate crystals deposited in poly(ε-caprolactone)-b-poly(ethylene glycol)-b-poly(ε-caprolactone) porous microspheres was developed by Liao et al. (2018) for skin wound healing. The porous structure of the microspheres offered additional anchor points for fibroblast attachment and growth, enhancing the cell growth in the hybrid hydrogel.

N-Carboxymethyl chitosan and alginate-based hydrogels were prepared by electrostatic interaction and divalent chelation with epidermal growth factor (EGF) by Hu et al. (2018b) for cell proliferation and wound healing activities. Their investigation suggested that the loading of EGF did not depreciate the mechanical properties of hydrogels. Moreover, the porous 3D structure of the hydrogels permitted sufficient loading and release of EGF and improved cell proliferation indicating that the hydrogel is an excellent candidate for wound healing applications. Carboxymethyl

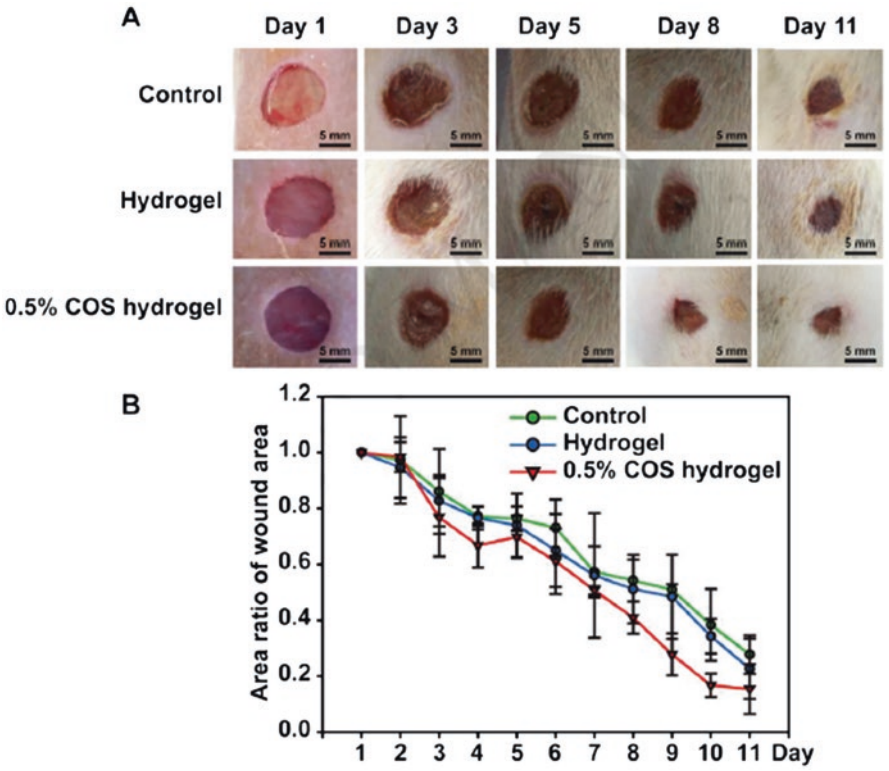

Fig 4.2 Photographs at each time point of 10 mm diameter wounds (**a**) and wound closure curves (**b**) demonstrating the accelerated healing for wounds treated with CMCS/alginate hydrogel and CMCS/alginate hydrogel with 0.5% COS. (Lv et al. 2018)

chitosan (CMCS)-alginate and CMCS-alginate-chitosan oligosaccharide (COS) hydrogels were synthesized by Lv et al. (2018) for in situ wound healing treatment. CMCS-alginate and CMCS-alginate-COS hydrogels demonstrated faster wound contraction, while the healing speed of CMCS-alginate-COS hydrogels was faster as compared with CMCS-alginate. At 11 days of treatment, both the hydrogels showed remarkable increase in thickness and integrity of epidermal tissue and increased construction of collagen fibres (Fig. 4.2.) (Wound healing and cytotoxicity from Lv et al. 2018).

Akbar and co-authors (2018) evaluated the in vivo anti-diabetic and wound healing potential of curcumin-loaded chitosan-alginate-maltodextrin-pluronic-based mixed polymeric micelles. They reported that after 2 weeks of treatment of wound with different formulations, effective wound healing responses in rats were observed. Shahzad et al. (2018) studied the wound healing activity of cefazolin nanoparticles of chitosan loaded into Na-alginate/pectin films crosslinked by calcium chloride. Their finding suggested that after 7 days the films were fairly protective against the growth of the secondary bacterial infections at the wound area. Kamoun et al. (2018)

have demonstrated Na-alginate/PVA hydrogels containing sodium ampicillin as an ideal candidate in wound care. It has also been reported that alginate- and gelatin-based biocomposite wafers containing silver sulfadiazine have a potential wound healing application (Boateng et al. 2015).

The main problem associated with chronic non-healing wounds is drug-resistant infection as the skin barrier functionality reduced. Also, biofilm formation due to the existence of aerobic and anaerobic bacteria produces a drug-resistant infection that escapes the host immune response. Tarusha and co-workers (2018) fabricated alginate hydrogels loaded with hyaluronic acid-lactose-modified chitosan and silver nanoparticles to stimulate wound healing and to regulate bacterial contamination of non-healing wounds. In vitro investigation revealed that hyaluronic acid released by the membrane is able to stimulate the wound healing whereas the silver nanoparticle exploits an effectual antibacterial activity against both planktonic bacteria and biofilms.

Amniotic fluid (AF) is enriched with a varied range of growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF) and transforming growth factor beta 1 (TGF- β1) that are ideal to promote cellular response and wound healing. AF has specified functions and applications like cell proliferation, migration and differentiation which have enormous effect on improvement of the wound healing process. Moreover, AF has revealed to enhance healing in bone, regeneration in tendon tissue and prevention of scar formation in nerve cells. Ghalei and co-workers (2018) fabricated alginate hydrogel-electrospun silk fibroin fibres to deliver AF to the wound site. Fibroblast culturing on the fabricated dressings demonstrated that cellular proliferation, spreading and secretion of collagen enhanced with increasing AF. Taken together, the results provided a novel bioactive dressing with great potentials for speeding up the healing process in severe wounds.

4.7.3 Tissue Engineering

Tissue engineering has emerged as a viable approach to treat disease or repair damage in tissues and organs. The ideal paradigm in tissue engineering is introduction of tissue grafts native to the wounded areas to facilitate the regenerative process. Tissue engineering mostly involves the in vitro seeding and proliferation of cells in a scaffold-based or injectable supports. To ensure that adequate cells reach the target tissue, it is important to have effective cell transplantation process that is capable of sustaining the survival of implanted cells while maintaining their function and improving their adhesion with the host (Sun and Tan 2013). The delivery of cells with the help of a biocompatible material has emerged as an efficient strategy. In this regard, alginate is a classic example because of its versatility and tunability. The potential of alginate as an artificial three-dimensional cellular matrix in a diversity of applications in regenerative medicine has been elucidated beautifully by Giri et al. (2012) and Sun and Tan (2013).

4.7.3.1 Bone Tissue Regeneration

Bone is a complex tissue that comprises of hydroxyapatite and collagen. Bone defects or fractures occur under various medical conditions such as osteoporosis, arthritis, neoplasm, congenital defects, etc. The current standard treatment is based on the use of autograft, allograft and xenograft. Despite the introduction of several augmentation techniques and bone graft materials, bone regeneration still remains a subject of clinical challenge. Autografts and allografts are inevitably associated with certain shortcomings such as donor site morbidity, risk of immune reaction and post-operative pain and infection (Venkatesan et al. 2015). Therefore, there has been a constant need for development of biomaterials that apart from restoring/repairing the damaged bone should also be structurally, functionally and mechanically equivalent to a healthy bone and favour cell adhesion, proliferation and differentiation for bone tissue regeneration. In this regard, alginate has been appraised as a promising biomaterial because of its biocompatibility, non-immunogenicity and biodegradability. A broad overview of alginate composites, their preparation and subsequent applications in bone tissue engineering has been vividly provided by Venkatesan et al. (2015) in their review article.

Over the years, natural polymer-ceramic composites have evolved to be a promising bone graft substitute. While the ceramic provide strength and osteoconductivity, the polymer imparts flexibility and resorbability. Recently, R. A. Popescu et al. (2018) have combined bioactive glass-ceramics with alginate-pullulan hydrogel for the synthesis of new biocompatible hydrogels for in vivo bone tissue regeneration. The proliferation rates for the fibroblast and osteoblast cell viability assays were excellent for the composites. Additionally, the histopathological results displayed good biocompatibility, thus validating their worth in bone regeneration applications. In another report by Tohamy et al. (2018), alginate-hydroxyethyl cellulose-hydroxyapatite composite scaffolds were potentially explored for enhanced in vitro bone regeneration. The authors witnessed higher protein adsorption, cell proliferation and cell viability for human mesenchymal cells. X. Zhang and co-workers (2018) prepared composite hydrogels of Na-alginate-akermanite-glutamate to promote irregular bone regeneration through stem cell recruitment. Their findings revealed no cytotoxicity and higher gene expressions in human bone marrow stromal cells after culturing with hydrogel extracts. The implanted hydrogel also assisted bone mesenchymal stem cell migration to the injured area via CXCR4 (C-X-C chemokine receptor type 4) elevation and stimulated osteogenic differentiation of these cells through the MAPK pathway. This study validated the hydrogels as competent materials for the regeneration of irregular bone cavities.

Clinically used supraphysiological dose of bone morphogenetic protein-2 (BMP-2) usually carries the risk of adverse effects. Thus, an injectable hydrogel composed of BMP-2-loaded recombinant collagen-based microspheres and alginate were developed by Mumcuoglu et al. (2018). BMP-2 doses of 10 μg, 3 μg and 1 μg per implant (50 μg/mL, 15 μg/mL and 5 μg/mL, respectively) were effectively injected subcutaneously in rats in a time- and dose-dependent manner for both ectopic and calvarial rat defect models. Lin et al. (2018) reported about the implantation of alginate fibre with diclofenac and bone cells coated with chitosan for bone regeneration

during inflammation. The outcome revealed that on days 7 and 10, when diclofenac was consumed and the concentrations of inflammatory compounds surged, the coating efficiently blocked the harmful compounds and protected the bone cells within the fibres. In another study by Chen and co-authors (2018c) the effect of an IPN of sodium hyaluronate-Na-alginate scaffold combined with berberine on osteochondral repair was investigated in vivo. The authors explored the mechanism of the osteochondral repair and found that the IPN could simultaneously regenerate not only the cartilage but also the subchondral bone and hence is a promising material for osteochondral defect regeneration. Hydrogels comprising of chitosan-alginate-hydroxyapatite have also been successfully designed and efficaciously utilized for bone tissue engineering therapies with amazing biocompatibility and proliferation with osteoblast cells (Kim et al. 2015; Sharma et al. 2016).

4.7.3.2 Cartilage Regeneration

Despite intensive research, regeneration of articular cartilage largely remains an unresolved medical concern due to their very limited reparative capacity. For cartilage treatment in tissue engineering, the implantation of autologous chondrocytes and stem cells have been explored in the pursuit of cartilage repair (Bidarra et al. 2014). There have been many recent examples where alginate has been employed for cartilage tissue regeneration.

Ruvinov and co-authors (2018) assessed the feasibility and long-term efficiency of a bilayered injectable acellular affinity-binding alginate hydrogel in a mini pig model of osteochondral defects and the outcomes were evaluated after 6 months. Macroscopical and histological evaluation of the defects treated with the hydrogel revealed about the effective reconstruction of articular cartilage layer, glossy surface and cellular organization hyaline tissue associated with noticeable deposition of proteoglycans and type II collagen. The authors concluded that the model showed promising potential of an injectable acellular growth factor-loaded affinity-binding candidate for effective repair and regeneration of articular hyaline cartilage (Fig. 4.3) (Chen et al. 2018c).

Three-dimensional cell printing is a unique technique that enables free-form fabrication of cell-laden hydrogel scaffolds with controllable features and interconnected pores for tissue engineering applications. Thus, bioink materials that are able to offer good printability and favourable cellular interaction are highly desirable. Recent literature has pointed towards the surge of 3D bioprinted alginate scaffolds for cartilage engineering. Yang et al. (2018a, b) constructed printed cartilage tissue using collagen type I or agarose mixed with Na-alginate to serve as 3D bioprinting bioinks by incorporating chondrocytes. The Na-alginate/collagen scaffold could noticeably facilitate cell adhesion and proliferation and improved the expression of cartilage-specific genes such as *Acan*, *Col2al* and *Sox9* than Na-alginate/agarose. The authors further observed that Na-alginate/collagen scaffold effectively suppressed the differentiation of chondrocytes and preserved the phenotype and hence is a promising biomaterial in cartilage tissue engineering. In a study by Markstedt and co-workers (2015), 3D bioprinted human chondrocytes with nanocellulose/alginate bioink for cartilage tissue engineering were developed. The bioinks enabled

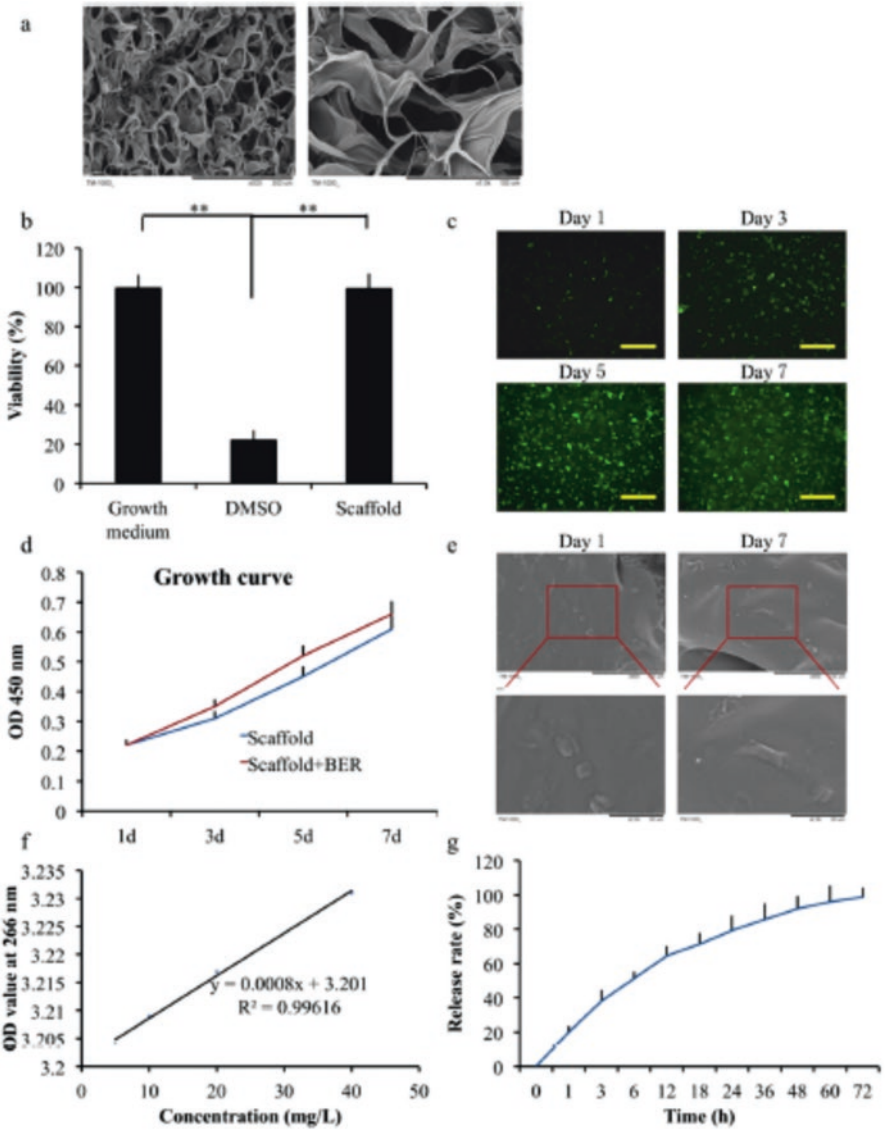

Fig. 4.3 Preparation and characterization of sodium hyaluronate and sodium alginate IPN scaffold. (**a**) Microscopic structure of the scaffold. (**b**) Cell viability in the presence of the scaffold. (**c**) Live-dead cell staining. Scale bars = 200 μm. (**d**) Cell proliferation assay. (**e**) SEM micrographs of BMSCs on the scaffold. (**f**) Standard curve of BER. (**g**) Controlled release profile of BER. (Chen et al. 2018c)

the printing of both 2D grid-like structures and 3D constructs. Additionally, the human chondrocytes bioprinted in the bioink exhibited a cell viability of 86% even after seven days of culture. This study established the efficacy of nanocellulose/alginate bioinks for 3D bioprinting of living tissues. Kundu et al. (2015) have developed 3D cell-printed scaffolds using layer-by-layer deposition of polycaprolactone and chondrocyte cell-encapsulated alginate hydrogel. The 3D cell-printed scaffolds were then implanted in the dorsal subcutaneous spaces of female nude mice. After 4 weeks, the retrieved implants revealed much enhanced cartilage tissue formation in the scaffold.

Several studies have also demonstrated the use of injectable hydrogels for cartilage regeneration purposes because of the ease of handling and complete filling of defect area through minimally invasive surgical instruments. In a study by Liao et al. (2017) an injectable 3D alginate hydrogel crosslinked by calcium gluconate-loaded porous microspheres was assessed for cartilage regeneration features. The authors reported that the defects could be completely healed by 18 weeks and the repaired chondrocytes regained a normal tissue structure. A series of injectable in situ self-crosslinking poly(L-glutamic acid)-alginate hydrogels were fabricated and rabbit chondrocytes were encapsulated in them (Yan et al. 2014). The results affirmed the injectability and rapid in vivo gel formation along with mechanical stability, cell growth and ectopic cartilage formation.

4.7.3.3 Cardiac Tissue Regeneration

Heart failure is one of the most common causes of death globally. Majority cases of heart failure have been due to myocardial infarction (MI) associated with the left ventricle. Since the adult heart lacks regenerative capacity, loss of myocardium is irreversible and ultimately leads to failure. Existing heart failure therapies aim to compensate for the insufficient and low intrinsic regenerative ability of the adult heart. In order to repopulate the areas of cell loss in the damaged hearts, cell regeneration has gained thrust (Bidarra et al. 2014). Alginate has been identified as one building block to accomplish the therapeutic regeneration of cardiomyocytes.

Liberski et al. (2016) have reviewed the current applications of alginate in cardiac regeneration and valve replacement techniques. In another review article by Ruvinov and Cohen (2016), the authors have summarized the versatile applications of alginate as a supporting cardiac implant after acute MI to its employment as delivery vehicles for stem cells and other bioactive molecules and/or regenerative factors to the heart. The preclinical and first-in-man clinical trials using alginate hydrogels leading to myocardial repair and tissue regeneration have been discussed in greater details.

Recently, Sondermeijer et al. (2018) reported the development of a porous, biocompatible 3D alginate scaffold covalently modified with RGDfK (Arg-Gly-Asp-D-Phe-Lys) peptide. Following implantation in the abdominal rectus muscles in rats, the authors observed that the scaffolds seeded with human mesenchymal precursor cells and patched to the epicardial surface of infarcted myocardium induced myocardial neoangiogenesis and significantly improved cardiac function. The

authors established this biomaterial as a potential strategy to deliver cells to myocardial infarct areas to improve neovascularization and cardiac function.

Rosellini and co-workers (2018) have lately fabricated a new class of porous scaffolds by integrating a protein (collagen or gelatin) with alginate for mimicking native ECM for cardiac tissue engineering applications. The gelatin-alginate scaffolds better mimicked the native tissues and exhibited superior mechanical properties. A high viability of the resulting cardiac constructs was observed from the scaffolds after culturing with neonatal rat cardiomyocytes. The authors strongly proposed the protein-alginate scaffolds as viable substitutes for application in cardiac tissue regeneration.

Cardiac stem cells (CSC) are a heterogenic group of cells concentrated in particular areas of heart such as the atria or pericardium. CSCs represent a logical cell type to be explored as a regenerative treatment option for tissues damaged due to MI. Since the isolation of CSCs is time consuming and expensive, O'Neill and co-authors (2018) proposed the incorporation of growth factor-eluting alginate microparticles into collagen-based scaffolds to promote the recruitment and expansion of CSCs. The alginate microparticles were encapsulated with two types of proteins, hepatocyte growth factor and insulin-like growth factor-1, and subsequently incorporated into the collagen matrix. The in vitro assays with isolated CSCs demonstrated that the sustained protein release (which extended up to 15 days) from the scaffolds resulted in motogenic and proliferative effect.

4.7.3.4 Liver Tissue Regeneration

Liver tissue engineering basically deals with the possibility of reproducing in total or in part the functions of the liver in order to treat acute or chronic liver disorders and, ultimately, create a fully functional organ to be transplanted or used as an extracorporeal device. The technological strategies in this direction are based on allocating hepatocytes/hepatocyte-like cells within a 3D structure to ensure their survival and maintain their functional phenotype. The recently published review article by Mazza et al. (2018) elucidates liver tissue engineering with precision in terms of implantable liver tissues to whole organ engineering.

A recent study carried out by Liu and co-authors (2018c) employed Ca-alginate gel sheets, embedded with liver cells (RLC-18) with the intention to imitate liver lobule tissue. The Ca-alginate sheets having hepatic lobule-shaped patterns were deposited onto a microelectrode device using electrodeposition and the viability of embedded cells exceeded 80%. The cell sheets were removed from the electrode substrate and stacked onto a 3D multilayered structure to mimic the morphology of liver lobule tissue. The authors concluded that the developed method could provide a new bottom-up paradigm to build 3D macroscopic liver tissue similar to that in vivo.

Developing 3D cell culture systems is necessary for investigating the mechanism of hepatocellular carcinoma (HCC) metastasis and screen therapeutic drugs. Sun et al. (2018) constructed decellularized liver matrix-alginate (DLM-ALG) hybrid gel beads for the 3D culture of HCC cells. DLM-ALG beads exhibited better activity of matrix metalloproteinases (MMPs) of HCCLM3 cells, including MMP2 and

MMP9 and urokinase plasminogen activator system. The findings of this study established DLM-ALG beads as potential in HCC research and subsequently in liver tissue engineering.

Injectable hydrogels synthesized from glycyrrhizin, alginate and calcium for 3D cell culture in liver tissue engineering has been reported by Tong et al. (2018). The hydrogels showed good biocompatibility and could maintain the viability, proliferation and liver function for longer periods of time. Moreover, the hydrogels enhanced the mRNA expression of cytochrome P450, which were key enzymes to the metabolization of hepatocytes and could be a potential 3D cell culture system for liver tissue engineering.

4.7.3.5 Nerve Tissue Regeneration

Neural regeneration research is designed to develop strategies for therapy for nerve damage incurred by disease or injury. In order to fabricate fully functional and biomimetic nerve substitutes, various kinds of polymeric-based scaffolds have been proposed. Artificial nerve grafts need to mimic the native extracellular matrix both structurally and mechanically in order to provide the appropriate environment for the neotissues. Furthermore, artificial nerve grafts need to be electrically conductive to support the electrical conduction of injured nerve during regeneration and enhance regeneration. The biomaterial further needs to induce appropriate chemical and physical signalling cues, which transduce into intracellular biochemical responses to moderate the cellular function. Alginate being a negatively charged polysaccharide has been widely applied to develop artificial constructs for peripheral nerve tissues (Prang et al. 2006).

Bioprinting Schwann cell-encapsulated scaffolds using alginate, fibrin, hyaluronic acid and/or RGD peptide were synthesized by Ning et al. (2018) for nerve tissue engineering. The printed scaffolds enhanced the alignment of Schwann cells inside scaffolds and provided haptotactic cues to direct the extension of dorsal root ganglion neurites, which has potential applications in nerve tissue engineering. A composite hydrogel of PAAm-graphene oxide-gelatin-Na-alginate for accelerating peripheral nerve regeneration was fabricated through in situ free radical polymerization (Zhao et al. 2018). The hydrogel displayed remarkable adhesion and proliferation of cultured Schwann cells. The results of this study validated the hydrogel for neural tissue engineering applications by promoting Schwann cell growth. In another study, Golafshan and co-workers (2017) developed graphene-Na-alginate-PVA scaffolds for engineering neural constructs. The scaffolds displayed superior electrical and mechanical properties with enhanced PC12 cell interaction. Overall, the developed scaffolds were promising devices for peripheral nerve regeneration. Wu et al. (2017) studied about the neural tissue engineering through chitosan-polylactide-alginate fibres. Nerve growth factor (NGF)-induced neurite extension of PC12 cells confirmed about the bioactivity of NGF released from fibres was well retained. Bu and co-authors (2018) have synthesized a conductive polymer of Na-alginate-carboxymethyl chitosan crosslinked with Ca^{2+} ions. The conductivity of the hydrogel was implemented by doping with polypyrrole. The conductive

hydrogel exhibited excellent biocompatibility and repair features as a bioactive biomaterial and is potent for neural tissue engineering applications.

In a novel methodology, Buyukoz et al. (2018) utilized a combined strategy of thermally induced phase separation and porogen leaching to create interconnected macropores and nanofibrous structures. Gelatin scaffolds integrated with nerve growth factor-loaded alginate microspheres were prepared by the aforementioned strategy. The scaffolds had good topologic and mechanical properties similar to brain tissue and pore structure suitable for cell growth and differentiation and can have potential applications in brain tissue engineering.

4.8 Conclusion and Future Perspectives

To summarize, alginate has been extensively utilized in drug/protein delivery or as building blocks for tissue repair and regeneration. Owing to their versatility, alginate IPNs have been tailored with the desired structures, properties and functions. Alginate-based biomaterials are promising substrates for tissue engineering applications with the advantage that both drugs and cells can be readily integrated into the scaffolding matrix. The surge in alginate bioinks in 3D bioprinting points to the greater utility and success of the biopolymer in tissue engineering. Successful exploitation of alginate-based biomaterials in different tissues and organs such as bone, cartilage, cardiac, liver and nerve suggests their promising future for repair and regeneration applications. The applications of alginate IPNs as wound healing dressings are manifold. Alginate has rendered itself as a hugely potent wound dressing agent.

Engineering of more alginate-based biomaterials endowed with precisely designed physical, chemical and biological properties should be carried out to mimic the environment of natural tissues. The design of such alginate IPNs could further revolutionize the applicability of alginate IPNs in the world of biomedicine.

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pH Sensed Interpenetrating Polymeric Network: Application in Drug Delivery

5

Brahmeshwar Mishra, Mansi Upadhyay, and Bharti Bakde

Abstract

In recent years pH-sensitive interpenetrating polymeric network (IPN) has emerged out as an excellent drug delivery vehicle in controlling the release of numerous therapeutics. Application of 'smart', stimuli sensed polymers and the topology of polymeric network of IPN in combination have made this possible. These intelligent delivery systems through mechanism of swelling, extent of cross-linking, concentration of polymeric ratio and change in pH of the environment or media release the entrapped drug at specific time and site in a programmed way. Due to variation in pH in different parts of the body in normal or pathological condition, preparation of such pH sensed IPN could be useful. The literature evidenced development of large numbers of intelligent IPNs with excellent and efficient results. This chapter highlights a brief overview on hydrogel, its translation to smart IPN and its application in delivery of several drugs belonging to the different classes or category.

Keywords

Hydrogel · Interpenetrating polymeric network · pH sensitive · Swelling · Drug release

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5.1 Introduction

With an increased discovery and development of novel drugs, the attention towards fabrication of advanced and smart drug delivery system has also increased (Creque et al. 1980). Following a conventional route of administration, when drug passes through tissues and liver, it undergoes biotransformation before reaching the systemic circulation that may result into degradation (chemical/enzymatic); thus significant portion of the drug is destroyed and the therapeutic level might not be achieved with the therapeutic dose (Orive et al. 2003). To overcome such adversities related to conventional dosage system that is markedly recognized for its low bioavailability, pharmaceutical engineers are looking for 'intelligent' drug delivery system that can sense and determine the signal produced from the disease and depending upon the extent and strength of that signal can release specific amount of drug to the site of action (Misra et al. 2008). Release of bioactive agents through these types of system could only be expected from controlled delivery system. Preparation of controlled drug delivery requires smart matrix or carrier that increases the drug bioavailability by releasing the drug at predetermined time for certain period and, thus, curtails the wastage of drug as well as the systemic and local side effects (Balmayor et al. 2011). Hydrogels, three-dimensional macromolecular structures, have always been an area of interest in the past and in the present pioneering research. It has occupied approximately all the research areas, for instance, branch of medicines, oncology, immunology, cardiology, biotechnology, pain and wound healing management, due to its attractive feature of retaining high water content and cross-linked network. Both these inherent properties of hydrogel ensure to accord excellent tissue flexibility (water content of 70–99% provides soft and elastic consistency) (Li et al. 2014) and tunable mechanical strength (ranging from 0.5 kPa to 5 MPa), thus paralleling the physical property with flexibility of the soft tissues in human (Li and Mooney 2016). Hydrogels came into existence after serendipitous discovery by Otto Wichterle and Drahoslav Lin in 1960 who designed a new hydrophilic polymer poly(2-hydroxyethyl methacrylate) (PHEMA) for ophthalmic application (Kopecek 2009). Since then, this drug delivery vehicle has shown now drastic changes from simple (generation I) to smart network (generations II and III). Generation I focussed on the milestone as set by Wichterle and Lim. It dealt only with development of simple network and chemical cross-linking of synthetic polymers specifically useful in ophthalmic preparation and drug delivery (Buwalda et al. 2014). After 1960, in the year 1968 Katchalsky et al. brought a revolution by converting chemical energy into mechanical energy and that is how a new era of stimuli sensed 'smart' or 'intelligent' hydrogel took place. Afterwards the focus of research on simple macromolecular hydrogel was shifted towards stimuli sensed hydrogel. The hydrogels that were included in generation II were pH sensitive, temperature sensitive, light sensitive, sound sensitive and electric current sensitive, etc., whereas generation III (mid-1990) emphasized on new techniques of cross-linking such as inclusion complex formation peptide interaction, metal-ligand interaction, etc., in order to strengthen the mechanical and thermal properties of hydrogel.

5.2 Overview on Interpenetrating Polymeric Network (IPN)

Polymers today are of great interest. The increased interest of pharmaceutical industries towards manufacturing of drug delivery vehicle at macroscale level has fuelled to synthesize numerous polymers (Ranade 1990). Although several polymers, either of natural or synthetic origin, are frequently used in drug delivery applications, multicomponent polymer mixtures are mostly preferred nowadays mainly because of their good mechanical strength (Klempner 1994). Interpenetrating polymeric network (IPN), an advanced class of hydrogel (generation II), is a type of multicomponent polymeric mixture, has grown promptly in field of drug delivery (Hoare and Kohane 2008) and is defined as concoction of two or more polymers (natural/synthetic individually or in combination), where at least one of the polymers is either synthesized or cross-linked in the immediate presence of each other (Sperling and Hu 2003). IPNs based on their cross-linking manner are classified as full IPN or semi-IPN. If both the polymers in a network are cross-linked, then a full IPN is formed; however if only one component, i.e. polymer in a network, is cross-linked leaving another one in linear form, it is termed as semi-IPN (Aminabhavi et al. 2015). Thus, IPN can be differentiated from simple mixture of polymers in terms of solubility; i.e. they do not dissolve but swell, as cross-linkers maintain the rigidity of the polymeric chain network that reinforces the swelling rather than solubility (Upadhyay et al. 2018a). A schematic presentation of full IPN, semi-IPN and polymer blend is presented in Fig. 5.1.

5.3 History of IPN: From Simple to Smart

Basis of IPN was led in 1914 by Aylsworth while preparing simultaneous interpenetrating polymeric network (SIN) of phenol and formaldehyde which was vulcanized with rubber and sulphur to make it thick and tough; thus the polymer network formed was utilized further for commercial purpose (Lohani et al. 2014;

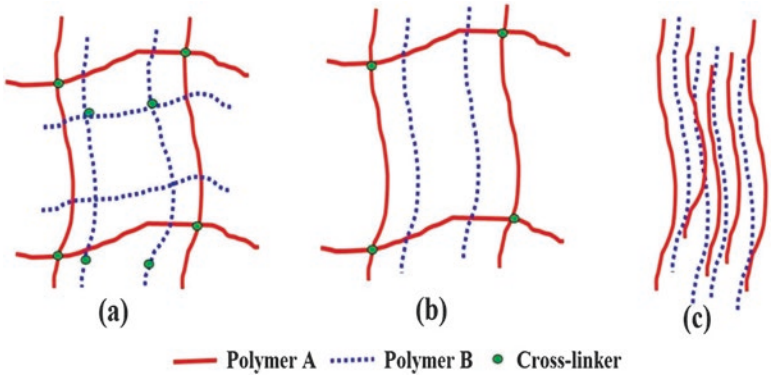

Fig. 5.1 Diagrammatic presentation of (**a**) full IPN, (**b**) semi-IPN, (**c**) polymer blend

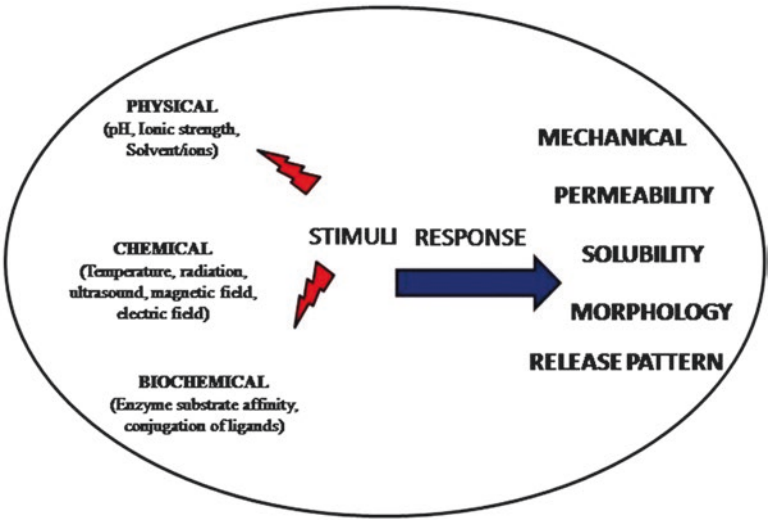

Fig. 5.2 Changes or response in the system due to the external application of stimuli

Sperling and Mishra 1996). Following the work of Aylsworth, several newly synthesized polymers also came into existence; for instance, Amos (1974) formed high impact polystyrene by graft copolymer synthesis of styrene-butadiene rubber (SBR) with polystyrene. In both the cases still the word IPN was not recognized, though both of them described their formed polymer network in their own languages and patented it. Afterwards researchers started seeking interest towards polymers and prepared other IPNs; for example, Solt et al. (1955) formed an ion exchange resin through IPN and Staudinger et al. prepared homo-IPN (Sperling 2012). These above-formed polymers were formed successfully but remain unnamed until John Millar in the year 1960 coined the term IPN during preparation of homo-IPN via styrene suspension polymerization (Millar 1960). Afterwards, series of IPNs appeared; for example, in the same year 1969, Frisch et al. and Sperling et al. developed latex and sequential IPNs, respectively, along with identification of topology of the elastomeric network of IPNs and its mechanical behaviour (Harrats et al. 2005). In 1971, Sperling and Arnts worked together to develop simultaneous IPN (Sperling and Arnts 1971). After recognition of polymeric network and their behaviour, the investigator turned their research towards synthesis of 'smart IPNs'. In the era of 2000s the fabrication and application of these systems were in great demand (Matricardi et al. 2013). The feature of smartness in IPNs is due to the 'intelligent' materials called as polymers (Culver et al. 2017). From the past few decades application of 'smart' polymers also termed as 'intelligent', 'stimuli-sensitive' or 'environmental-responsive' polymers have gained much attention in drug delivery and biomedical applications (Huh et al. 2012). Stimuli sensed polymers are the polymers that are sensitive to certain physical (temperature, radiation, ultrasound, magnetic and electric field, etc.), chemical (pH, ionic strength, etc.) and

biochemical (enzyme substrate affinity, conjugation of ligands, etc.) triggers and applying any of the above stimuli to the polymers leads to a noticeable change either in the morphology or in the solubility or release pattern of the active molecule or drug from the delivery vehicle or molecular bond arrangement (Fig. 5.2).

The changes exhibited by 'smart' polymers are reversible in nature and can be returned to their initial state, i.e. to their original shape and size after removal of the stimuli; that is why they are also referred as 'intelligent' polymers.

The present chapter focusses on novel interpenetrating polymeric network composed of pH sensed polymers and their application in drug delivery.

5.4 pH Sensed Polymers and Their Release Mechanism

pH-sensitive polymers exhibit change in their arrangement or configuration depending upon the pH of the surrounding environment. This transition could either result into swelling or shrinkage of the system (Xie et al. 2017). The backbone of these polymers contains acidic (generally carboxylic or sulfonic groups) or basic functional groups (amine group). Once in contact with the aqueous vehicle of suitable pH and ionic strength, they dissociate and generate some fix ions on the polymeric network leading to the swelling or shrinkage of the polymeric system (Sakthivel et al. 2015). Figure 5.3 illustrates change in the swelling and shrinking behaviour of anionic and cationic polymers.

The commonly used acidic and basic polymers used in drug delivery and biomedical applications are presented in Table 5.1.

While fabricating a drug delivery system the pH of gastrointestinal tract (GI) must be taken into consideration. The variation in the pH ranging from stomach to intestine as well as change in the pH during chronic condition such as wound or in

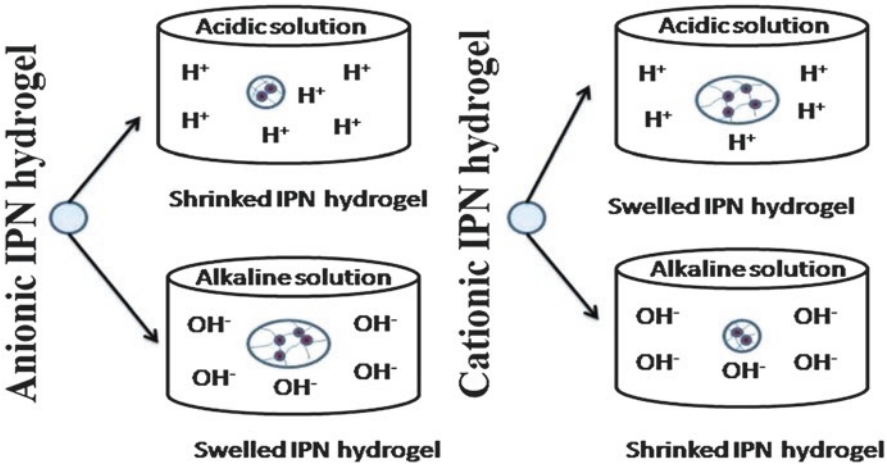

Fig. 5.3 Change in the polymeric structure of IPN hydrogel at acidic and alkaline pH

Table 5.1 Examples of pH-sensitive polyacidic and polybasic polymers

pH-sensitive polymers	Polyacidic	References
Anionic group	**Synthetic polymers**	
Carboxylic group	Poly(acrylic acid) (PAA)	Liu et al. (2010)
	Poly(methacrylic acid) (PMMA)	Chen et al. (2005)
Sulfonic group	2-Acrylamido-2-methyl propyl sulfonic acid	Gad (2008)
	2-Methacryloxyethylsulfonic acid	Centomo et al. (2012)
	Ethylenesulfonic acid	Breslow and Kutner (1958)
	Styrenesulfonic acid	Kang et al. (1992)
	2-Methylpropylsulfonic acid	Szilágyi and Zrínyi (2005)
	Sulfoxyethyl methacrylate	Rannard et al. (2007)
	Propionic acid	Yin et al. (2006)
	Natural polymers	
	Alginic acid	Kim and Lee (1992)
	Hyaluronic acid	He et al. (2013)
	Carboxymethyl cellulose	Cha et al. (2012)
	Carboxymethyl dextran	Zhang et al. (2005)
	Polybasic	
Cationic group	**Synthetic polymers**	
Amine group	Poly(2-diethylaminoethyl methacrylate (PDMAEMA)	Li et al. (2005)
	Acrylamide	Şen et al. (2000)
	Aminoethyl methacrylate	Emileh et al. (2007)
	Dialkyldimethylammonium chloride	Colomer et al. (2012)
	Natural polymers	
	Chitosan	Qu et al. (2000)
	Poly(lysine)	Choi et al. (1995)
	Poly(histidine)	Lee et al. (2003)

a deadly disease such as cancer differs from each other. Thus, proper selection of the smart polymers should be done (Schmaljohann 2006). A variation in pH along GI tract and in different physiological conditions is exhibited in Table 5.2.

Generally, the release of drug from such polymeric matrix follows combined effect of polymer swelling and diffusion (Costa and Lobo 2001; Singh et al. 2015). The polymeric networks in non-swollen condition remain normal and without pores; however when swelled in the presence of surrounding media and also depending upon the functional groups, polymer network forms pores and allows diffusion of the drug slowly in a controlled manner (Vashist et al. 2014). For instance, a drug encapsulated within anionic polymer shows minimum swelling in the stomach due to non-ionization of the acidic group. The polymeric network remains close to each other and resist the release of drug but as soon as the delivery vehicle enters the alkaline or neutral region of the intestine the pH range rises above the pKa, the same acidic pendant group now ionizes resulting generation of electrostatic repulsive force

Table 5.2 pH value of various cellular and tissue components (Rofstad et al. 2006; Watson et al. 2005)

Cellular components/tissue	pH
Stomach	1.0–3.0
Intestine	7.0–7.4
Duodenum	4.8–8.2
Colon	6.8–7.0
Early endosome	6.0–6.5
Late endosome	5.0–6.0
Lysosome	4.5–5.0
Golgi apparatus	6.4
Tumour	7.2–6.5
Blood	7.35–7.45

among the ionized group resulting swelling of the polymeric network and allowing drug to ooze or diffuse from the pores. With contrast to anionic polymers, a cationic polymer that contains basic functional groups behaves opposite to that of the acidic polymers (Almeida et al. 2012)**.** Biologically, anionic polymers are preferred in case of oral drug delivery whereas cationic polymers are used for drug delivery to the inflamed tissues that typically possess low pH (Schoener and Peppas 2012).

5.5 Development of pH Sensed IPNs and Their Application in Different Classes of Drug

Smart IPNs are composed of smart polymers and possess a unique property to respond quickly to a sudden change in the pH or temperature thus, modifies the release of drug in accordance. The literature is flooded with the use of wide variety of pH-activated smart materials that have been extensively used and successfully translated the simple IPN into a smart IPN. The widely explored polymers such as poly(methacrylic acid), poly(acrylamide), poly(acrylamidoglycolic acid), poly(2-diethylaminomethyl methacrylate), poly(acrylic acid), etc., are used in the preparation of intelligent IPNs in combination with other polymers, for example, poly(ethylene glycol), chitosan, gelatin, poly(vinyl alcohol), etc., to deliver several therapeutics in different ailments or disease. Some examples of therapeutics and their delivery through IPN matrix composed of pH sensed polymers are discussed below.

5.5.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

With respect to controlled, extended and targeted delivery of NSAIDs, pH-sensitive IPNs are the most explored drug delivery vehicle. Some commonly used NSAIDs that have been delivered through pH sensed IPNs are summarized in Table 5.3.

Table 5.3 Examples of NSAIDs delivered through pH-sensitive IPN hydrogel

Drug	Parent polymer	Cross-linker	Drug release at pH	References
			Acidic alkaline	
Ketoprofen	Polyacrylamide, gum ghatti and sodium alginate	Calcium chloride and glutaraldehyde	17% 83%	Boppana et al. (2015)
Ketoprofen	Polyacrylamide, tamarind seed gum and sodium alginate	Calcium chloride and glutaraldehyde	18% 83%	Boppana et al. (2016)
Ketorolac	Poly(vinyl alcohol) and carboxymethyl cellulose	Glutaraldehyde	40%; 60% 99.6%	Kondolot Solak and Er (2016)
Nifedipine	N-Succinyl chitosan and sodium alginate	Calcium chloride	11.6% 74%	Dai et al. (2008)
Diclofenac	Poly(vinyl alcohol) and poly(acrylic acid)	Glutaraldehyde	3.76% 100%	Kurkuri and Aminabhavi (2004)

5.5.1.1 Ketoprofen

Ketoprofen (KPF), a propionic acid derivative with antipyretic and analgesic effect, is also used in inflammatory and joint disorders (Carbone et al. 2013). The drug on oral administration absorbs quickly through GI tract and has short elimination half-life of 2 h and thus shows incomplete absorption and serious adverse effect. Hence, a controlled release of KPF is required. To overcome the problems associated with KPF, Bopanna et al. (2015) attempted to prepare pH-sensitive IPN microbeads using polymers sodium alginate and polyacrylamide grafted gum ghatti by dual cross-linking of the polymers with calcium chloride and glutaraldehyde for gastro-protective controlled release of KPF. The formed IPN microbeads showed excellent reversible pH sensitivity that was assured through swelling study performed at two different pH, i.e. 1.2 and 7.4. In acidic solution, the carboxylic group of the polymeric matrix undergoes protonation leading to the shrinkage of the microbeads whereas in alkaline solution the ionizable carboxylic group deprotonated resulting into collective electrostatic repulsive force among the ionized group, thus swelling of the microbeads. Further, drug release of KPF from pH-sensitive polymeric microbeads, showed controlled release of 83% till 12 h at pH 7.4. The result of in vivo pharmacokinetic study revealed higher area under the curve (AUC) and elimination half-life ($t_{1/2}$) of the developed IPN microbeads in comparison to pure KPF is a proof of concept that the prepared IPN microbeads composed of pH-sensitive polymers were capable of enhancing the biological half-life and improving the systemic circulation of the poorly absorbed KPF.

In order to understand further effect of some new natural grafted polymer on same model drug KPF, Boppana et al. (2016) performed one more experiment involving preparation of pH-sensitive polyacrylamide grafted tamarind seed polysaccharide and sodium alginate. The grafting and synthesis of tamarind seed gum

with polyacrylamide was done by microwave irradiation technique. The developed dual cross-linked (calcium chloride and glutaraldehyde) IPN of alginate and grafted polymer exhibited pulsatile swelling and shrinking with respect to change in pH. The cumulative drug release of 83% at pH 7.4 and 18% at 1.2 is a proof that the formed microbeads are more sensitive towards targeting maximum drug to the intestine region rather than stomach.

Novel pH sensed IPN hydrogel beads of polyacrylamide grafted xanthan gum and sodium carboxymethyl cellulose, fabricated by Kulkarni and Sa (2008), also showed altered swelling and release behaviour with a change in the pH from 1.2 to 7.4. The grafting of xanthan gum with polyacrylamide was done by radical polymerization technique followed by preparation of IPN beads by cross-linking grafted xanthan gum and sodium carboxymethyl cellulose with aluminium chloride.

5.5.1.2 Ketorolac Tromethamine

Ketorolac tromethamine (KT), another therapeutic agent which belongs to the class of NSAIDs, is commonly used for moderate to severe pain management (Berger and Zaghi 2011). However, its short elimination half-life and variable dosing frequency for different dosage form such as for tablet, administration of drug should not exceed more than five days whereas for intravenous or intramuscular dosage form not more than two days. Due to such variable pattern for the administration of drug, Solak and Er (2016) developed a pH-sensitive IPN microsphere of PVA and carboxymethyl cellulose for the controlled delivery of KT. The microspheres were prepared by emulsion cross-linking method. The drug release of KT entrapped within pH sensed polymer was performed at three pH values of 1.2, 6.8 and 7.4 and was found to be highest at pH 7.4 (99.6%) in comparison to the release of drug at pH 1.2 (40%) and pH 6.8 (60%), suggesting that IPN delivery vehicle could be a useful delivery device for controlled/sustained release of drug for targeted delivery to the colon or intestine region.

5.5.1.3 Nifedipine

Nifedipine is a short-acting dihydropyridine calcium channel blocker (Snider et al. 2008). It is associated with several side effects such as tachycardia, myocardial and cerebrovascular ischemia, etc.; thus, its extended delivery must be developed (Gibbons et al. 2003). Phadke et al. (2015) by utilizing grafting technology synthesized a grafted polymer acrylamide grafted chitosan copolymer (AAm-g-CS) in the presence of potassium persulfate as an initiator and interpenetrated it with PVA by using cross-linker glutaraldehyde. To make the formulation pH sensitive and also to extend the drug release, the microspheres were coated with sodium alginate. The pH-sensitive IPN microspheres prepared by water in oil emulsion method exhibited maximum drug entrapment (96%) with maximum drug release (93%) for 14 h depending upon the composition of IPN mixture, extent of cross-linking and quantity of acrylamide.

Another experiment performed by Dai et al. (2008) for the controlled delivery of nifedipine by blending the pH sensed N-succinyl chitosan and alginate IPN hydrogel beads was studied by varying the ratio of polymeric blend, ratio of drug to

polymer, amount of cross-linker and gelation time. Through study it was found that the release of drug at pH 1.5 was comparatively low (11.6%) than at pH 7.4 (74%), suggesting the pH-stimulated IPN hydrogel beads to be a suitable carrier for its delivery to the intestinal tract.

5.5.1.4 Diclofenac Sodium

Diclofenac, a phenylacetic derivative, is an extensively used analgesic, antipyretic and anti-inflammatory drug (Altman et al. 2015). It is highly acidic drug (pKa = 4) with high risk of local irritation and varying dosage regimen shows adverse reaction, for example, peptic and gastric ulcer, renal dysfunction, hypersensitivity reaction, etc. (Sallmann 1986). To avoid the above problems, a sequential pH-sensitive IPN of poly(vinyl alcohol) and poly(acrylic acid) prepared by Kurkuri and Aminabhavi (2004) demonstrated controlled release of diclofenac from the formed microspheres to the intestine. The factors responsible for the slow and prolong release of the drug were found to be pH of the medium, degree or extent of cross-linking and the amount of drug loading.

In another study, Wang et al. (2009) with an attempt to reduce the side effects and dosing frequency of diclofenac developed a series of pH-sensitive hydrogel beads consisting of chitosan-g-poly(acrylic acid)/attapulgite/sodium alginate (CTS-g-PAA/APT/SA). The role of attapulgite, polymeric clay was to enhance the mechanical strength. pH sensitivity of the prepared hydrogel beads was examined by the result of in vitro release study. It was found that cumulative release of diclofenac from the beads was 3.76% at pH 2.1 and 100% within 24 h at pH 6.8. However in case of pH 7.4 a noticeable result was observed, i.e. 100% cumulative release within 2 h. The obtained result suggested that the release of the drug was swelling and polymeric clay dependent.

5.5.2 Cytotoxic Agents

From the past few decades application of ‘intelligent’ polymers for oral drug delivery in the area of oncology has been widely studied. Few of them are discussed below.

5.5.2.1 5 -Fluorouracil

5-Fluorouracil (5-FU) is an extensively used anticancer agent against colorectal cancer. Its existence and application in oncology field is approximately more than 50 years ago when it was developed by Heidelberger et al. (1957). Due to poor oral absorption and intra-patient variability, a controlled release preparation of 5-FU for colon delivery via sequential IPN hydrogel composed of natural polymer konjac glucomannan (KGM) and cationic pH-sensitive polymer PMMA by Xu et al. (2013) was developed. The preparation of formulation was done by pouring a solution of monomeric methacrylic acid with cross-linker N,N′-methylenebisacrylamide and dropping the formed mixture into the dried KGM gel. Swelling study revealed the

pH sensitivity of the formed IPN hydrogel. Further, the in vitro release behaviour of 5-FU was found to be in controlled manner due to swelling and enzymatic degradation caused by enzyme Mannaway 25 L (enzyme available in colon region) present in the dissolution media during the study suggested the hydrogel bead to be suitable for colon-specific drug delivery.

5.5.2.2 Capecitabine

Capecitabine, an oral chemotherapeutic and a prodrug of 5-FU, was developed to mimic the intravenous infusion of 5-FU (Meulenaar 2013). The drug is commonly used for colon cancer and breast cancer (Schellens 2007). The prescribed twice daily dosing (1250 mg/m^2) and short biological half-life (0.5–1 h) exhibits adverse effects such as bone marrow depression, cardiotoxicity, diarrhoea, etc. Thus, a controlled release dosage form of capecitabine is required that can reduce dose as well as dosing frequency (Upadhyay et al. 2018b). Alange et al. (2017) developed pH-sensitive spray dried microspheres using hydrolysed polyacrylamide-g-carboxymethylcellulose sodium (PAAm-g-NaCMC) for the targeted colon delivery of the capecitabine by free radical polymerization technique. The pH sensitivity of the polymer was done by swelling study. The entrapment efficiency of the microspheres was found in the range of 70.98–94.41%. A comparative drug release study performed for the microspheres composed of pure sodium carboxymethyl cellulose failed to retard the drug release in a simulated dissolution media of stomach and intestine whereas the microbeads prepared with pH sensed polymer showed extended release of drug up to 24 h.

5.5.2.3 Doxorubicin

Doxorubicin is a wide-spectrum antineoplastic antibiotic agent that belongs to the class of anthracycline obtained from the bacteria *Streptomyces* (Patel and Kaufmann 2012). Doxorubicin is given intravenously as a single dose which should not be increased more than 400 mg; otherwise it will lead to severe cardiac damage (Lum et al. 1985; Weiss 1992). So far, nanotechnology has shown successful effort towards targeted delivery of doxorubicin. Livatag and Doxil are the best examples. Both these nanoformulations have reduced the multidrug resistance; however, their high dosage, treatment duration and route of administration are same as that of free drug. Thus, a novel polymeric dosage formulation must be considered for the controlled delivery of doxorubicin (Gabizon 2001). Semi-IPN hydrogel fabricated with novel biodegradable polysaccharide Salecan and synthetic polymer PMMMA developed by free radical polymerization and cross-linked with N,N′-methylenebis(acrylamide) exhibited pH-dependent controlled drug delivery of doxorubicin. The release of doxorubicin from the IPN hydrogel was pH dependent showing accelerated release at pH 7.4 whereas low at pH 5.0 (Qi et al. 2015). The cellular uptake of hydrogel-loaded doxorubicin by HepG2 and A549 cells during cytotoxicity and cellular uptake studies showed reduction in the proliferation of cancer cell indicated that the prepared pH-responsive semi-IPN hydrogel can result into a promising and effective drug delivery vehicle against cancer.

5.5.2.4 Curcumin

Curcumin, a polyphenolic compound extracted from the Indian spice 'haldi' or *Curcuma longa*, is a natural antineoplastic agent (Nobili et al. 2009). During 1987, National Cancer Institute tested numerous natural agents for anticancer activity and among them only curcumin exhibited potent antitumour effect (Park et al. 2013). To date, application of curcumin has shown effective and improved result in various solid tumours (Patel et al. 2010). However, its hydrophobicity, poor bioavailability, high dose and repeated dosing pattern show undesirable pharmacokinetic properties (Ammon and Wahl 1991; Madhavi and Kagan 2014). IPN nanogel of curcumin encapsulated within natural polymer gelatin and poly(acrylamidoglycolic acid; AGA) and fabricated by free radial emulsion polymerization technique showed stable and controlled release for 4 days of curcumin from IPN nanogel. The release was pH dependent and increased with the increase in hydrophilic AGA content. The pH sensitivity of the nanogel was confirmed by measuring the mean hydrodynamic diameter of the nanogels by differential light scattering method at different pH varying from 1.2 to 5.0 that showed low diameter and 5.0–8.5 exhibited high mean diameter of the nanogel based on the concept of protonation and deprotonation, respectively. The anticancer activity performed on HCT-116 colorectal cancer cell line demonstrated enhanced tumour cell reduction, suggesting this novel IPN nanogel composed of intelligent polymer can be an effective carrier for cancer treatment (Rao et al. 2015).

5.5.3 Antidiabetic Agents

5.5.3.1 Insulin

Insulin is a peptide hormone secreted by the β-cells of pancreatic islets of Langerhans. The role of insulin is to maintain the normal blood glucose level by facilitating cellular glucose uptake (Wilcox 2005). However, the most critical problem with this protein nature drug is that it has to be administered intravenously because of tissue impermeability and short biological half-life. Further, in chronic condition even consumption of multiple injections from week to month which may remain lifetime is also suggested that could result into high toxicity and poor patient compliance (Wu and Jin 2008). A sustained polymeric oral delivery of insulin can be a promising strategy. Mukhopadhyay et al. (2013) demonstrated successful oral delivery of insulin to intestine by pH-sensitive N-succinyl chitosan grafted polyacrylamide hydrogel. The prepared hydrogel showed 38% and 76% loading and entrapment efficiency of insulin, respectively. Maximum release of insulin at intestinal pH (98%) than stomach pH (26%) confirmed its excellent pH activity and more affinity towards intestinal tissue. The per oral in vivo pharmacokinetic study performed in diabetic mice showed successful lowering of blood glucose level with increased bioavailability of approximately 4.43%. Thus, the developed pH-sensitive poly(acrylamide) and chitosan grafted hydrogel was found to be non-toxic and acted as suitable carrier for oral delivery of insulin.

5.5.3.2 Glipizide

Glipizide is a second-generation sulfonylurea hypoglycaemic agent. Its poor oral absorption, short-acting biological half-life and practically insoluble in aqueous solution show bioavailability problem. pH-sensitive hydrogel of gelatin and poly(methacrylic acid) loaded with glipizide and cross-linked with glutaraldehyde exhibited higher swelling in alkaline pH in comparison to acidic pH confirming the polymers to be pH sensed through swelling and deswelling property. The drug release over a period of 12 h showed sustained release of glipizide from the IPN delivery vehicle (Gupta et al. 2007).

5.5.4 Antibiotics

5.5.4.1 Ciprofloxacin

Ciprofloxacin is a wide-spectrum fluoroquinolone antibiotic with elimination half-life of 4 h. The drug is useful in treatment of typhoid, osteomyelitis, gonorrhoea and infectious conditions such as septicaemia, urinary tract and respiratory, etc. (Herrlin et al. 2000). To extend the plasma half-life, Kajjari et al. (2011) blended acrylamide grafted guar gum with chitosan and cross-linked it with glutaraldehyde. The formed IPN microsphere showed 74% entrapment of ciprofloxacin with an extended release over 12 h at alkaline pH 7.4.

5.5.4.2 Clarithromycin

Clarithromycin is a macrolide antibiotic used specifically in the treatment of peptic ulcer and upper respiratory tract infections, for example, pneumonia, chronic bronchitis, maxillary sinusitis, etc. (Ramteke et al. 2006). The recommended high dose of 500 mg twice daily causes adverse effect. To minimize the dose and sustain the release chitosan and PVP-based semi-IPN was prepared by Vaghani and Patel (2011). The hydrogel exhibited 97% drug content. The developed hydrogel containing chitosan (2%w/v) and PVP (4%w/v) demonstrated 100% drug release over 12 h in gastric environment.

5.5.4.3 Amoxicillin

Amoxicillin is a broad-spectrum short-acting antibiotic. It is useful in curing peptic ulcer due to *Helicobacter pylori* (Mahady et al. 2005). Risbud et al. (2000) formed semi-IPN porous and non-porous dried hydrogel membrane prepared by cross-linking the polymers chitosan and PVP with glutaraldehyde. Porous or freeze-dried IPN membrane with pore diameter 39.20 μm showed more swelling than non-porous or air-dried IPN membrane. Also, the in vitro release of 73% of amoxicillin from freeze-dried membrane till 3 h at pH 1.0 was found to be superior in comparison to the air-dried membrane that exhibited only 33% in the condition which suggested that the prepared freeze-dried membranes are effective for controlled delivery of ciprofloxacin in acidic media.

With a concept of dual cross-linking Ekici and Saraydin (2007) fabricated a novel IPN hydrogel made up of chitosan, PVP and PAA (C-P-AAc) and cross-linked it with glutaraldehyde and MBA in varying concentration of 0.5%w/w, 1.0% w/w and 2.0% w/w for gastrointestinal delivery of amoxicillin that exhibited pH-dependent release higher at acidic pH and, thus, concluded that the formed pH-sensitive hydrogel can serve as a potential device for targeted delivery of amoxicillin to the stomach region.

5.5.5 Antitubercular Drug

5.5.5.1 Isoniazid

Tuberculosis is the most lethal and prevalent infectious disease worldwide (Shishoo et al. 2001). The antitubercular drug isoniazid is extensively used as first-line treatment in the disease (Shafran et al. 1996). Presystemic metabolism of the drug, associated severe toxic effects and poor patient compliance call for the development of a controlled delivery of isoniazid. Sullad et al. (2010) designed a novel pH sensed hydrogel of acrylic acid grafted guar gum with PVA for the controlled release of isoniazid. The process of grafting of guar gum with acrylic acid was done by free radical polymerization and the IPN microbeads using PVA and acrylic acid grafted guar gum were prepared by water in oil emulsion cross-linking method in the presence of cross-linker glutaraldehyde. The prepared drug-loaded microbeads were of 10 μm diameter. The result of drug release study showed an extended profile of 8 h of the drug into both pH 1.2 (62%) and 7.4 (81%) depending upon extent of cross-linker, drug loading and swelling efficiency of the polymeric matrix.

A novel experimental work by Angadi et al. (2011) also demonstrated controlled release behaviour of isoniazid from stearic acid-coated IPN microsphere of chitosan and gelatin. The IPN microsphere was prepared by emulsion cross-linking method. The work was an attempt to develop a pH-sensitive and gastroprotective controlled release system of the model drug. Coating of stearic acid prevented burst release in the stomach while releasing maximum isoniazid to the intestine over 30 h.

5.5.6 Biomolecules (Enzyme and Protein)

Advancement in delivery of nucleic acid such as DNA and RNA through recombinant technology has provided an ease in delivering bioactive molecules such as proteins and enzymes. These bioactive molecules undergo rapid enzymatic degradation and acidic denaturation when administered orally; that is why intravenous route is preferred most. However, this route of administration is costly as well as exhibits poor patient compliance. Thus to protect these acid labile substances and to deliver them through the most convenient way, i.e. by oral drug delivery, application

of biomaterials was in extreme use. Several pH-sensitive IPNs composed of natural polymers or in combination with synthetic polymers have shown successful result in delivering the protein and enzymes orally. Few of them are discussed below.

5.5.6.1 Bovine Serum Albumin

With an attempt to deliver bovine serum albumin orally and in a controlled way, Gong et al. (2011) developed a dual cross-linked (calcium and sulfate ion), pH-sensitive IPN of alginate and N-α-glutaric acid chitosan (GAC). The beads loaded with model protein bovine serum albumin showed diverse drug release pattern at varied pH. For instance, at pH 1.2 less than 18% of the protein was released; however the release was found to be higher, i.e. 100% at pH 6.8 and 7.4 over 7 h, suggesting the fabricated dual cross-linked and pH-sensitive IPN polymeric carrier could act as an efficient oral delivery vehicle for proteins.

Yang et al. (2013) reported an IPN hydrogel of methoxy poly(ethylene glycol) grafted carboxymethyl chitosan (mPEG-g-CMC) and alginate for the intestinal targeted delivery of bovine serum albumin. The process of pegylation was done to enhance the solubility of chitosan. The protein-loaded IPN hydrogel showed drug loading efficiency of 32%–63% due to variation in polymer concentration. The release of bovine serum albumin from the IPN hydrogel was found to be slow in acidic media pH 1.2 while improved at pH 7.4 suggesting it to be promising delivery system for intestine region.

5.5.6.2 Streptokinase

Streptokinase is an extensively used thrombolytic agent (Banerjee et al. 2004). To understand the effect of swelling and deswelling on the controlled release behaviour of the protein drug, Park et al. (2001) developed a pH-sensitive poly(L-lysine) and polyethylene glycol methacrylate-based semi-IPN for regulated drug release at specific pH. The semi-IPN hydrogel prepared by free radical polymerization method showed reversible swelling and deswelling behaviour with respect to change in pH that was further affected by the concentration of the polymers poly(L-lysine) and polyethylene glycol methacrylate. The release of drug from the IPN hydrogel was also studied that exhibited more than 40% drug release at pH 9 whereas less than 20% at pH 5 over 7 h, suggesting the prepared tool to be useful in achieving regulated and desirable drug release with a slight change in pH.

5.6 Patents on pH-Sensitive IPNs

Based on the novelty and scientific works performed related to synthesis of pH-sensitive IPNs, few inventions have also been patented and are illustrated in Table 5.4.

Table 5.4 List of patents granted related to pH-sensitive IPNs

Patent No.	Title	Inventor	Original assignee	Patent granted year	References
US4931287A	Heterogeneous interpenetrating polymer networks for the controlled release of drugs	You H. Bae, Teruo Okano W. Kim	University of Utah	5 June, 1990	Bae et al. (1990)
US5904927A	Drug delivery using pH-sensitive semi-interpenetrating network hydrogels	Mansoor M. Amiji	North eastern University, Boston	18 May, 1999	Amiji (1999)
US5316774A	Blocked polymeric particles having internal pore networks for delivering active substances to selected environments	Robert P. Eury, Rajesh Patel	Advanced Polymer Systems, Inc., Redwood City, California	31 May, 1994	Eury and Patel (1994)
US5580929A	Interpenetrating polymer network phase transition gels	Toyoichi Tanaka, Franck main, Etsuo Kokufuta, Masahiko Annaka	Massachusetts Institute of Technology, Boston, MA	3 Dec, 1996	Tanaka et al. (1995)
US5651979A	Apparatus and method for delivering a biologically active compound into a biological environment	Eyal S. Ron, Schiller	Gel Sciences, Inc., Bedford, MA	29 July,1997	Ron et al. (1997)
US6451346B1	Biodegradable pH/ thermoresponsive hydrogels for sustained delivery of biologically active agents	Subodh Shah, Weiguo Dai	Amgen Inc., Thousand Oaks, CA (USA)	17 Sep, 2002	Shah and Dai (2002)

(continued)

Table 5.4 (continued)

Patent No.	Title	Inventor	Original assignee	Patent granted year	References
US6103865A	pH-sensitive polymer containing sulfonamides and its synthesis method	You Han Bae; Sang Yeob Park	Kwangju Institute of Science and Technology, Kwangju, Rep. of Korea	15 Aug, 2000	Bae and Park (2000)
US20040001892A1	Tunable semi-interpenetrating polymer networks (SIPNS) for medicine and biotechnology	Kevin E. Healy, Ranee A. Stile	The Regents of the University of California, Oakland, CA; Northwest University, Evanston, IL	1 Jan, 2004	Healy and Stile (2011)

5.7 Future Challenges for pH Sensed IPN-Based Drug Delivery System

Despite successful results of pH sensed IPN in delivering various kinds of drugs of diverse category such as anticancer, antidiabetic, antipyretic and analgesic, antitubercular, etc., the clinical transition of this delivery system has not made yet. Potent cytotoxic effect and high molecular weight of polymers utilized in the synthesis process might be the possible reasons for blocking the progress of smart IPNs commercially. Unfortunately, these polymers do not degrade actively and tend to accumulate within body, thus associated with neural and renal toxic effect. Other important factor that must be considered while developing this drug delivery system is the proper combination selection and appropriate ratio of polymers and cross-linkers because the release of drug from such systems depends upon swelling and extent of cross-linking; thus, a skilled knowledge is required.

5.8 Conclusion

IPNs are one step ahead drug delivery vehicle over hydrogels and converting IPNs into smart and programmable device using intelligent polymers is another achievement in area of drug delivery. Development of pH-sensitive polymers and utilization of this sensitivity to deliver the drug at specific site with desired and controlled release of therapeutics in human body is the foremost need that will result into reduced dose and dosing frequency and also reduced toxicity. Already numerous

drugs have proved their site specificity release owing to the pulsatile swelling of the polymers with respect to the environmental pH; still there are some other factors, for instance, molecular weight and slow degradation of the polymers that has to be considered in order to bring this smart polymeric IPN completely in practice as well as for commercial utilization.

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IPN Dendrimers in Drug Delivery

6

Keerti Jain, Neelesh Kumar Mehra, Vineet Kumar Jain, and Narendra Kumar Jain

Abstract

Instead of enormous development in biomedical field, the treatment of severe ailments like tuberculosis, visceral leishmaniasis, systemic fungal infections, HIV and cancer still faces noteworthy confronts like severe toxicities, resistance and patient non-compliance, etc. To overcome these problems interpenetrating polymeric nanomaterials like liposomes, nanoparticles, dendrimers, carbon nanotubes and quantum dots are continuously being explored to improve therapeutic index of available medicine as well as new drug entity. This review exhaustively summarizes the dendrimer-mediated advancements in the drug delivery as well as possible avenues where dendrimers could be exploited to improve the therapeutic effectiveness of currently available treatment strategies. Dendrimers are a new class of interpenetrating polymer network (IPN) with unique properties like well-defined size and structure, versatility, water solubility, multivalency and internal hydrophobic cavities, which rendered these nanostructures an emerging carrier in biomedical and drug delivery applications. Classically, these well-defined nano-architectures have been explored as nanoscaffolds and nano-containers to conjugate and deliver drugs, genes, oligonucleopeptides, imaging agents, siRNA, aptamers, etc. In spite of tremendous research efforts over nearly three decades, the stakeholders are confronted with

IPN: Interpenetrating polymer network

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certain fundamental issues related to dendrimer applications. In this review, we propose to dwell upon the unexplored facets of dendrimer research with possible applications in drug delivery.

Keywords
Dendrimers · Drug delivery · Potentials · Toxicity · Polymers and drug

6.1 Introduction

Dendrimers are emerging as promising interpenetrating polymer network (IPN) nanomaterials for biomedical applications ascribed to their salient characteristics including well-defined macromolecular architecture, precise molecular weight, multivalent surface functionalities, high degree of branching and internal cavities (Hutnick et al. 2017; Zhou and Lu 2014; Jain et al. 2015a). Dendrimers represent a group of IPN having hyperbranched globular architecture, which are designed to develop well-defined structure with controlled and precise geometry, size, shape and molecular weight. Dendrimers were first introduced as synthetic protein in 1985 and term was coined by Prof. Donald A. Tomalia (Tomalia et al. 1985). Dendrimers are made up of mainly three architectural components as shown in Figs. 6.1 and 6.2, i.e. core, interior branches and surface functional groups.

The presence of surface functional groups makes dendrimers a perfect platform or scaffold for a number of different cargos including drug, imaging agents, contrast agents, nucleic acids, proteins, peptides, antibodies, diagnostic agents and targeting ligands, etc. (Hsu et al. 2017; Kaur et al. 2017a, b; Ghalandarlaki et al. 2014). In

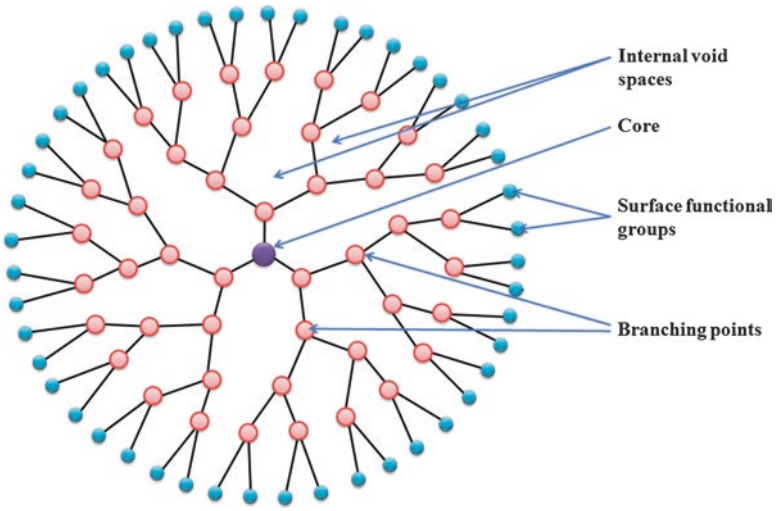

Fig. 6.1 General structure of dendrimer

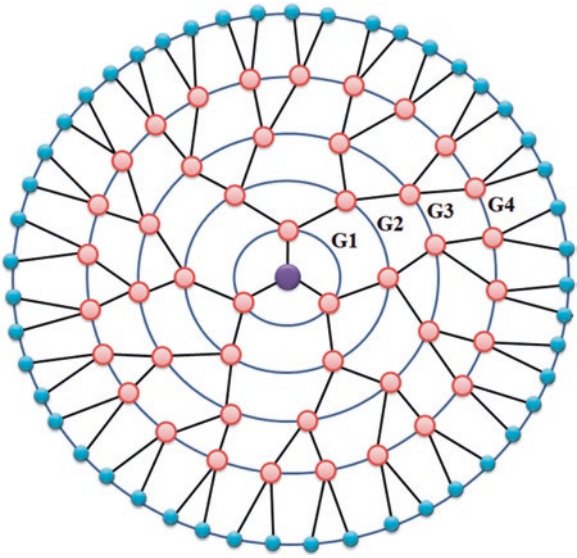

Fig. 6.2 Schematic presentation of different generations of dendrimer

past few decades dendrimers have emerged as idyllic drug delivery system attributed to well-defined geometry and unique architecture, biocompatibility, residence time, pharmacokinetics and pharmacodynamics. Dendrimers can serve as host for a variety of guest molecules owing to the presence of hydrophobic internal voids and hydrophilic surface groups. They have been explored as drug delivery system to increase drug solubility, permeability, stability, sustained release and targeted delivery with reduction in toxicity. The presence of a number of surface functional groups also makes possible attachment of a number of targeting moieties to deliver the drug specifically to diseased site. Another important feature of dendrimers is their ability to cross the biological membrane to deliver drug into intracellular compartments via different transcellular processes including endocytosis (Edagwa et al. 2014; Falanga et al. 2014; Shcharbin et al., 2017; Kaur et al. 2017a, b).

One more distinct characteristic of dendrimeric architecture is the presence of internal voids and highly branched surface functional groups, which are generally hydrophobic and hydrophilic, respectively. This amphiphilic nature of dendrimers assists in its solubilization property. Further presence of voids enables entrapment of hydrophobic moieties as well as large number of surface functional groups makes them ideal template for functionalization of drug, diagnostic agents, imaging agents and targeting moiety, etc. (Hinman et al. 2017; Shcharbin et al. 2017; Edagwa et al. 2014).

6.2 Synthesis of Dendrimers

The highly branched, polyvalent, monodisperse polymers, dendrimers, have generated unremitting attention as nanoscaffold for specialized applications in the field of technology including medicines. The multivalency, well-controlled architecture,

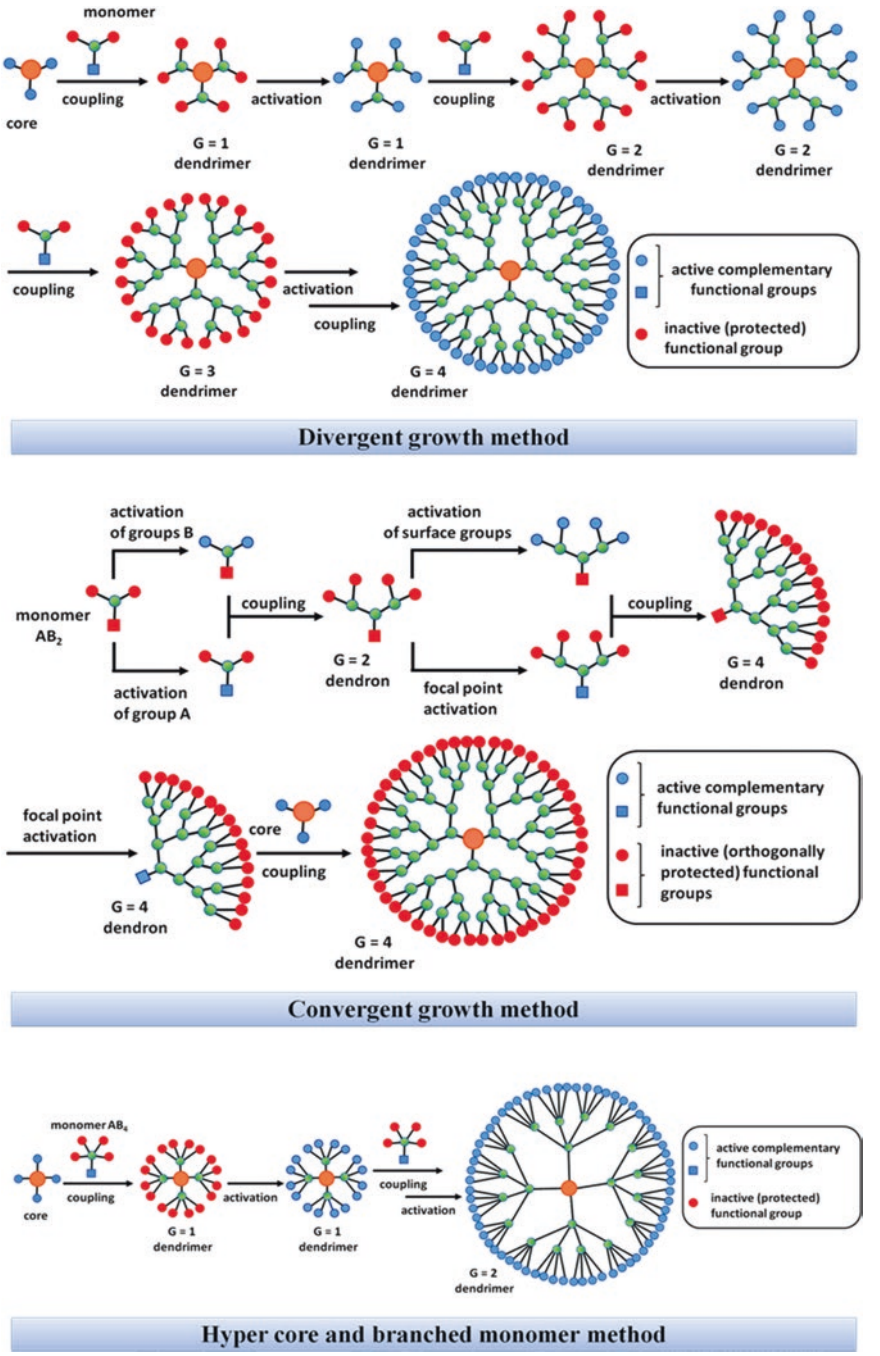

Fig. 6.3 Schematic presentation of general methods of synthesis of dendrimers. (Reproduced with permission from Sowinska and Urbanczyk-Lipkowska 2014)

tailor-made surface groups and smartly engineered internal voids of dendrimers arise due to their synthesis methods, which convert simple monomer units into multifunctional hyperbranched macromolecular architecture of dendrimers with different surface morphologies and internal structures (Ganneau et al. 2017; Sowinska and Urbanczyk-Lipkowska 2014). Although conventionally dendrimers are being synthesized by convergent and divergent growth method yet scientists are exploring various methods to synthesize dendrimers with distinct architecture, robust molecular properties and, most importantly, defined biological properties. Orthogonal reactions and molecular synthesis are two alternative approaches, which could help in synthesizing dendrimeric architectures made of repetitive building blocks with robust structures and defined functions (Yang 2016; Liu et al. 2015). Basically, most of the dendrimers have been synthesized mainly, divergent and convergent growth methods, which are shown graphically in Fig. 6.3. The other methods used to synthesize dendrimers include double exponential growth method, hyper cores and branched monomer method and click chemistry (Kesharwani et al. 2014; Kaur et al. 2016; Singh et al. 2016).

6.3 Types of Dendrimers

Dendrimers containing different core molecules, branching monomer units and surface functionalities have been synthesized and investigated for their drug delivery applications, which could be classified as follows.

6.3.1 Dendrimers Based on Monomers

(a) Poly(amidoamine) or PAMAM dendrimers.
(b) Poly(propylene imine) or PPI dendrimers.
(c) Poly(amidoamine-organosilicon) or PAMAMOS dendrimers.
(d) Polyether dendrimers.
(e) Polyester dendrimers.
(f) Polyether imine dendrimers.
(g) Poly-L-lysine (PLL) dendrimers.
(h) Citric acid dendrimers.
(i) Melamine dendrimers.
(j) Phosphate dendrimers.
(k) Triazine dendrimers.

6.3.2 Dendrimers Based on Macromolecules

(a) Carbohydrate dendrimers (mannose dendrimers, maltose dendrimers).
(b) Glycopeptide dendrimers.
(c) Polyethylene glycol (PEG) dendrimers.
(d) Peptide dendrimers (glutamic acid dendrimers).

6.3.3 Surface-Engineered Dendrimers

(a) Acetylated dendrimers.
(b) Amino acid (arginine, ornithine, lysine)−/peptide (RGD, gH625)-conjugated dendrimers.
(c) Antibody (HER2 monoclonal antibody)-conjugated dendrimers.
(d) Carbohydrate (mannose, galactose)-conjugated or glycosylated dendrimers.
(e) Dendrimers conjugated to macrophage activators [muramyl dipeptide (MDP), tuftsin].
(f) Drug-conjugated dendrimers.
(g) Epidermal growth factor (EGF)-conjugated dendrimers.
(h) Folic acid- or folate-conjugated dendrimers.
(i) PEG-conjugated or PEGylated dendrimers.
(j) SiRNA-conjugated dendrimers.

6.3.4 Specialized Dendrimers

(a) Amphiphilic dendrimers.
(b) Chiral dendrimers.
(c) Domino dendrimers.
(d) Hybrid dendrimers.
(e) Liquid crystalline dendrimers.
(f) Micellar dendrimers.
(g) Multilingual dendrimers.
(h) Multiple antigen peptide dendrimers.
(i) Nanoparticle-cored dendrimers.
(j) Tecto-dendrimers (Jain and Jain 2014a, 2015; Singh et al. 2016; Kaur et al. 2016; Mehra et al. 2015a).

Domino or self-immolative dendrimers are designed to release their end groups through a domino-like chain disintegration initiated by single cleavage, which could be catalysed by specific enzyme to work as single triggered multi-prodrug unit (Amir et al. 2006, 2007). Sagi et al. (2008) designed self-immolative polymers with polyurethane backbone, which could be disassembled sequentially into its building blocks upon initiation by a triggering event at the polymer head to develop highly sensitive molecular sensors with large signal-to-noise ratios (Sagi et al. 2008). Domino dendrimers could be explored as promising targeted drug delivery systems, for example, in treatment of cancer disease, or may be investigated as biosensor for diagnosis (Wang et al. 2016; Amir et al. 2006, 2007; Gnaim and Shabat 2014).

Zhou and Lu (2014) reported the advantages of a dendritic architecture based on polyhedral oligomeric silsesquioxane-cored nanoglobules having higher number of surface functional groups with well-defined 3D structure and comparatively more

compact morphologies at same generation number for the delivery of drugs, genes and imaging contrast agents. Zhang et al. (2014) designed a modular strategy to synthesize Janus dendrimers with both hydrophilic and hydrophobic groups having the ability to self-assemble as onion-like dendrimersome vesicles with defined number of internal bilayers and controllable size. The objective of developing these dendrimersomes was to develop a nanocarrier, which could mimic the structure of biological membrane and could form uniform vesicles in order to develop promising nanomedicines (Zhang et al. 2014).

Shcharbin et al. (2015) exhaustively reviewed the phosphate-based nanomaterials including nanoparticles, dendrimers, liposomes, graphenes, quantum dots and fullerenes as promising biocompatible tool for application in the field of nanomedicine (Shcharbin et al. 2015). In 2015, Wei et al. reported nanomicellar drug delivery system based on amphiphilic dendrimers. These amphiphilic dendrimers were composed of hybrid molecules containing a hydrophobic lipid moiety, which could self-assemble to form micelles in aqueous medium and a hydrophilic dendrimeric polymer. These nanomicellar dendrimers were designed to develop a hybrid system having properties of lipids as well as dendrimers, i.e. micelle formation ability, large void spaces, high drug payload, stability and mechanical strength (Wei et al. 2015).

6.4 Properties of Dendrimers

The well-controlled unique architecture of dendrimers as nanocarriers renders them some specific advantages, which are presented briefly in Table 6.1.

Table 6.1 Properties of dendrimers and advantages

Properties	Associated advantages
Nanospace	An environment for bioactives
Biocompatible functional groups	Reduced toxicity of payload
Well-defined architecture	Monodispersity
Controllable surface functionalities	Brilliant drug delivery vehicle, delivery of diagnostic and imaging agents, targeting
Globular shape and nanometric size	Mimic the biological components like protein, haemoglobin, etc. important in determining biological and rheological properties
Hydrophilic surface groups, hydrophobic internal cavities	Solubilization of hydrophobic drug molecules
Monodispersity or nearly monodisperse or low polydispersity	Reproducible pharmacokinetic behaviour
A large number of multivalent surface functional groups than any polymeric material for a given molecular weight	Make possible to attach several molecules separately or simultaneously like drugs, imaging agents, cell-penetrating peptides, targeting groups and/or solubilizing moieties. Theranostic applications

6.4.1 Surface Charge

As we know dendrimers are composed of three architectural components, namely, core, interior branching units and surface groups. Depending on the nature of the surface groups dendrimers carry positive, negative or neutral charges. Surface charge plays important implications on the therapeutic applications as well as on negative aspects of dendrimers as a vehicle for bioactives. The salient characteristics of dendrimers in regard of surface charges are as follows:

1. Cationic dendrimers (PPI and PAMAM) form complexes with DNA, due to negative charge of DNA.
2. The overall positive charge of dendrimer-DNA complex allows interaction with negatively charged biological membranes, which results in membrane destabilization and endocytosis followed by efficient internalization of complex.
3. Cationic charge of dendrimer (due to amine groups present at periphery) is also associated with cytotoxicity, haemolysis, drug leakage, immunogenicity and RES uptake.
4. Dendrimers are soluble in polar and protic solvents because of the surface charge.
5. Microvascular extravasation of dendrimer is influenced by size and molecular weight, charge and molecular geometry.
6. At low pH region positively charged dendrimers showed extended conformation due to electrostatic repulsion whereas at neutral pH back folding occurs due to hydrogen bonding between the uncharged tertiary amines in interior and the positively charged surface amines. At higher pH, cationic dendrimers acquire globular structure due to higher degree of back folding because of weak "interdendron" repulsive forces.
7. Since biological membranes are negatively charged, the positively charged dendrimers interact with these membranes and elicit toxicities such as cytotoxicity, haemolytic toxicity and in vivo toxicity whereas negatively and neutrally charged dendrimers elicit lesser toxicity because of little or no interaction with biological membranes (Barraza et al. 2017; Diaz et al. 2018; El-Sayed et al. 2001; Nguyen et al. 2017; Shcharbin et al. 2017; Soni et al. 2015).

Surface charges of dendrimers have important impact on the design as well as therapeutic applications of dendrimers. This can be demonstrated by the fact that toxicity associated due to terminal-NH_2 groups and multiple cationic charges of dendrimers limits the extensive pharmaceutical and biomedical applications of dendrimers (Mehra et al. 2016; Agashe et al. 2006; Malik et al. 2000; Jain et al. 2015d). Dendrimers with positively charged amine surface groups including PPI, PAMAM and PLL exert significant in vitro cytotoxicity due to their surface cationic groups. Evidence regarding dendrimer safety is conflicting since there are reports of concentration and generation-dependent toxicity of free amine groups present at periphery of dendrimers. Chen and coworkers investigated the cytotoxicity of cationic melamine dendrimers having surface groups like amine, guanidine, carboxylate, sulphonate or phosphonate and concluded that cationic dendrimers were much more cytotoxic than anionic or PEGylated dendrimers (Harada et al. 2013; Chen et al.

2004). This phenomenon is not only applicable to dendrimers but also other cationic macromolecules, which also causes destabilization of the cell membrane and results in cell lysis (Fischer et al. 2003; Jain et al. 2015b). So surface charge is an important property of dendrimers and hence a possible tip to scientists working in the areas of dendrimeric research.

6.4.2 Biocompatibility of Dendrimers

As discussed earlier, the end group present on to the dendrimer periphery is a leading parameter in determining the toxicity and it could be better understood from the following points:

1. Dendrimers with peripheral amine groups (PAMAM and PPI dendrimers) generally display concentration-dependent cytotoxicity and haemolytic toxicity (Roberts et al. 1996; Malik et al. 2000; Jevprasesphant et al. 2003).
2. Dendrimers restraining neutral or anionic surface functional groups have been found to elucidate much less toxicity and haemolysis (Malik et al. 2000; Padilla De Jesús et al. 2002).
3. Toxicities of dendrimers can be overcome by partial or complete modification of the periphery of the cationic dendrimer with negatively charged or neutral groups (Malik et al. 2000; Jevprasesphant et al. 2003; Chen et al. 2004).

For the in vivo applications of dendrimers, dendrimers must fulfil the following requirements:

1. They should be non-toxic.
2. Able to cross biological membranes.
3. Ability to circulate in the body for the time required to produce clinical efficacy.
4. Ability to target some specific sites.
5. Rapid biodegradation or renal elimination rate.

All these points are influenced by the architectural components of dendrimers including core, interior branches and surface groups and as well as by molecular weight of dendrimers. For biocompatibility, the interaction of dendrimers with biological membrane should not result in disruption of cell and cell membrane. When cationic dendrimers interact with negatively charged biological membrane, it results in damage of cell membrane followed by cell lyses, which finally results in toxicity of dendrimers. Although few reports are available on the in vivo behaviour of dendrimers, yet in vivo toxicity correlates reasonably well with in vitro toxicity of dendrimers. The following features have been derived from the in vivo investigation of dendrimers:

1. Neutral dendrimers like polyester dendrimers have been found to be non-toxic both in in vitro and in vivo studies.

2. Generally mice can tolerate low doses of cationic PAMAM dendrimers irrespective of the fact that the surface of dendrimers has been modified or not.
3. Degradation of dendrimers by hydrolytic enzymes renders them promising biodegradable carriers for bioactives. Biodegradability is important in preventing the bioaccumulation and toxic effects of dendrimers (Heyder et al. 2017).
4. Dendrimers are biopermeable and this attribute makes them potential drug delivery vehicles.
5. The extravasation time of dendrimers increases with increase in generation and molecular weight of cationic PAMAM dendrimers having surface amino terminals.
6. In the biopermeable studies, anionic dendrimers were found to exhibit faster transfer rate than other polymeric systems (Yellepeddi and Ghandehari 2016; Heyder et al. 2017; Jain et al. 2015d).

6.4.3 Biological Properties

In the scenario of growing interest in biomedical application of dendrimers, it is necessary to understand the biological properties of dendrimers for predicting in vivo fate of this multifaceted carrier. As explained earlier, because of the cationic charge dendrimers interact with biological membranes and cause toxicity. The interaction of dendrimers with negatively charged biological membrane results in nanohole formation followed by membrane disruption, thinning and erosion (Heyder et al. 2017; Jain et al. 2010, 2015d).

For use as drug delivery vehicle, dendrimers must satisfy certain requirements which include non-toxicity, permeability through biological barriers, ability to circulate in the body, clinical efficacy and targeting specificity followed by rapid elimination rate or biodegradability. For this the biocompatibility and pharmacokinetics of dendrimers are important factors but only very limited amount of research dealing with these subjects has appeared. Roberts et al. (1996) investigated the biological behaviour of 3.0G, 5.0G and 7.0G PAMAM dendrimers. In this study, only seventh generation of PAMAM dendrimers elucidated in vivo toxicity, while the in vitro toxicity was concentration- and generation-dependent, with the seventh generation being more toxic than the third or the fifth generation. The authors ascribed the polycationic nature of the dendrimers for the observed toxicity. Duncan and coworker reported that amine-terminated PAMAM dendrimers exhibited the haemolysis and cytotoxicity whereas carboxylate terminated did not (Duncan and Malik 1996).

Dendrimer toxicity in biological system is generally characterized by:

(i) Haemolytic toxicity.
(ii) cytotoxicity,
(iii) Haematological toxicity.
(iv) Immunogenicity.
(v) In vivo toxicity.

Interaction of positive charge of dendrimers with proteins of erythrocyte and other biological membrane causes changes in protein conformation; hence toxicity of dendrimers is generation- and concentration-dependent. Increase in generation results in increased number of surface groups with consequent increase in cationic charge which leads to increased interaction with membrane proteins (Singh et al. 2016; Klajnert and Bryszewska 2001).

6.4.4 Pharmacokinetics and Biodistribution

Bioavailability, toxicity and efficacy of dendrimers depend on drug release kinetics, tissue accumulation profile and elimination rate. All these parameters can be investigated by performing the pharmacokinetic and biodistribution studies (Lee et al. 2005; Bajwa et al. 2016a, b; Kaur et al. 2017a, b).

Some important implications in this regard are summarized below:

1. As the macromolecules are retained in tumour vasculature via enhanced permeation and retention effect, it will be important to determine the blood circulation half-life of dendrimers for passive tumour targeting.
2. Uncharged or negatively charged dendrimers with high molecular weight have long circulation time.
3. On intraperitoneal or intravenous administration polycationic PAMAM dendrimers exhibit fast clearance from bloodstream with accumulation either in liver, spleen, kidney or pancreas.
4. Engineering of surface amino groups with hydrophilic polyethylene oxide chains or acetyl group decreases the liver uptake via steric stabilization of dendrimer surface or masking of positive charge.
5. PAMAM dendrimers with negatively charged surface show longer circulation time with significant liver accumulation.

In vitro and in vivo toxicity, immunogenicity, biodistribution and pharmacokinetics of dendrimers have been studied by various researchers. A plethora of research is undergoing to investigate the therapeutics crossing various cell membranes or biological barriers with the aid of dendrimers. A low tissue deposition with rapid serosal transfer rates was observed in crossing adult rat intestine with anionic 2.5 and 3.5G PAMAM dendrimers whereas the transport of G3.0 PAMAM and surface-modified (with lauroyl chains) G3.0 PAMAM across Caco-2 cell monolayers followed endocytosis-mediated cellular internalization (Yang and Kao 2006). In a thorough study Kannan et al. (2004) observed that cellular entry of dendritic polymers depends on the functional end groups and molecular mass. This study was performed with human lung epithelial carcinoma cells and authors suggested that faster cellular entry of cationic 3.0 and 4.0G PAMAM dendrimers was attributed to fluid-phase pinocytic route, which is the consequence of electrostatic interaction of cationic dendrimers with negatively charged epithelial cells, while the slow entry of

neutral 3.0G PEG dendrimers and polyol dendrimers was ascribed to non-specific adsorption of dendrimers to the cell membrane followed by endocytosis.

Dendrimers are being extensively investigated for drug delivery applications and have consequently been explored to improve pharmacokinetic profile of therapeutic agents. Conjugation of galactose to surface of dendrimers renders no change in biological half-life ($t_{1/2}$) and elimination rate constant (K_e) of primaquine phosphate compared to plain PPI dendrimers with decrease in peak plasma concentration (C_{max}) and initial drug levels owing to slower rate of release. Mean residence time (MRT) and area under plasma drug concentration curve (AUC) were also found to increase compared to parent drug-loaded dendrimers (Bhadra et al. 2005). Biological half-life of indomethacin was increased by 3.14 times with increase in MRT and decrease in clearance with folate-conjugated dendrimers as compared with plain dendrimers. It was observed that conjugation of polyethylene oxide and doxorubicin to dendrimers led to tumour targeting via EPR effect in the later stage of tumour whereas in early stage tumour, greater drug and dendrimer elimination was observed because of the lesser development of vasculature for effective tumour targeting (Chandrasekar et al. 2007). Loading of antiviral drug lamivudine in mannosylated dendrimer reduced the dose requirement by 2.3-folds (Dutta and Jain 2007).

Sakthivel et al. (1999) explored the biodistribution of lipidic dendrimer consisting of 16 lipidic amino groups synthesized from protected glycine, lysine and 2-amino-tetradecanoic acid adopting solid-phase peptide synthetic methods followed by acetylation with tritiated acetic anhydride in pyridine. Uptake by gut epithelial tissue was studied after a single oral dose administration by gavage in female Sprague Dawley rats. In distribution studies 3%, 1.5%, 0.1%, 0.5%, 15% and 5% of dendrimers were observed in blood, liver, spleen, kidneys, small intestine and large intestine, respectively. In lymphoid and non-lymphoid small intestine 4% and 0.3% of dendrimer were measured after 12 h, respectively, suggesting that the total percentage of the administered dose absorbed through the lymphoid tissue was comparatively greater than through the non-lymphoid tissue of the small intestine with respect to organ weight after 3 and 24 h (Sakthivel et al. 1999).

Kojima et al. (2010) investigated the influence of the generation of the PAMAM dendrimer and the molecular weight of PEG in PEGylated PAMAM dendrimers on the biodistribution. As PEGylation is known method to improve the blood retention, this phenomenon is also true with dendrimers. In this study, authors synthesized three types of PEGylated-l-lysine-bearing polyamidoamine dendrimers (PEG2k-Lys-PAMAM (G4), PEG5k-Lys-PAMAM (G4), PEG2k-Lys-PAMAM (G5)) consisting of 4.0 and 5.0 generations of dendrimers and PEG chains with 2 kDa and 5 kDa molecular weights. Simultaneously an acetylated-l-lysine-bearing dendrimer was also synthesized as a non-PEGylated dendrimer. For tracking, dendrimers were labelled with radioactive indium-111 bifunctional diethylenetriaminepentaacetic acid (pSCN-benzyl-DTPA) which was bound to the epsilon-amino group of lysine. In results, PEGylated dendrimers showed longer blood retention and lower accumulation in other normal organs such as the kidneys than the non-PEGylated dendrimers, and this retention was found to increase with the higher generation and the longer PEG chains (Kojima et al. 2010).

Kaminskas et al. (2015) investigated the lymphatic pharmacokinetics and antitumour activity of PEGylated polylysine dendrimers conjugated to MTX and observed lower lymph node targeting with dendrimers after intravenous administration. Instead of low retention in lymph node, the growth of lymph node metastases was inhibited (Kaminskas et al. 2015). Although it is expected and even being investigated that dendrimers could serve as perfect drug delivery vehicles, yet the structural heterogeneity (generation and surface groups) could result into significant variation in pharmacokinetics which could be a major stumbling block in clinical translation of dendrimers (Zhou et al. 2014). Hence these problems could be alleviated by designing precisely controlled dendrimer-drug conjugates.

6.5 Characterization of Dendrimers

Dendrimers are synthesized using step by step controlled sequences. Characterization is a crucial part in designing of appropriate dendrimeric system for drug delivery, which gives information about chemistry, structure, morphology, size and monodispersity of dendrimers. Various analytical techniques have been employed till date for the characterization of dendrimer synthesis as well as their application in drug delivery. A summary of various characterization techniques used for dendrimers is presented in Table 6.2 (Caminade et al. 2005; Kesharwani et al. 2014).

Physicochemical properties of 5.0G PAMAM dendrimers with different terminal groups, hydroxyl-surface polymer G5-OH and amino-surface polymer G5-NH_2, were studied by Nourse et al., using the techniques of absorption spectroscopy, SEC, SDS-PAGE, density measurement, measurement of sedimentation velocity and sedimentation equilibrium (Nourse et al. 2000). Tsutsumiuchi et al. studied the formation of ion complexes between PAMAM dendrimer HCl salt and poly(L-glutamic acid) sodium salt in water and phosphate buffers by pH, turbidity and viscosity measurements and electrophoresis analysis (Tsutsumiuchi et al. 2000). Shi et al. separated and examined PAMAM dendrimers of different generations with carboxyl, acetyl and hydroxyl terminal groups and a folic acid dendrimer conjugate (dendrimer-FA) using reverse-phase high-performance liquid chromatography (HPLC) (Shi et al. 2006). Myc et al. synthesized and characterized PAMAM nanodevice in which folic acid was conjugated as a targeting molecule and a caspase-specific FRET-based agent (PhiPhiLux G1D2) as the apoptosis-detecting agent to analyse the degree of apoptosis in targeted cells. The number of tertiary and primary amino groups in 5.0G PAMAM dendrimers was determined by potentiometric titration. Gel permeation chromatography (GPC) was used to define defects in analysed structure. HPLC analysis showed the removal of free folic acid after membrane filtration purification of the acetylated PAMAM with attached folic acid (Myc et al. 2007).

Cason et al. used UPLC analysis for monitoring PAMAM dendrimer surface transformations and product quality. On comparing the results with HPLC it was found that the application of UPLC increased the average number of theoretical plates and reduced retention times of analytes while improving the resolution

Table 6.2 Characterization techniques for dendrimers

Characterization	Analytical technique	Information obtained
Physical characterization	Intrinsic viscosity	Physicochemical properties of dendrimers
	Refractive index	
	Differential scanning calorimetry	
	Dielectric spectroscopy	
Spectroscopic and spectrometric techniques	UV-visible spectroscopy	Confirmation of synthesis and purity of dendrimers.
	Infrared (IR) spectroscopy	Analysis of chemical changes that take place at the surface of dendrimers during the synthesis and surface engineering may be analysed
	Raman spectroscopy	Information about the structure, dynamics, reaction state, physical and chemical properties
	Nuclear magnetic resonance (NMR) spectroscopy	
	Mass spectrometry	
	Fluorescence spectroscopy	
	Chirality, optical rotation, circular dichroism	
	X-ray diffraction	
Scattering methods	Laser light scattering	Quantitative information on size, shape and structure of dendrimers
	Dynamic light scattering	
	Static light scattering	
	Small-angle X-ray scattering (SAXS)	
	Wide-angle X-ray scattering (WAXS)	
	Small-angle neutron scattering (SANS)	
Microscopic methods	Electron microscopy	Size, shape and surface morphology of dendrimers
	Scanning electron microscopy (SEM)	
	Transmission electron microscopy (TEM)	
	High-resolution transmission electron microscopy (HRTEM)	
	Atomic force microscopy (AFM)	
	Confocal laser scanning microscopy (CLSM)	
Chromatographic methods	Size exclusion chromatography (SEC)	Purification of dendrimers
	High-performance liquid chromatography (HPLC)	Removal of unloaded cargo
	Ultra-performance liquid chromatography (HPLC)	Separation of surface-engineered dendrimers

Table 6.2 (continued)

Characterization	Analytical technique	Information obtained
Electrical procedures	Electrophoresis	Charge determination
	Electrochemistry	Determination of isoelectric point
	Electron paramagnetic resonance	Purification of dendrimers
		Determination of radicals formed in chemical reactions and completion of chemical reactions
Miscellaneous	X-ray photoelectron spectroscopy	Formation of different generation dendrimers
	Sedimentation	Conversion of half generation to full generation of dendrimers
	Dipole moments	Physicochemical properties of dendrimers
	Titrimetry and chemical tests for determination of number of NH_2 end groups of PAMAM and PPI dendrimers	

capability to discriminate surface variances in dendrimers (Cason et al. 2008). Later, immunoimaging scanning probe microscopy was used to determine biotinylated G4 PAMAM dendrimers and it was observed that this biorecognition technique could provide low-level detection of dendrimers with high specificity, good accuracy and reproducibility (Cason et al. 2012). A new POMAM hybrid dendrimers constructed from poly(propylene imine) core and poly(amidoamine) shells was synthesized by Majoros et al. (2008). The developed dendrimers were characterized by HPLC, GPC, NMR and noncontact atomic force microscopy (AFM). Tulu et al. synthesized water-soluble dendrimers based on poly(propylene oxide) amines and characterized them by elemental analysis, GPC, Fourier transform infrared spectroscopy (FT-IR), ^{1}H and ^{13}C NMR (Tulu et al. 2009). Li et al. (2015) developed EGF-dendriplexes for tumour targeted delivery. The formation of these dendriplexes was characterized using gel retardation assay, zeta size and zeta potential.

For analytical characterization of dendrimers nuclear magnetic resonance-mass spectroscopy (NMR-MS), gel permeation chromatography (GPC), high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC), fast atom bombardment-mass spectrometry (FAB-MS) and electrospray ionization mass spectrometry (ESI-MS), UV-VIS spectroscopy (Sharma et al. 2003), Fourier transform infrared spectroscopy (FT-IR), MALDI-TOF-MS, electron and atomic force microscopy, small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), dynamic light scattering, potentiometric titration (Ambade et al. 2005), size exclusion chromatography (SEC) and electrophoresis, polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (CE) (Shi et al. 2006; Sharma et al. 2003) are some most widely used current techniques. HPLC has been used for the analysis of PAMAMs and derivatives and other dendritic polymers such as poly(propylene imine) and polyether dendrimers (Shi et al. 2006; Kesharwani et al. 2014).

Riechers et al. (2015) determined the conformation of individual PEG molecules in PEGylated PAMAM dendrimers using atomic force microscopy (AFM) and scanning tunnelling microscopy (STM). AFM imaging techniques rendered the view of dendrimer core, individual dendrimers and PEG extensions as well as provided the accurate measurement of height (Fig. 6.4). Further STM images also made possible high-resolution visualization of PEG surface groups (Fig. 6.5). Both the imaging techniques showed uniform distribution of PEG chains on dendrimer surface with a distance of 4.5 nm from core. Finally, authors concluded that this kind of sound piece of information on dendrimeric architecture could improve the drug delivery efficiency of dendrimers (Riechers et al. 2015).

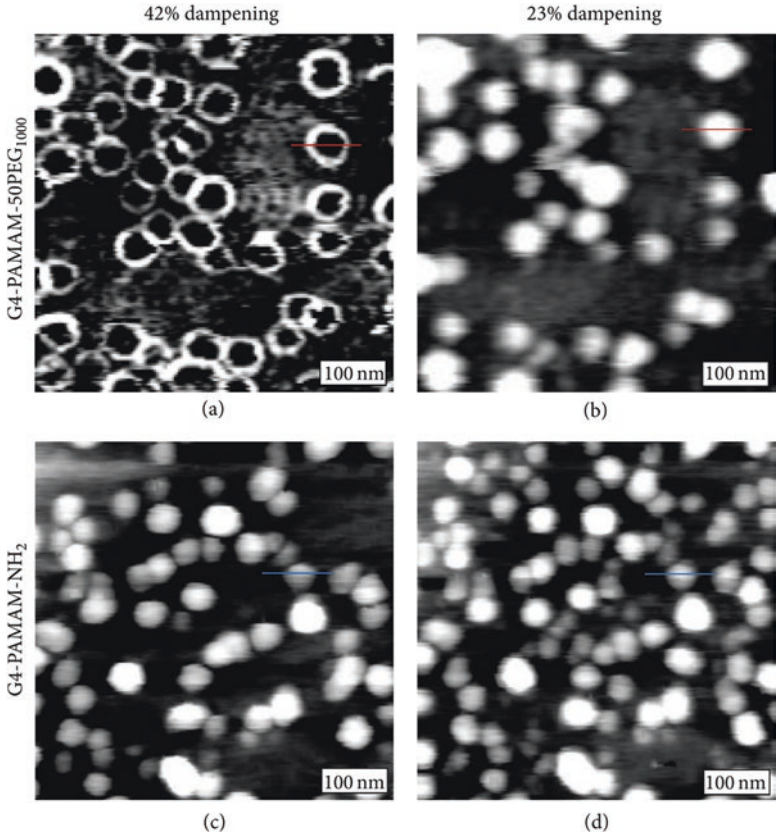

Fig. 6.4 AFM tapping mode imaging of G4-PAMAM-50PEG$_{1000}$ dendrimers. 300 × 300 nm^2 topographic images of G4-PAMAM-50PEG$_{1000}$ acquired at a damping set point of 42% (**a**) and 23% (**b**). 300 × 300 nm^2 AFM topographic images of G4-PAMAM-NH$_2$ at damping set points of 42% (**c**) and 23% (**d**). Cursor profile 1 is a representative G4-PAMAM-50PEG$_{1000}$ and G4-PAMAM-NH$_2$ dendrimer imaged with 42% dampening as indicated in (**a**) (red) and (**c**) (blue). Cursor profile 2 is a representative G4-PAMAM-50PEG$_{1000}$ and G4-PAMAM-NH$_2$ dendrimer imaged with 23% dampening as indicated in (**b**) (red) and (**d**) (blue). (Reproduced with permission from Riechers et al. 2015)

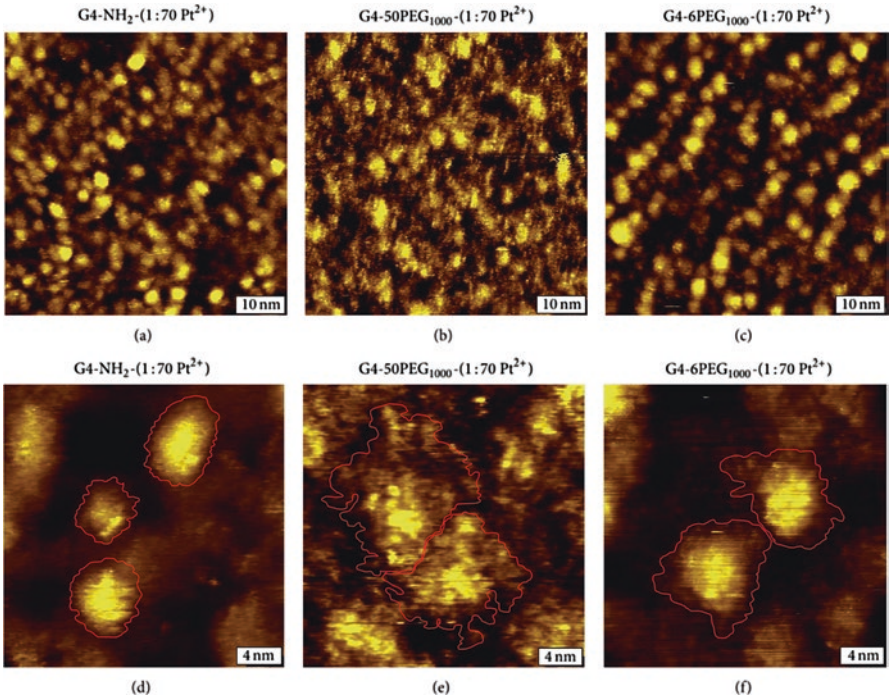

Fig. 6.5 High-resolution characterization of dendrimers with varying degrees of PEGylation. (**a**) A 60 × 60 nm^2 STM topographic image of G4-PAMAM-NH_2. (**b**) A 15 × 15 nm^2 STM topographic image of G4-PAMAM-NH_2 with three representative dendrimer contact areas highlighted in red. (**c**) 60 × 60 nm^2 image of G4-PAMAM-50PEG_{1000}. (**d**) 15 × 15 nm^2 image of G4-PAMAM-50PEG_{1000} with two representative dendrimer/PEG contact areas highlighted in red. (**e**) 60 × 60 nm^2 image of G4-PAMAM-6PEG_{1000}. (**f**) 15 × 15 nm^2 image of G4-PAMAM-6PEG_{1000} with two representative dendrimer/PEG contact areas highlighted in red. All images were acquired at 0.7–0.9 V and 20–30 pA. (Reproduced with permission from Riechers et al. 2015)

The presence of a large number of functional groups on the surface of dendrimers makes surface modification possible to create new conjugates and complexes of dendrimers for various biomedical applications. Hence various analytical methodologies are required to confirm the formation of these conjugates and complexes as well as to ensure purity of product (Cason et al. 2008). PAMAM dendrimer solutions may be polycationic or polyanionic in nature, depending on their structure, terminal modifications and solution conditions, such as solvent, pH, temperature and concentration. They can be analysed by electrophoretic methods. Polyacrylamide gel electrophoresis (PAGE) provides semi-quantitative information about their purity and electrophoretic mobility (Shi et al. 2005).

A new electrophoretic method based on the dynamic coating of the capillary for the separation of seven generations of PAMAM dendrimers at pH 7.4 was developed (Sedláková et al. 2006). The separation of compounds possessing amino groups by capillary zone electrophoresis (CZE) suffers from the interaction of

solutes with the capillary wall, which results in the absence or incomplete separation and retention in the capillary. However, positively charged PAMAM dendrimers strongly bind to the silica surface of the capillary so that they cannot be separated at neutral pH. The best separation was obtained with the system containing polyethylenimine (PEI) at a concentration of 0.05% (*w/v*) for the lower generations. Comparing two buffers, phosphate and Tris-phosphate, better resolution was obtained with the Tris-phosphate buffer. The strong influence of PEI as a dynamic capillary wall modifier was confirmed as it had a great impact on the separation/resolution of individual generations. It also improves the separation at acid pH (Sedláková et al. 2006).

Shi et al. synthesized surface-modified ethylenediamine-core PAMAM succinamic acid dendrimers (PAMAM-SAHs) and core-shell tecto(dendrimer) carrying succinamic acid termini. For characterization of these modified dendrimers, PAGE, size-exclusion chromatography (SEC), potentiometric acid-base titration and capillary zone electrophoresis (CZE) were used. PAGE showed that the relative mobilities of 2.0G to 7.0G dendrimers decreased with the increasing number of generations. The electrophoretic mobilities of individual generations of PAMAM polyanions were analogous, indicating that the separation mainly depends on their approximately identical charge/mass ratio. A CZE method was established for PAMAM-SAHs and applied to a core-shell tecto(dendrimer) too. The similar electrophoretic mobilities for all generations indicated that the polyanionic PAMAMs are not adsorbed on the surface of the quartz capillary; thus only the charge/mass ratio and the electroosmotic flow influence the separation. The E5(E3.SAH)n tecto-dendrimer (numbers by E refer to the generation of dendrimers) had a lower electrophoretic mobility, which was consistent with its lower charge/mass ratio (Shi et al. 2005). Weber et al. developed amino-terminated carbosilane dendrimers to protect and transport small interfering RNA (siRNA) bind to dendrimer via electrostatic interactions. Stability and the strength of the complex were tested by performing heparin competition assays on agarose gel electrophoretic system and PAGE system. Complex was resistant to degradation by RNase and carbosilane dendrimer had a protective effect on siRNA in the presence of RNase (Weber et al. 2008).

A combination of capillary electrophoresis and mass spectrometry (CE/MS) appears to be a perfect technique for the separation and identification of basic dendrimers. Stöckigt et al. used the online coupling of capillary electrophoresis with a sector mass spectrometer via an electrospray ionization (ESI) source to separate and identify polydisperse dendrimeric diaminobutane (DAB)-based polynitriles [DAB-dendr-$(CN)_8$] and by-products resulting from the synthesis. The samples consisted of two main fractions characterized by different electrophoretic mobilities. Increasing the pH from 3 to 7 changed the selectivity. CE/MS offers the possibility to detect closely related compounds and isomers (Stöckigt et al. 1996). A simple electrophoresis method was developed by Sharma et al. for assessing purity of PAMAM dendrimers. Simple modifications of analytical conditions allowed separating any charged, water-soluble dendrimers of varied shapes and dimensions during one analysis. PAMAM dendrimer separation was studied under basic and acidic environment. Electrophoresis under acidic environment increased resolution and

sensitivity. The use of low temperature (4 °C) for separation and post-electrophoresis manipulations led to enhanced dendrimer separation (Sharma et al. 2003).

6.6 Molecular Modelling of Dendrimers

In recent years molecular modelling techniques are being used as promising tools to predict the properties of nanomaterials with defined and repetitive branching architecture, terminal functionality like dendrimers and their interactions at molecular level to design optimum drug delivery system. Currently a number of studies have been performed or being performed to optimize dendrimers as promising drug delivery systems by modelling their interaction with bioactives such as drugs, proteins, nucleic acids and biological membranes. In these studies dendrimer size and surface have been observed as critical parameters, which could be tuned to enhance their performance as drug carriers. Molecular modelling and computational studies could support experimental results by providing valuable insights about dendrimer structure and possible molecular interactions at the molecular level. The progress in computational simulation techniques and molecular modelling could provide basis to improve prediction and understanding about the drug delivery properties of dendrimers (Bugno et al. 2015; Martinho et al. 2014).

6.7 Drug Delivery Applications

The three-dimensional macromolecular architecture of dendrimers shows enormous uniformity in molecular weight, monodispersity, size, shape and surface functional groups, and these salient characteristics of dendrimers assist in designing of biodegradable dendrimers for delivery of bioactives and have been explored in various pharmaceutical and biomedical applications. Nanomedicine is continuously being investigated for targeted delivery for therapeutic agents in order to improve treatment efficacy of both infectious and non-infectious diseases (Jain and Jain 2012, 2013; Wu et al. 2015).

6.7.1 Hybrid Nanocomposite Dendrimers

Various scientific studies have suggested that the complexes of dendrimers with other nanomaterials could result in hybrid nanocomposites having superior drug delivery efficacy as compared to dendrimers or other nanomaterials, alone. These hybrid nanocomposites include combination of dendrimers with materials like CNTs, nanoparticles, liposomes, quantum dots, etc. (Kesharwani et al. 2014). Brunetti et al. (2015) reviewed the applications and advances of nanoparticle-cored dendrimers as drug delivery vectors and reported that these hybrid nanocomposites could serve as promising drug delivery platform with properties like stability, tunable membrane properties and high payload, etc. (Brunetti et al. 2015).

6.7.2 Intracellular Drug Delivery

Dendrimers interact in different ways with drug molecule. Chanvorachote et al. (2015) investigated the mechanism of interaction of lipopeptide antibiotic daptomycin with PAMAM dendrimers using fluorescence spectroscopy. The interaction between daptomycin and dendrimer was reflected as a change in fluorescence spectrum and, finally, it was observed that daptomycin interacted mainly with cationic surface amino groups of PAMAM dendrimers via its ionized aspartic acid residue (Asp-9) (Chanvorachote et al. 2015). In HIV arena dendrimers have been explored to deliver antiretroviral drug to macrophages and lymphocytes, which are the reservoirs of HIV (Edagwa et al. 2014). Dendrimers can hasten the delivery of bioactives across biological membrane by modulation of tight junctions, increasing paracellular transport of small molecules and translocation across epithelial barriers (Souza et al. 2015). Wolfbeis (2015) reviewed the applications of nanomaterials including dendrimers in fluorescent imaging of tissues and cells.

6.7.3 Drug Delivery to Cancer Cells

Cancer is a group of malignant diseases characterized by uncontrolled growth of abnormal cells, which can metastasize, i.e. invade the other tissues. Mortality rates due to cancer are expected to increase in coming years according to reports of the World Health Organization (Estanqueiro et al. 2015). Current treatment protocol of cancer, particularly chemotherapy, suffers from the limitations, mainly resistance and serious adverse effects. Applications of nanotechnology, particularly nanocarriers, for example, quantum dots, carbon nanotubes, dendrimers, polymeric nanoparticles, lipidic nanocarriers (solid lipid nanoparticles, lipid drug conjugates, liposomes, etc.), polymer drug conjugates, transfersomes, ethosomes, niosomes and polymeric micelles, etc., are being investigated to improve treatment of cancer by site-specific delivery of anticancer agents to spare the normal cells that will reduce the toxic effects and higher concentration of anticancer drug to cancer cells that will improve efficacy, finally resulting in improved therapeutic index (Estanqueiro et al. 2015; Mehra et al. 2014, 2015a, b; Jain et al. 2013, 2014b Prabhu et al. 2015; Soni et al. 2015).

The presence of numerous extensions from central core leads to a number of modifiable surface functionality and internal void spaces in the architecture of dendrimers allow for either conjugation or encapsulation of anticancer bioactives (Prabhu et al. 2015). Khatri et al. (2014) conjugated methotrexate (MTX) with polyamidoamine (PAMAM) dendrimers via amide bond between the amine groups of dendrimer and the carboxylic groups of the MTX using a dicyclohexylcarbodiimide coupling reaction to investigate their cytotoxic effect on uterine sarcoma cells, MES-SA. The resultant conjugate showed increased anticancer activity towards MES-SA cells in comparison with free MTX. MTX, a potent inhibitor of dihydrofolate reductase, has been explored for the treatment of cancers and inflammatory disorders including gout (Wong and Choi 2015). Nanocarriers have been

investigated for delivery of MTX with different targeting ligands like folic acid. Further, it has been observed that MTX-conjugated dendrimers bind to the folic acid receptors with three to four-fold affinity in comparison to free MTX. Wong and Choi (2015) finally suggested that dendrimeric nanoparticles could be developed as promising delivery system for methotrexate.

6.7.4 Therapeutic Activity of Dendrimers

Dendrimers have been explored for their therapeutic effect and drug delivery efficacy by many scientists. They have also been reported to potentiate the therapeutic activity of various medicinal agents (Mollazade et al. 2013; Liu et al. 2014). In our laboratory we also explored dendrimers for simultaneous immunostimulatory activity and targeted drug delivery as well as for dual attack on cancer cells by antiangiogenic dendrimers and controlled delivery of anticancer drugs (Jain and Jain 2014b; Jain et al. 2014a, 2015b, c). Further dendrimers including surface-engineered dendrimers have also shown intrinsic therapeutic activity including anti-inflammatory, antimicrobial, wound healing, anti-angiogenic, anticancer, antiviral and immunostimulatory activity, etc. (Liu et al. 2014; Peng et al. 2013). In addition dendrimers have been also found to show therapeutic benefit in bone mineralization, tissue repair and cartilage formation, etc. (Gajbhiye et al. 2009). Dendrimers have shown promising drug delivery potentials in HIV as well as intrinsic anti-HIV activity by restraining HIV and host cell attachment or by inhibiting proliferation of HIV after internalizing into infected host cells (Peng et al. 2013). Silver nanoparticles have been observed to show anti-inflammatory, antimicrobial and wound healing activity. The combination of dendrimers with silver nanoparticles potentiated the anti-inflammatory activity (Kang et al. 2014; Liu et al. 2014).

6.7.5 Photodynamic Therapy (PDT)

PDT is one of the promising treatment strategies for treatment of cancer based on the activation of a photosensitizer (PS) on absorption of specific wavelength light leading to the generation of reactive oxygen species (ROS), which causes local tissue damage through a cascade of cellular and molecular events. Currently, application of PDT is limited by poor water solubility, aggregation tendency (resulting into reduced ROS generation and reduced efficacy of PDT), poor penetration through skin and severe side effects on systemic administration of PS like 5-aminolevulinic acid (ALA). To combat these problems related to PDT scientists are exploring various strategies including nanotechnology such as liposomes, nanoparticles, dendrimers, polymeric conjugates and polymeric micelles for targeted delivery of PS (Avci et al. 2014). Kim et al. (2014) examined the photosensitizing properties of different generations of porphyrin dendrimers and observed the 3.0G porphyrin dendrimers best among 1.0G–3.0G porphyrin dendrimers in terms of in vitro phototoxicity. Pereira et al. (2014) investigated galactodendrimers for delivery of

phthalocyanine, which is a photosensitizer, and observed it as a potential photodynamic therapeutic agent for treatment of bladder cancer when surrounded by galactodendrimers. Later Setaro et al. (2015) designed phthalocyanine dendrimers which were negatively charged. These phthalocyanine dendrimers were active as photosensitizer and able to generate singlet oxygen from molecular oxygen after activation.

6.7.6 Combination Therapy in Cancer

Although targeted treatments to combat cancer using nanomedicines may lead to dramatic regressions, yet, regrettably, the responses are frequently transitory due to resistant cancer cells, which could be tackled by one of the challenging strategies, i.e. combination drug therapy, also known as cocktail therapy. Combined therapy is very important in treatment of cancer due to complexity and severity of disease, toxicity associated with most of the anticancer drug and emergence of resistance (Conniot et al. 2014). Dendrimers have been investigated for dual therapy of cancer either by use of chemotherapeutic agents and some other therapy like anti-angiogenesis, PDT, etc., or use of multiple antineoplastic agents (Mignani et al. 2015). Targeted delivery of anticancer agent with anti-angiogenic dendrimers has resulted in potentiated anticancer activity of drug, doxorubicin (Jain et al. 2014a; Jain and Jain 2014b). Khodadust et al. (2014) observed increase in biocompatibility, anticancer efficacy and reduction in toxicity on combined targeting of anticancer drug, doxorubicin, with dendrimers and magnetic nanoparticles. In this study, researchers designed PAMAM dendrimer-coated iron oxide nanoparticles for delivery of doxorubicin and increased anticancer activity was obtained in cell lines and tumour-bearing mice.

6.7.7 Delivery of Peptides

Nanomaterials including polymeric conjugates, polymeric nanoparticles, lipidic nanoparticles, polymeric micelles, inorganic materials, dendrimers and nanotubes are being investigated as promising delivery vehicles for peptide-based therapeutic and immunogenic agents (Skwarczynski and Toth 2014). Dendrimers made up of gallic acid-triethylene glycol have shown applicability as promising delivery vehicle for drug, genes and peptides (Sousa-Herves et al. 2014). Falanga et al. (2014) investigated the interaction of amide-based dendrimers functionalized with the membrane-interacting peptide gH625 derived from the herpes simplex virus type 1 (HSV-1) envelope glycoprotein H with liposomes. In this study authors explored the mechanism of interaction of dendrimers with cells and intracellular drug delivery by examining their interactions with liposomes made of phosphatidylcholine and cholesterol using fluorescence spectroscopy, isothermal titration calorimetry and surface plasmon resonance. Dendrimers functionalized with gH625 peptide showed high affinity for the membrane bilayer with interaction by non-active translocation mechanism without pore formation (Falanga et al. 2014).

6.7.8 Ophthalmic Delivery

Dendrimers are being explored for enhancement of the ocular bioavailability of drugs attributed to their ability to modulate tight junction, translocate through the epithelial membranes and increase the paracellular transport of drug. Previously carboxyl- and hydroxyl-terminated dendrimers have been explored by researchers to increase retention of pilocarpine and its ocular bioavailability (Vandamme and Brobeck 2005; Souza et al. 2015). Scientists have observed that iontophoresis of nanocarriers can sustain drug delivery and maintain therapeutic concentrations of bioactive in the eye. Souza et al. (2015) investigated the ocular delivery of dexamethasone from 4.0G PAMAM dendrimers with iontophoresis to determine the effect of iontophoresis on PAMAM penetration and distribution of dexamethasone into the cornea as well as to elucidate the effect of dendrimers and/or iontophoresis on transcorneal absorption via ex vivo and in vivo studies. FITC-labelled anionic and cationic PAMAM dendrimers increased the corneal uptake as observed in confocal microscopy, improving the ocular transport. Iontophoresis increased the transport of dendrimers penetrated across the epithelium into the cornea by 3.0, 5.6 and 2.9 folds for dexamethasone-loaded 3.5G PAMAM dendrimers, dexamethasone-loaded 4.0G PAMAM dendrimers and dexamethasone, respectively, in ex vivo studies, whereas in in vivo studies the rate of corneal transport increased by 6.6, 2.5 and 2 folds, respectively (Souza et al. 2015). The results of this investigation suggest that dendrimers could be developed as promising drug delivery system for sustained and targeted delivery to the eye.

6.7.9 Solubilization

Curcumin has shown strong pro-apoptotic and anti-proliferative activity but its poor aqueous solubility, instability and poor bioavailability limit its use in cancer treatment (Yallapu et al. 2011; Debnath et al. 2013; Wang et al. 2013). Conjugate of dendrimers with curcumin increased the water solubility of curcumin with effective anticancer activity as observed against breast cancer cell lines, SKBr3 and BT549, and induction of apoptosis mediated by caspase-3 activation (Debnath et al. 2013). Further Yallapu et al. (2011) investigated the interaction of dendrimer-based nanoformulations of curcumin with serum proteins, human red blood cells and cancer cells in order to assess its clinical applications using UV-visible spectroscopy, particle size analysis, zeta potential, Western blot, haemolytic and haemocompatibility studies. The dendrimeric nanoformulations of curcumin displayed enhanced uptake by cancer cells but simultaneously also showed higher haemolytic toxicity with significant binding capacity towards plasma proteins due to interaction of cell membrane with positively charged amino surface leading to destabilization of cell membrane followed by cell lysis (Yallapu et al. 2011). Later Wang et al. (2013) investigated the acetylated PAMAM dendrimers to increase water solubility, stability and bioavailability of curcumin. These acetylated dendrimers showed sustained release for curcumin with significant anticancer activity towards A549 cells with generation of reactive oxygen species, mitochondrial membrane potential and cell

apoptosis. From these studies as well as from previous studies it is clear that dendrimers could be successfully explored for delivery of poorly soluble drugs.

6.7.10 Nucleic Acid Delivery

Efficient and safe gene delivery is a critical part in designing of gene delivery vehicles. After viral vectors various polymeric materials including dendrimers have been examined as gene delivery vector. Polymeric gene delivery vectors suffer from the problems of polydispersity, heterogeneity and non-site-specific delivery necessitating the development of nanomaterials with defined architecture and dendrimers have been emerged as promising material in this regard owing to their nearly monodisperse architecture, precisely defined molecular weight, uniform size and shape and, most importantly, multivalent surface functional groups (Jain et al. 2012). Currently various scientists are exploring dendrimers as potential nonviral vectors for the efficient delivery of drugs and nucleic acids to the brain and cancer cells due to their highly branched macromolecular architecture with surface functionalities, which could be modified strategically and a large number of internal void spaces (Somani and Dufès 2014).

Li et al. (2015) designed a tumour-targeted self-assembled nano-complex of PAMAM dendrimers, DNA and epidermal growth factor (EGF) via electrostatic interactions for delivery of nucleic acid. The cytotoxicity of these nano-complexes was found to decrease on incorporation of EGF into dendrimers as determined by MTT assay. The in vitro cell line studies with HepG2 cells and in vivo biodistribution studies with xenograft tumour model in mouse showed that EGF-dendriplexes showed higher uptake and preferential distribution into EGFR-positive cancer cells showing promising applicability of dendriplexes in gene transfection with high efficiency (Li et al. 2015). Brunner et al. (2015) developed dendritic siRNA for transfection of neuronal cells exploiting ligand-receptor interactions, which could open the new pathway to treat incurable diseases.

6.7.11 Oral Delivery

Oral delivery, one of the most promising strategies to achieve patient compliance, is restricted by poor solubility characteristics, less permeability, poor bioavailability, high molecular weight, instability of drug into gastrointestinal tract and gastric irritability of drug. Micro- and nanosized carrier systems like microspheres, microcapsules, polymeric nanoparticles, lipidic nanocarriers like lipid-drug conjugates, solid lipid nanoparticles, liposomes, micelles, micro- and nanoemulsions, nanocrystals, self-nanoemulsifying drug delivery systems, dendrimers and carbon nanotubes are continuously being investigated for oral delivery of various bioactives including anticancer drugs, anti-infective drugs, low molecular weight heparins, peptides, vaccines and nucleic acids, etc. (Bagre et al. 2013; Pathak and Raghuvanshi 2015).

Dendrimers serve as promising oral delivery vehicle by traversing through epithelial barriers. They can translocate by transcellular as well as paracellular route by modulation of tight junctions. The permeability of dendrimers across biological membranes depends on their surface charges (Avaritt and Swaan 2014). Bharatwaj et al. (2014) examined the mechanism of uptake and cellular transport PAMAM dendrimers across pulmonary epithelium with solid lipid nanoparticles and suggested that blending of nanomaterials could improve the drug delivery efficacy. Madaan et al. (2014) suggested that PAMAM dendrimers could increase the oral bioavailability of a drug belonging to Biopharmaceutical Classification System (BCS) class II, i.e. quercetin.

6.8 Retrospect of Dendrimers: Toxicity and Safety Issues

Although dendrimer research in the field of drug delivery is showing significant advantages, yet few reports have also identified their negative aspects like potential toxicity. The nanometric size and high surface charge density make dendrimer penetrate through the biological membrane but this nanoscale size range also facilitates non-specific interactions with biological components having size range in nanometres, for example, plasma membranes, nucleus, mitochondria, endosomes, proteins and nucleic acid, etc. (Fig. 6.6). Nanoscale size range of dendrimers made them nanocarriers of substantial interest in cell transfection strategy and is being developed for in vivo gene delivery owing to their ability to interact with or permeate through biological membranes. But on the other hand the non-selective uptake of these nanocarriers renders them vulnerable to cause toxicities including cytotoxicity, haemolysis, immunogenicity, haematological toxicity and in vivo toxicity (Oddone et al. 2016; Lee et al. 2008; Jain et al. 2010). At this juncture it appears approximate to discuss the cytotoxic behaviour of dendrimers, the mechanism induction of cytotoxicity, haemolytic toxicity, haematological toxicity, immunogenicity and in vivo toxicity of dendrimers.

6.8.1 Cytotoxicity

Cytotoxicity of an agent means being toxic to cells that finally results in cell death. Dendrimer-mediated cytotoxicity depends not only on the core of dendritic framework but is also strongly influenced by the nature of numerous end groups on dendrimer periphery. The cationic dendrimers like PAMAM and PPI with terminal primary amines are more toxic than neutral or anionic dendrimers.

The characteristic features of cytotoxicity of dendrimers may be summarized as follows:

1. Cationic dendrimers are much more toxic than dendrimers containing neutral or anionic dendrimers.
2. Toxicity of dendrimers is influenced by generation and concentration.

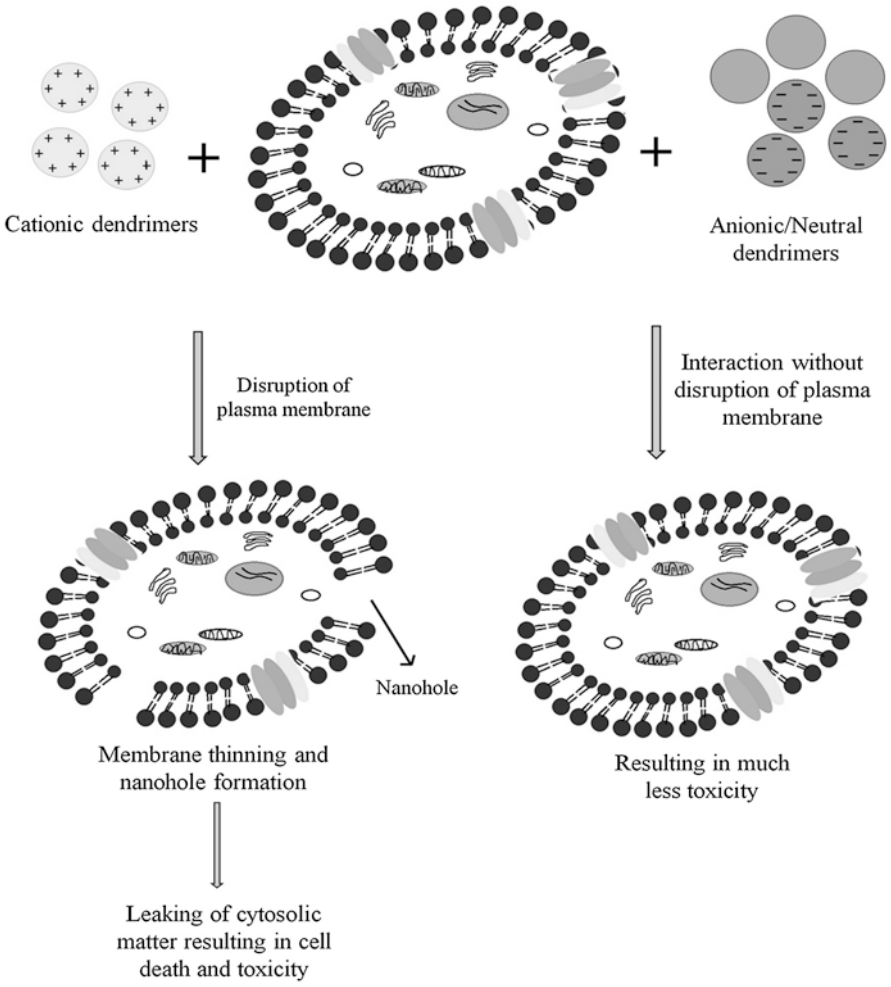

Fig. 6.6 Different surface groups of dendrimers and their toxicity

3. Increase in generation amplifies the surface groups, which results in increased interaction with biological membranes (except cationic PPI dendrimers whose toxicity is generation independent).
4. Toxicity of cationic dendrimers can be lowered by partial or complete modification of surface groups.

Cytotoxicity of different type of dendrimers has been investigated against different cell lines. In study on Chinese hamster lung fibroblast V79 cells and male Swiss-Webster mice, Roberts and associates (Roberts et al. 1996) observed that PAMAM

dendrimers showed dose- and generation-dependent haemolysis and cytotoxicity whereas 5.0G and lower generations did not show the toxicity. 6.0G and higher-generation PAMAM dendrimers show high toxicity attributed to highly polycationic nature of high-generation PAMAM dendrimers. On the other side, 5.0G and lower generations caused fewer problems in in vitro application owing to lesser toxicity. PAMAM dendrimers showed significant cytotoxicity in Caco-2, B16F10, CCRF and HepG2 cell lines. Similarly PPI dendrimers showed considerable cytotoxicity in B16F10, CCRF, COS-7 and HepG2. A difference between PPI and PAMAM dendrimers was noted, and PAMAM dendrimers showed the generation-dependent haemolysis and cytotoxicity whereas toxicity of PPI dendrimers was generation dependent, although the toxicity of both types of dendrimers was found to be concentration and time dependent (Agashe et al. 2006; Jevprasesphant et al. 2003; Malik et al. 2000). Later PPI dendrimers, PEG-conjugated PPI dendrimers and PPI dendrimers with peripheral neutral acetamide groups were studied for cytotoxicity and membrane disruption on cultured human umbilical vein endothelial cells (HUVEC). In the result of these studies it was observed that the plain PPI dendrimers demonstrated drastic time-dependent changes in the plasma membrane permeability and prominent cytotoxicity (Stasko et al. 2007). The cytotoxicity of the cationic dendrimers is the result of interactions between negatively charged biological membranes and the positively charged dendrimer surface. This interaction favours the adherence of dendrimers to the cell membrane followed by damage of cell membrane and cell lysis. Neutrally charged dendrimers, for example, polyester dendrimers, have been found non-toxic both in vitro and in vivo, because the neutral surface of dendrimers does not interact with biological membrane adversely (Padilla De Jesús et al. 2002; Ihre et al. 2001).

It is well known that toxicity of dendrimers is due to their surface cationic charge; hence dendrimers may be tailored to make biologically acceptable by modifying the surface with anionic or neutral group. This phenomenon is also applicable to other types of dendrimers such as dendrimers based on melamine.

6.8.2 Haemolytic Toxicity

Including cell membranes the surface cationic groups of dendrimers also interact with RBCs and this interaction finally results in haemolysis. Haemolytic toxicity of dendrimers is determined by applying the following procedure. Briefly, dendrimers are mixed with RBC suspension and this mixture is incubated for definite time intervals at 37 °C, and the incubated mixture of dendrimers and RBC is centrifuged at 3000 rpm for 15 min. Finally, supernatant is analysed at 540 nm spectrophotometrically against blank (normal saline solution). Percentage haemolysis is calculated against the absorbance factor of 100% haemolytic sample in distilled water (Nguyen et al. 2017; Oddone et al. 2016; Agrawal et al. 2007).

Plethora of research is available on the haemolytic toxicity studies of dendrimers. Bhadra et al. (2003) evaluated 4.0 G PAMAM dendrimers for delivery of an anticancer drug 5-FU and found its haemolytic toxicity to be ~15.3–17.3% for amine-terminated dendrimers and negligible for carboxylic acid-terminated half-generation dendrimers. These observations were later supported by another study in which these dendrimers were found to exhibit haemolytic toxicity up to 18%, close to the value reported by Bhadra and coworkers (Asthana et al. 2005). In lower generation, 3.0 G PPI and PAMAM dendrimers were found to induce haemolysis above a concentration of 1 mg/ml (Malik et al. 2000). However marked haemolysis was observed with 4.0 and 5.0 G PPI dendrimers in the study performed with the objective to evaluate potential of dendrimer for liver-targeted delivery of primaquine phosphate. The authors found 35.7% haemolysis with 4.0 G PPI dendrimers and 49.2% haemolysis with 5.0 G PPI dendrimers (Bhadra et al. 2005). Agashe et al. (2006) also investigated the haemolytic toxicity of 5.0 G PPI dendrimers and observed 34.2 ± 0.2%, 51.6 ± 0.3% and 86.2 ± 0.6% haemolysis with 5.0 G PPI dendrimers at concentration of 1 mg/ml after incubation for 1, 2 and 4 h, respectively. Poly-L-Lysine (PLL) dendrimers were also found to induce haemolysis in vitro due to the presence of peripheral amino groups (Agashe et al. 2006). Agrawal et al. (2007) reported 14.1 ± 1.02% and 12.22 ± 2.4% haemolysis, respectively, with 4.0 G and 3.0 G PLL dendrimers loaded with chloroquine phosphate (Agrawal et al. 2007).

6.8.3 Immunogenicity

A few reports are available on the immunogenic properties of dendrimers (Roberts et al. 1996; Agashe et al. 2006; Huang et al. 2017; Dobrovolskaia 2017). Roberts et al. (1996) investigated the immunogenicity of PAMAM dendrimers in Chinese hamster lung fibroblast V79 cells and male Swiss-Webster mice with in vitro toxicity, in vivo toxicity, immunogenicity and biodistribution. In this study authors determined the immunogenicity of 3.0G, 5.0G and 7.0G PAMAM dendrimers by immunoprecipitation and Ouchterlony double diffusion assay and observed no apparent immunogenicity for these generations in the dose range of 0.1–0.0001 μM. Similarly 5.0G PPI dendrimers were found to be unable to provoke any detectable humoral immune response using ELISA for monitoring antibody titre in Balb/C mice under the experimental conditions (Agashe et al. 2006). From these studies it is clear that dendrimers show no more or only weak immunogenicity. So it could be assumed that dendrimers are treated by the host immune system as indigenous agent by biological system and it is an encouraging sign for its proposed drug delivery calibre. Still there is need of extensive research in this area to provide corroboration by unequivocal data to arrive at any significant supposition.

6.8.4 Haematological Toxicity

Interaction of dendrimers with RBCs does not induce haemolysis only but also influences haematological parameters ascribed to polycationic nature of the unmodified dendrimers. The effect of dendrimers on different blood parameters including white blood corpuscles (WBCs), red blood corpuscles (RBCs), haemoglobin (Hb), haematocrit (HCT) and mean corpuscular haemoglobin (MCH) has been determined by various researchers using different analytical and chemical methods. Agashe et al. (2006) observed a significant decrease in RBC count, a substantial increase in WBC count, a decrease in Hb content and MCH value and a considerable difference in HCT value between control and PPI dendrimers using Erma particle counter method. RBC count, Hb, HCT and MCH values were found to decrease drastically to 4.43 ± 1.15, 8.26 ± 1.39, 27.06 ± 3.16 and 18.82 ± 1.06 from normal values of 7.15 ± 1.37, 14.89 ± 1.35, 41.69 ± 5.51 and 20.76 ± 1.35, respectively. Later Agrawal et al. (2007) also observed a significant increase in WBC count and decrease in RBC count, with 4.0 G PLL dendrimers. From these studies we may conclude that the cationic dendrimers have shown significant impairment in haematological parameters and this underlines the necessity to devise some strategy to render them more biocompatible.

6.8.5 In Vivo Toxicity

Only few researches are available on the in vivo toxicological behaviour of dendrimers. In vivo toxicity studies are vital to prove the safety of dendrimer to gain GRAS (generally recognized as safe) status. Roberts et al. (1996) studied in vivo toxicity of 3.0, 5.0 and 7.0 G PAMAM dendrimers in Swiss-Webster mice. In the result of this study the authors observed that only 7.0 G PAMAM dendrimers produced potential biological complications. Subsequently Rajananthanan et al. (1999) studied the immunopotential properties of 5.0 G dendritic polymer-based novel molecular aggregate formulation in mice. In the result the authors found these dendrimeric aggregates to be non-toxic to mice and unable to produce antigen-specific antibody response. In the in vivo biodistribution studies of ^{125}I-labelled PAMAM dendrimers, Malik et al. (2000) determined the toxicological profile of dendrimers and observed that after *i.p.* and *i.v.* administration cationic PAMAM dendrimers were rapidly cleared from circulation whereas only 0.1–1.0% of the dose was recovered in blood 1 h post administration. Neerman et al. (2004) performed a detailed study to elucidate in vivo behaviour of melamine dendrimers. The authors administered 2.5, 10, 40 and 160 mg/kg of melamine dendrimers in mice intraperitoneally to determine acute toxicity of dendrimers and observed 100% mortality in 6–12 h post injection of 160 mg/kg dose. Increase in liver enzyme activity indicated the hepatotoxicity at the dose of 40 mg/kg. At subchronic doses of dendrimers (2.5 and 10 mg/kg), mortality and renal damage was not observed. So a detailed study of in vivo deposition and toxicological behaviour of dendrimers is needed to establish possible clinical application of dendrimers in biological system.

6.9 Engineering of Dendrimers: Functionalization and Designing of Biodegradable and Biocompatible Dendrimers

Nanotechnology is continuously being explored in the field of medicine as well as making remarkable progress in terms of increase in therapeutic efficacy and reduction in side effects. The incredible physicochemical and biological properties, which make nanomaterials unique also, cause them to interact non-specifically with biological components resulting into potential toxicities (Bagre et al. 2013; Jain et al. 2010, 2014b).

PAMAM dendrimers are one of the most explored classes of dendrimeric polymers in delivery of various bioactives like drug, gene, contrast, etc. In spite of promising results in the field of drug delivery, the biosafety of the PAMAM dendrimers is a crucial aspect that needs to be investigated carefully as an important parameter for toxicity and biological applications of dendrimers. Lin et al. (2015) explored the biosafety or biocompatibility of dendrimers by assessing the effects of 5.0G PAMAM dendrimers with amine, hydroxyl or carboxyl surface groups on the secondary structure and conformation of immune molecules using UV-visible, fluorescence and circular dichroism spectroscopies as well as on complement activation and antigen-antibody reaction determined by enzyme-linked immunosorbent assay (ELISA) and agglutination assay. In this study carboxylic group-terminated 5.0G PAMAM dendrimers impaired red blood cell (RBC) antigen-antibody reaction at a concentration of 10 mg/mL. Finally, from the results the authors concluded that the effects of the PAMAM dendrimers on immune molecules depend on their bulk structure and surface groups as the PAMAM dendrimers could affect the secondary structure and conformation of γ-globulin and could inhibit the complement activation (Lin et al. 2015).

Namazi et al. (2014) synthesized the biodegradable and biocompatible peptide dendrimers to stimulate artificial proteins having globular architecture. These dendrimers were synthesized as ABA-type triblock copolymers (glutamic acid dimethyl ester-poly (ethylene glycol)-glutamic acid dimethyl ester) using PEG 600 as core and glutamic acid as branching units by liquid-phase peptide divergent growth approach. These dendrimers were found to be 20–100 nm in size as determined through transmission electron microscope (TEM). These dendrimers were also concluded to be biocompatible delivery system for controlled drug delivery (Namazi et al. 2014).

He et al. (2015) reported the synthesis of amine-terminated 5.0G PAMAM dendrimers functionalized with fluorescent marker, fluorescein isothiocyanate (FITC) using thiourea linkage and cyclic arginine-glycine-aspartic acid (RGD) peptide through polyethylene glycol (PEG) spacer and consecutively remaining amine terminals of dendrimers were acetylated. These surface-engineered dendrimers were investigated for encapsulation and targeted delivery of an anticancer agent doxorubicin (Dox). The scientists finally concluded that these RGD conjugated multifunction dendrimers elicited the targeted delivery of Dox to αvβ3 integrin-overexpressing cancer cells with sustained release and high drug payload.

6.10 Conclusion

The lucid designing of optimum nanocarriers is the most challenging aspect of controlled and targeted drug delivery. Although, nanotechnology led to development of nanomaterials for drug delivery, which seems to be very promising in current scenario, yet it is necessary to check their in vivo performance as well as effect on biological and environmental components. Dendrimers are revolutionizing the ultimate prospects for highly specific controlled drug delivery. They have shown their promising advantages in each part of drug delivery from solubilization to theranostic applications, from delivery of drug molecule to siRNA. The next goal is to translate this inception into clinical benefits to provide reliable, effective and advanced treatment options for different ailments particularly for severe and fatal diseases like cancer, AIDS, autoimmune diseases and neurodegenerative disorders for which at present no reliable therapy is available. To be successful at drug delivery, the dendrimers shall have to prove safety while ruling out the toxicity. Large-scale production of such dendrimers will further enhance their therapeutic application economically. Consider the enormous possibilities of therapeutic potential of dendrimers; the available literature indicates only a tip of the iceberg.

Conflict of Interest The authors report no conflict of interest related to manuscript.

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7 Bioprotein Based IPN Nanoparticles as Potential Vehicles for Anticancer Drug Delivery: Fabrication Technology

Md. Mehedi Hasan, Adib H. Chisty, Mohammed Mizanur Rahman, and M. Nuruzzaman Khan

Abstract

The ever growing interest in bioactive biopolymers originated from natural sources and advances in extraction and purification of protein have led to the development of protein-polymer based targeted drug delivery system. Among them, interpenetrating polymer network (IPN) based nanoparticles have gained great attention in the last decades, mainly due to their biomedical applications. IPN nanoparticles based drug delivery system is basically designed to deliver drugs at a predetermined rate for a desired period of time with minimum fluctuation. A number of reports on the IPN based drug delivery systems showed that these carriers have emerged as a novel drug carrier in controlled delivery system. This chapter aims to give an overview of the recent design concepts of IPN based nanoparticles, methods of synthesis, some natural polymers especially protein widely used for IPN and also covers recent advances in IPN based nanoparticles system for pharmaceutical applications as well as in anticancer drug delivery system.

Keywords

Interpenetrating polymer network · Drug delivery · Bioprotein · Controlled release

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7.1 Introduction

The recent extensive advances in polymer chemistry allow the state of art design of biodegradable and biocompatible polymer based nanoparticles for targeting the drug into the desired site. The proper placing of a drug delivery system in the site of action for maximizing drug availability and minimizing the side effects and thus optimizing the effect of pharmacotherapy are the main research focus. Controlled drug delivery systems offer numerous benefits over conventional dosage forms, which include reduced adverse reaction, toxicity and frequency of dosing with improved efficacy, patient compliance and convenience (Liu et al. 2007).

In the past few years, polymer nanoparticles evolved as a potential carrier in drug delivery system and have attracted much attention and undergone many investigations in the field of biomedical due to their wide range of applications including their surface area, size, optical and magnetic properties, and biological transport that are brought into the perspective of drug delivery. The growing demand for improved polymer properties has paved the development of the blending of polymer mixtures. The blend materials from natural and synthetic sources alone are not always able to meet the complex demands of the biomaterials. In order to improve the poor biological performance and mechanical strength, a new class of polymers has been introduced which are based on blending of either natural or synthetic polymers alone or in combinations. The use of natural polymers especially bioprotein is valuable due to their nontoxicity, low cost, biodegradability, biocompatibility and safety although their mechanical properties are often poor while, the success of synthetic polymers relies on their broad range of mechanical properties.

An interpenetrating polymer network (IPN) is a blending of two or more polymers in a network with at least one of the polymers synthesized in the presence of another (Hu 2014). This results in realization of physically crosslinked network when polymer chains of the second system are entangled with or penetrate the network formed by the first polymer. Each individual network retains its individual properties, so synergistic improvements in properties like strength or toughness can be seen (Stanciu 2003). IPN can be distinguished from the other multiple systems through their bicontinuous structure. They are usually formed by crosslinking of two polymers that are in intimate contact but without any chemical contact and yield a material with improved properties depending on the composition and degree of crosslinking (Kudela 1987). IPN offers synergistic effect by sharing the properties of both the polymers subsequently avoiding the limitations of natural as well as synthetic polymers. It is expected to have a better capability for the controlled release of drugs under physiological conditions.

IPN based drug delivery system is designed to deliver drugs in zero-order pattern with minimum fluctuation. The concept of IPN goes back at least as far as 1914 when the first interpenetrating polymer network was invented by Aylsworth (Aylsworth September 1914). For the first time, the term IPN was introduced by Miller in 1960s in a scientific report about polystyrene networks (Millar 1960).

The field of IPN has expanded dramatically since that time. Advances in polymer science have led to the development of several novel drug delivery systems. Among them, IPNs have shown superior performances over the conventional individual polymers and, consequently, the ranges of applications have grown rapidly for such class of materials. The advanced properties of IPNs have attracted considerable attention in pharmaceutical field especially in the area of drug delivery. These biocompatible, nontoxic, and biodegradable polymer networks are now acquiring unique place in delivering bioactive molecules, particularly in controlled and targeted drug delivery applications. Various research investigations have shown that a variety of drugs can be delivered effectively via IPN based delivery systems. The idea of IPN nanoparticles as drug carriers may be employed to modify or to control the drug distribution at the tissue, cellular, or subcellular levels. IPN nanoparticles can be either nanospheres or nanocapsules depending on the method of preparation. Nanospheres are polymeric matrix systems in which the drug is dispersed within the polymer throughout the particle. On the contrary, nanocapsules are vesicular systems, which are formed by a drug-containing liquid core (aqueous or lipophilic) surrounded by polymeric; thus nanocapsules may be considered a reservoir system.

IPNs are prepared by some most commonly used bioproteins, which has been reported to be suitable for drug delivery system. Bioproteins available in nature are inherently biocompatible and nontoxic. Most commonly utilized proteins are gelatin, silk fibroin, silk sericin, and soy protein. Interestingly, our blood plasma can easily adapt them, which made them suitable for many pharmacological applications. Drugs loaded in the scaffolds, nanoparticles or IPNs are being used extensively in biomedical nowadays. The low mechanical properties of bioproteins can be resolved by either incorporating or by being incorporated in another synthetic or natural polymer such as starch, chitosan, alginate, polyacrylonitrile, etc. which can provide higher mechanical strength.

In this chapter, we will discuss the advances in fabrication technology of bioprotein based IPN nanoparticles and drug delivery system; particularly in the treatment of cancer patients. In the first part of this chapter, we will discuss the major challenges in anticancer drug delivery system. Then we will discuss about common bioprotein based IPN system in the second part. In the third part, the major fabrication techniques and application of IPN nanoparticles will be discussed.

7.1.1 Classification of IPN

IPN can be classified in several ways. Depending on their chemical bond, they can be divided in three groups as (a) covalent semi-IPN; when two separate polymer systems that are crosslinked form a single polymer network, it is called covalent semi-IPN. (b) Noncovalent semi-IPN; in noncovalent semi-IPNs only one of the polymer systems is crosslinked. (c) Noncovalent full IPN. A noncovalent full IPN is one in which two separate polymers are crosslinked independently.

7.2 Major Challenges in Anticancer Drug Delivery

In addition to the numerous approaches of treatment, cancer chemotherapy is considered as one of the major therapeutic methods to fight cancer. The objective of the ideal cancer chemotherapy is to deliver the precise amount of drug with anticipated controlled rate and sufficient long duration of time to the site of action (cancer cells) while preventing the normal cells to obtain the desired therapeutic response. To achieve this goal, drug delivery systems must hold enough amount of drug and root out the problems like drug resistance based on cellular or noncellular mechanism, reformed biodistribution, biotransformation as well as secretion of anticancer drugs from the body. The delivery systems should meet the requirements like sustained circulation (which can be obtained by PEGylation), sufficient tumour accumulation (by considering enhanced permeability and retention (EPR) effect), uptake by tumor cells (by active targeting) and organized drug release (by optimizing delivery system) with a profile matching the pharmacodynamics of the drug.

7.2.1 Biodistribution of Drug

While administrating conventional anticancer therapeutics through intravenous systems, drugs are distributed properly all over the body via the bloodstream, and affects both malignant and rapidly dividing normal cells of the bone marrow, gut, lymphoid tissue, spermatogenic cells, fetus, and hair follicles (Brown and Links 1999). Nevertheless, prominent limitations like severe side effects, high patient risks, repeated treatments, altered biodistribution of drug, and the acquisition of multidrug resistance (MDR) by the cancer cells were observed in such treatments (Brigger et al. 2012).

7.2.2 Multidrug Resistance (MDR)

Acquisition of drug resistance by cancer cells, considered as one of the potential factors influencing the success of cancer chemotherapy, could be attained following the noncellular as well as cellular mechanisms. The noncellular resistance, caused by poorly vascularized tumor regions, can effectively reduce drug access to the tumor thereby protecting cancerous cells from cytotoxicity. In addition to this, tumors in acidic environment can also possess a resistance mechanism against basic drugs enabling them to get ionized preventing their diffusion across the cellular membrane where high interstitial pressure and low microvascular pressure are also responsible for retarding or impeding extravasation of molecules. On the contrary, cellular mechanism involves a decrease in sensitivity and intracellular accumulation of drugs by overexpression of the plasma membrane P-glycoprotein (P-gp) which repels drugs from the cell. While entering into cell, such drugs combine with P-glycoprotein to form transmembrane channel which further uses the energy of adenosine triphosphate (ATP) hydrolysis to pump drugs out of the cells. To

overcome the limitations, Krishna and Mayer (2000) administrated P-gp inhibitors along with encapsulated anticancer drugs in nanoparticles preventing P-gp-mediated multidrug resistance.

7.2.3 Clearance by Reticuloendothelial System (RES)

Rapid blood clearance by the reticuloendothelial system (RES), composed of monocytes and macrophages located in reticular connective tissue, is also considered as one of the major problems in cancer therapy. Such cells usually remove pathogens, cellular debris as well as foreign substances from the bloodstream. However, the particle size, hydrophobicity, surface charge and composition of system also influence the clearance profile of the delivery system (Juliano 1976). Addressing the problems, Blume and co-workers (1990) proposed a coating of synthetic PEG (polyethylene glycol) to increase the surface hydrophilicity of liposome inhibiting protein adsorption and liposomes opsonization (Klibanov et al. 1990).

7.2.4 Hydrophobicity of Anticancer Drugs

Hydrophobicity of anticancer drugs is another most promising problem in cancer chemotherapy. Due to limited water solubility along with the unavailability of effective biocompatible delivery system, the use of various potential anticancer drugs as chemotherapeutic agents gets restricted. In addition to the problems associated with solubility, toxicity of anticancer drugs towards cancer as well as normal cells leads to the development of an intravenous (i.v.) formulation rather than oral design, however, the i.v. formulation must hold the drug within the system providing sustained release to reduce the contact of anticancer drugs to those of normal cells. In order to obtain such objectives ascribed above, the i.v. formulation should be soluble in aqueous media which is mostly rare in case of anticancer drugs. For instance, paclitaxel, one of the most successful anticancer drugs, is highly hydrophobic with a limited water solubility (less than 0.5 mg/L). For the solubilization of paclitaxel an approach by Si-Shen et al. (Feng and Chien 2003) reported the use of adjuvants like Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol which were much more effective in solubilizing process though another prominent study by Weiss and coworkers (1990) found limitations regarding severe side effects including hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity, hyperlipidemia, abnormal lipoprotein patterns, erythrocyte aggregation, and peripheral neuropathy while using such additives. Development of novel delivery systems for these molecules without using organic solvents and adjuvants would be one of the major approaches to overcome the obstacles stated above. Considering these, Farokhzad et al. (2006) and Cuenca et al. (2006) reported the use of porous materials in nanoscale offering a great potential and prominent approach to deliver targeted drug together with controlled releasing technology. Lu et al. (2007), working with mesoporous nanoparticles as a drug delivery system for camptothecin (a hydrophobic

anticancer drug to overcome poor water solubility), utilized camptothecin (CPT) into the pores of fluorescent mesoporous silica nanoparticles (FMSNs) and delivered the drug into a variety of human cancer cells to induce cell death, a route suggesting that the mesoporous silica nanoparticles might be used as a carrier overcoming the insolubility problem of many chemotherapeutic drugs. Prior to that, the use of CPT derivatives (modification of CPT with chemical modification to develop water solubility) were limited due to loss of their effectiveness towards stomach (Litvak et al. 1999), colon (Takiguchi et al. 1994), neck (Abigerges 1995), bladder (Keane et al. 1998) as well as breast (Miller et al. 2004) owing to chemical modification.

7.2.5 Nano-Carriers in Cancer Chemotherapy

Problems associated with cancer chemotherapy can be avoided through the proper implementation of nanotechnology based targeted cancer chemotherapy including nanocapsules, nanoparticles, nanorods, nanofibers, nanocrystals, nanotubes, stealth nanoparticles, liposomes, stealth liposomes, pH sensitive liposomes, temperature sensitive liposomes, etc. A study by Du and co-workers found (Du et al. 2011) the use of nanocarriers effective for cancer affected tissues due to enhanced permeability and retention (EPR) effect (Peer et al. 2007; Torchilin 2007; Farokhzad and Langer 2009). Such delivery provides selective and effective localization of pharmacological active moiety at pre-identified target in therapeutic concentration while restricting its access to nontarget sites therefore minimizing toxicity, maximizing therapeutic index together with developing the biodistribution of drug which is the foremost concern in success of cancer chemotherapy. Targeting to only cancer cells by nanodevices can successfully be attained by surface engineering where as the characteristics of both, i.e., the cancer including highly disordered leaky vasculature, high hydrostatic pressure, high requirements for nutrition, angiogenesis, Arg-Gly-Asp tripeptide (RGD) based strategy, EPR effect and the presence of over-expressed receptors also affect the selective targeting procedure. For example, in a study by Stella et al. (2000) folate grafted to PEGylated cyanoacrylate nanoparticles showed ten-fold better affinity for the folate binding protein (FBP) than free folate. Similar results were found when poly (ε-caprolactone) nanocarrier loaded with tamoxifen was administrated to mice (Kakde et al. 2011). Congruent to previous studies, nanocarriers loaded with anticancer drugs also showed greater retention time within the affected areas (Shenoy et al. 2004). In addition, several studies on nanovehicle system found nanoparticles to conglomerate all the advantages of other innovative carriers while reducing the associated problems. Prominent research works by Pinto and Müller (1999), Dingler et al. (1999) and Demirel et al. (2001) reported the development of Solid Lipid Nanoparticles (SLN) formulations for various application routes (i.e., parenteral, oral, dermal, ocular, pulmonary, rectal) and thoroughly characterized in vitro and in vivo. Among the various routes, better results were obtained in case of parenteral applications regarding excellent physical properties, protection of incorporated drugs from degradation, controlled drug release, and site-specific targeting.

7.3 Bioprotein Based IPN Nanoparticles

Some of the proteins founds in nature, such as gelatin, silk fibroin, silk sericin, soy protein, are inherently biocompatible and nontoxic. They are easily adapted by our blood plasma which made them suitable for many biomedical applications. Drugs loaded in the scaffolds, nanoparticles or IPNs of these bioproteins are being used extensively in biomedical field nowadays. Though bioproteins are biocompatible but most of them have low mechanical properties which doesn't allow its full utilization in drug delivery. This problem can be resolved by either incorporating or by being incorporated in another synthetic or natural polymer which can provide higher mechanical strength such as alginate, polyacrylonitrile, etc. This section describes about the IPNs prepared by some most commonly used bioproteins which has been reported to be suitable for drug delivery system.

7.3.1 Gelatin

Gelatin is produced by partial hydrolysis of native collagen. It is an important hydrocolloidal polypeptide and has widespread application in food, photographic and pharmaceutical industries by fulfilling various roles as a stabilizer, thickener, texturizer, etc. (Hasan et al. 2018). Hydrophilic chelating groups contain an acrylamide polymer poly(acrylamidoglycolic acid) (PAGA) used extensively in biomedical and separation fields (Nagaraj et al. 1987). In a recent study, curcumin encapsulated pH sensitive gelatin–acrylamidoglycolic acid (AGA) IPN-nanogels were studied for anticancer drug delivery purpose (Rao et al. 2015). IPN-nanogels were synthesized by free radical emulsion polymerization. *N*, *N*′-Methylenebisacrylamide (BIS) and glutaraldehyde crosslinkers were used for the crosslinking of AGA and gelatin, respectively, leading to the fabrication of nanogels (Fig. 7.1). Curcumin is a yellow

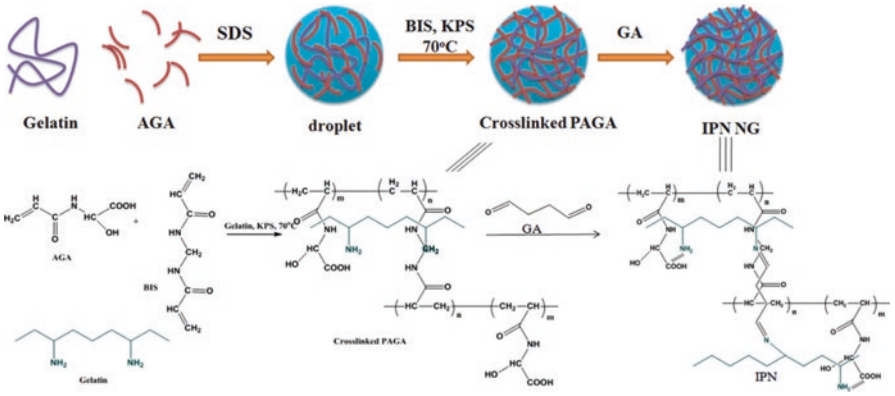

Fig. 7.1 Schematic representation of formation of IPN-nanogels; reaction represents crosslinking of BIS with AGA via covalent bond in gelatin, then glutaraldehyde (GA) are crosslinked with gelatin via imine linkages (Rao et al. 2015)

bioactive compound of the Indian spices, turmeric, has antiproliferative activity against many different types of cancer cells, including cancers of the colon, prostate, and breast (Chen and Huang 1998; Aggarwal et al. 2007). But the poor solubility, bioavailability, and rapid degradation in physiological pH hinders its application as anticancer drug. In this study, the hydrophobic curcumin was encapsulated successfully into hydrophilic IPN-nanogels. Normal fibroblast cell line and HCT-116 human colon cancer cells were studied for finding the biocompatibility of the pristine and curcumin encapsulated nanogels. It has been found that encapsulation enhanced the bioavailability and dispersion of the curcumin due to the complex network of IPN of gelatin and AGA. The encapsulation efficiency of these IPN-nanogels drug formulations ranged from 42% to 48%. Curcumin encapsulated nanogels showed negative and positive cytotoxicity towards normal fibroblast and HCT-116 cancer cell line. Because of the enhancement of dispersion encapsulated curcumin showed higher anti-cancer cell activity compared to pristine curcumin. The results suggest that these new type of IPN-nanogels are a good carrier for colorectal cancer drug delivery.

In an another study (Jain et al. 2009), moderately hydrophobic polyacrylonitrile and hydrophilic gelatin cryogel-IPN was prepared at subzero temperature by a combination of free radical crosslinking polymerization of acrylonitrile (AN) and simultaneous gelation in presence of *N*,*N*′-methylenebis(acrylamide) (MBAAm) and covalent crosslinking of gelatin with glutaraldehyde as crosslinkers, respectively. The interpenetrating cryogel was formed by single step freezing of both the precursors simultaneously. It was possible to achieve a supermacroporous and rigid scaffold of interpenetrating network with both high porosity and high mechanical strength due to the application of lyophilization and resulted in dense pore walls formed by cyroconcentration of polymers (Lozinsky et al. 2001). As the concentration of gelatin was varied, surface smoothness and the structure of pores present in the pore walls also varied. Cryogel-IPNs became more spongey and elastic with the increase of gelatin concentration by the virtue of hydrophilic nature of gelatin. Interconnected pores of cyrogel-IPNs allowed fast transportation of solvent and low resistance to flow of water. Total monomer concentration, crosslinking density, pore wall thickness, temperature at which the gels are prepared affect the rapidity of solvent transportation. Rapidity was also affected by the thickness of the gel, i.e., the distance between the outer boundaries to central parts of cryogel, larger the distance slower the rate of swelling and shrinkage due to slow rate of mass and heat exchange due to increased distance (Lozinsky et al. 2001; Plieva et al. 2005).To ensure the biocompatibility Chinese hamster ovary (CHO) cells were allowed to grow for a period of 7 days over the polyacrylonitrile (PAN)–gelatin cryogel-IPN scaffolds. CHO cells adhered well over the surface of the cryogel-IPN scaffolds and secreted extracellular matrix which was spread all over the matrix (Lozinsky et al. 2001; Xue et al. 2002; Plieva et al. 2005; Srivastava et al. 2007).

7.3.2 Silk Proteins

Silk fibroin (SF), a hydrophobic protein biopolymer obtained from *Bombyx mori* (silkworm) (Altman et al. 2003), has been extensively categorized as noninflammatory and highly biocompatible for various cell types (Hakimi et al. 2007). This biopolymer has been intensively used in the preparation of IPN hydrogels. It is a fibrous protein of silk fiber and consists of heavy (350 kDa) and light (25 kDa) chain polypeptides, connected by a disulfide link (Zhou et al. 2000). The regenerated fiber has been considered as a candidate for biomaterials owing to its good mechanical strength in the wet state, biocompatibility for the growth of cells, and high resistance against enzymatic degradation (Gotoh et al. 1997; Ha et al. 2003; Jin and Kaplan 2003; Nazarov et al. 2004). Various polymers have been used for making IPNs with silk fibroins. Synthesis of multicomponent materials like IPNs of Poly(*N*-isopropylacrylamide) (PNIPAAm) and SF was developed by Gil and Hudson (2007) to improve the deswelling kinetics by suppressing the skin layer formation.

Polymers of acrylamide (AAm) and its derivatives are well known for their hydrophilic and inert nature that makes them suitable for applications in medical and pharmacy (Rosiak et al. 1983). Semi-IPNs of poly acrylamide (PAAm) and SF were prepared by simultaneous free radical polymerization method (Mandal et al. 2009). In this study, semi-IPN was prepared by mixing organic fibroin protein and aqueous AAm solution in different ratios in presence of *N*,*N*,*N*′ ,*N*′ -tetramethylethylenediamine (TEMED) activator and ammonium persulfate initiator. The solutions turned to gel during incubation at 37°C due to *N*,*N*′-Methylenebisacrylamide crosslinker. Resulted crosslinked semi-IPNs were semi-transparent yellowish color due to fibroin content. They were soft and elastic and had a smooth slippery surface. Fourier-transform Infrared (FTIR) results indicated the intermolecular interactions between silk fibroin and polyacrylamide during gelation and hydrogel formation (Haider et al. 1993). Prepared semi-IPNs had higher mechanical strength which overcame the disadvantages of silk fibroins. It was because of the formation of intense fibroin bonding and well entrapment of it within 3D network of polyacrylamide, resulting in matrix stability and minimal release of fibroin protein into the medium. Biocompatibility of the IPNs was evaluated against AH927 feline embryo fibroblast cells and positive results indicated the compatibility of the semi-IPNS.

In an another study, poly(vinyl alcohol) (PVA) a particularly advantageous polymer for attaching cell signaling molecules or drugs via numerous hydroxyl groups present on the backbone (Bryant et al. 2004) has been used with silk fibroins to produce semi-IPNs by photopolymerization of precursor and blend solutions. Different weight ratios were selected to study the optimal composition of semi-IPNs for biomedical purpose. The vinyl groups react to generate an insoluble, but water-swellable, crosslinked network via a photopolymerization process, in the presence of a Irgacure-2959 photoinitiator and UV irradiation light source (Martens and Anseth 2000). Due to irradiation PVA chains containing multiple vinyl groups

are chemically crosslinked and the fibroin chains interdiffuse and become physically entangled within the PVA 3D network (Mandal et al. 2009; Liu and Chan-Park 2010). Incorporation of silk fibroin resulted in colored and brittle IPNs compared to pristine PVA. These were found biocompatible while tested against human fibroblast and Human keratinocytes (HaCaT) cell lines. Release of model compound proved the potential of semi-IPNs for drug release purpose. It was also reported that drug loading and release rate could be moderately controlled by varying the PVA and SF concentrations.

Silk sericin, another natural protein derived from silkworm, is a water soluble globular protein. It has been used by Wu et al. in the preparation of IPN hydrogels with PNIPAAm (Wu et al. 2006) and Polymethyl methacrylate (PMMA) (Wu et al. 2010), the last one being a fast pH-responsive hydrogel.

7.3.3 Fibrin

Fibrin is a polymerized form of a soluble 340 kDa protein named fibrinogen. Fibrinogen polymerized into fibrin through the action of thrombin in the presence of calcium. Fibrinogen can be isolated from a patient's own blood, making possible the formation of an autologous scaffold (Ye et al. 2000). Fibrin-based gels produced from blood molecules are biodegradable and biocompatible (Ariëns et al. 2002). They also support cell adhesion, proliferation, stem cell differentiation and, importantly, capillary formation (angiogenesis) (Ahmed et al. 2008; Janmey et al. 2008). But poor mechanical properties of fibrin gels render its applicability in biomedical purposes. Lower mechanical strength makes them susceptible to contraction/compaction by cells and rapid degradation by proteases. Consequently, cell-seeded fibrin gels often shrink during in vitro culture (Syedain et al. 2009) and/or degrade prematurely prior to or soon after transplantation (Meinhart et al. 1999). One way to eliminate the limitations of fibrin is to make IPNs of it with other biocompatible natural or synthetic polymers. Recent studies suggested that incorporation of another polymer into fibrin gels leads to better shape stability and mechanical strength. Studies on IPN hydrogels composed of fibrin and polyethylene oxide (PEO) demonstrated improved mechanical properties while maintaining cell growth (Akpalo et al. 2011). The PEO network was synthesized in an aqueous buffer medium, and the fibrin gelation was carried out upon UV irradiation so that PEO/Fibrin IPNs were obtained. Another study reported a fibrin–alginate IPN as a dynamic matrix for the growth of ovarian follicle cells (Shikanov et al. 2009). Follicle growth was facilitated by the degradation of fibrin and alginate phase remained to provide structural support. The follicles cultured were meiotically more competent than in alginate alone. In another study IPN hydrogels composed of fibrin and hyaluronic acid (HA) for tissue engineering applications were reported. HA is a non-sulfated glycosaminoglycan found primarily in the extracellular matrix (ECM). Due to its low immunogenic, biodegradable properties, and their versatility in chemical modification, it has been widely used as tissue engineering scaffolds (Masters et al. 2005; Prestwich 2011). In this study fibrin–HA IPN was prepared by

chemically crosslinking HA network and entangling fibrin in the matrix. Human umbilical vein endothelial cells (HUVECs) were cultured for biocompatibility analysis. Result showed that IPN had improved mechanical properties of fibrin gels while retaining the ability to promote cell proliferation but cell proliferation decreased with the degree of crosslinking.

7.3.4 Soy Protein

Renewable plant protein soy protein is 60–70% of the total soybean protein (Shewry et al. 1995). It is naturally abundant and regarded as an ideal biomaterial for biomedical, tissue engineering scaffold and drug delivery applications due to its good biodegradability and biocompatibility, ready availability, high thermal stability and noncytotoxicity (Vaz et al. 2003; Zheng et al. 2007; Liu et al. 2008). Recently, soy protein composites blended with environmentally sensitive polymers have been developed for cell cultures, drug carriers and wound dressing materials (Vaz et al. 2004; Snyders et al. 2007). Addition of stimuli sensitive polymers endow the composites with intelligent features and resulted in new potential applications in the biomedical and pharmaceutical fields (Liu et al. 2009). Novel temperature-sensitive interpenetrating polymer network of soy protein/Poly(*N*-isopropylacrylamide) (PNIPAAm) was prepared (Liu and Cui 2011). PNIPAAm is a typical temperature sensitive hydrogel which has been used with many other natural polymers for biomedical applications (Zhang et al. 2005; Mallikarjuna Reddy and Ramesh 2008). In this study, Glutaraldehyde and *N*,*N*′-methylenebisacrylamide as crosslinking agents and ammonium persulfate as initiator was used and properties such as network structure, miscibility dynamic swelling and deswelling kinetics and temperature sensitivity of IPNs were investigated. IPNs have shown good miscibility and thermal stability. Soy protein and BIS contents were the crucial parameters to obtain the expected properties such as swelling ratio, water retention capacity, network structure and temperature sensitivity. The release behavior and the release mechanism of a model protein, bovine serum albumin (BSA), were also investigated in detail (Liu and Cui 2011). The results indicated that the release behavior of BSA had strong temperature dependence and the release percentage of BSA could be controlled by modulating the amount of soy protein or crosslinking agent. The results indicate that the hydrogels may have potential applications in the field of biomedical materials such as in the controlled release of drugs.

7.4 Fabrication Techniques

Several methods have been reported to prepare IPNs from protein polymers. Fundamental free radical crosslinking reaction of polymers behind all these processes occurs either by chemical or irradiation in presence of an initiator. Though irradiation can mitigate the use of chemical crosslinkers but loaded drug may

degrade in contact with radiation. In this section several methods have been described by which IPNs of protein have been produced and isolated.

7.4.1 Desolvation or Coacervation

Coacervation/desolvation is the most commonly used method for the preparation of protein nanoparticles (Lohcharoenkal et al. 2014; Cheng et al. 2016). In addition to conformation changes in protein structure, the desolvation process reduces the solubility of the protein leading to phase separation. The size of nanoparticles in the coacervate can be controlled by controlling processing variables. After nanoparticles are formed, they are crosslinked by agents such as glutaraldehyde and glyoxal which leads to the formation of IPN nanoparticles (Langer et al. 2003). This desolvation is based on the differential solubility of proteins in different solvents as a function of solvent polarity, pH, ionic strength, and presence of electrolytes (Thiering et al. 2001; Lohcharoenkal et al. 2014). In case of albumin, antisolvent acetone produced smaller nanoparticles compared to ethanol. Moreover, the increase in antisolvent/solvent ratio results in rapid extraction or diffusion of the solvent into the antisolvent phase. This limits the growth of particles and smaller nanoparticles are formed (Wang et al. 2008).

Langer et al. (2003) studied processing parameters that influence the formation of Human serum albumin (HAS) nanoparticles. Furthermore, the pH value of the protein solution prior to desolvation has an impact on the resulting particle size and yield due to higher probability of protein coacervation at net-zero surface charge at the isoelectric point (Fuchs et al. 2010). It was found that by changing the initial pH, prior to desolvation step, nanoparticles ranging from 100 to 300 nm can be produced and higher pH leads to formation of smaller particles (Ko and Gunasekaran 2006).

Gelatin nanoparticles can be prepared by dissolving gelatin in an aqueous solution (pH 7), followed by changing the solvent composition of water and alcohol, and gradual addition of ethanol (Coester et al. 2000; Kaul and Amiji 2004; Jain et al. 2008). Denaturation and hydrophobicity of protein molecules also control the size of nanoparticle. Although β-lactoglobulin (BLG) and bovine serum albumin (BSA) have same p*I* but due to lower hydrophobicity BLG produces smaller nanoparticles (∼130 nm) than BSA (Ko and Gunasekaran 2006) and denaturation of BLG prior to phase separation further reduced the particle size of BLG nanoparticles to approximately 60 nm (Chen et al. 1994). In desolvation process rigidity of the nanoparticles can be increased by increasing the degree of crosslinking agent. Lysine residues in the protein involve in crosslinking. This crosslinking causes the stabilization of the protein nanoparticles, reduces enzymatic degradation, drug release from the nanoparticles (Langer et al. 2008; Nahar et al. 2008) and leads to the formation of denser but smaller particles (Nahar et al. 2008). But due to their toxic nature, it is essential to remove the crosslinkers as completely as possible thereafter (Wang and Uludag 2008). To stabilize the hydrophobic proteins such as gliadin and legumin during phase separation surfactants are required. Addition of surfactant in this case

increases product yield without alternating the particle size (Irache et al. 1995). Recently radiation has been used for crosslinking of some elastin-derived nanoparticles where α-elastin aggregates were generated by increasing the temperature and then irradiated with ^{60}Co gamma rays (Neradovic et al. 2004). Drugs can be loaded into particles by various processes such as surface adsorption and entrapment method during desolvation process. Efficiency of the method of loading varies from protein to protein and it also depends on drug properties as well as other factors such as drug/polymer ratio (Merodio et al. 2001). Casting evaporation has been used to form crosslinking polymers to synthesize IPN. For example, IPNs of gelatin and dextran have been prepared by this method in presence of glutaraldehyde as a crosslinker agent. In this process gelatin was added to crosslinker solution of glutaraldehyde and dextran was added.

7.4.2 Emulsion or Solvent Extraction

The emulsification and solvent extraction methods for production of nanoparticle proteins required an aqueous buffer solution. Emulsification of an aqueous solution of protein particle is done in oil by a high-speed homogenizer or ultrasonic shear and nanoparticles are formed at the water/oil interface (Fig. 7.2). Surfactants such as phosphatidylcholine and Span 80 are added as stabilizers to produce nanoparticles (Sussman et al. 2007; Yang et al. 2007). A nanosuspension is obtained after solvent evaporation and particles can be stored after freeze-drying (Xiao et al. 2009). This method has been used for the production of various protein nanoparticles including albumin, gelatin, and whey protein. Both chemical, by glutaraldehyde, and thermal crosslinking can be done in emulsion process. IPN-nanogels of gelatin and PAGA have also been prepared by using emulsion polymerizations. A double emulsion technique involving w/o/w emulsions allows encapsulation of proteins and hydrophilic drugs (Mayer et al. 2005).

Though single emulsion crosslinking technique based on w/o emulsion is used extensively but recently w/w emulsion method has also been developed to form IPN

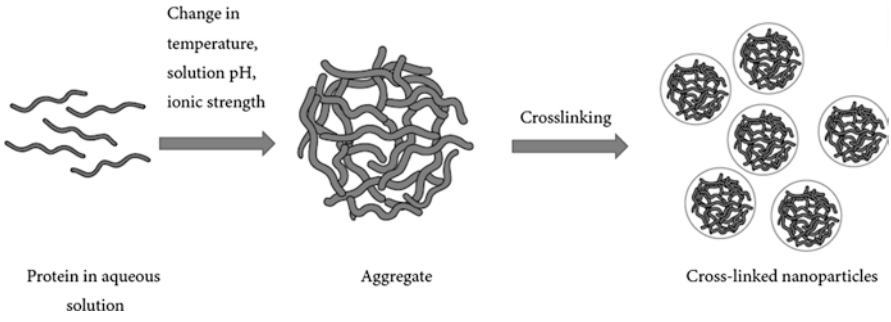

Fig. 7.2 Preparation of protein IPN particles by coacervation or desolvation method (Lohcharoenkal et al. 2014)

(Bhattacharya et al. 2013). The main advantage of w/w emulsion method is that there is no use of organic solvents which might leave toxic residue that is incompatible with IPN biomaterials. Difference between these two methods is how they are prepared. In w/o emulsification method an aqueous phase is added to oil phase to prepare w/o emulsion after dissolving the water soluble materials in aqueous phase by stirring until a homogenous solution is made at specific temperature (Banerjee et al. 2010). But in the case of w/w emulsion technique an aqueous solution of water soluble polymers is emulsified as a dispersed phase in an aqueous solution of another polymer that acts as continuous phase. Then the dispersed polymer phase is crosslinked to form IPN network (Bhattacharya et al. 2013). Miniemulsion and inverse miniemulsion techniques are also used for the preparation of semi- or full IPNs. In this technique small stable droplets are prepared in a continuous phase by the application of high shear stress (Landfester 2006). First constituent polymers are obtained by sonication using specific initiator then one of the polymers is crosslinked with crosslinking agent which produces a semi-IPN and finally another crosslinking agent for another polymer is added to form full IPN. High shear stress applied during the process may cause degradation of miniemulsion through coalescence. To prevent this degradation a surfactant and a costablizer are added that are soluble in dispersed phase but insoluble in continuous phase. Koul et al. (2011) synthesized novel IPN nanogels composed of poly(acrylic acid) and gelatin by inverse miniemulsion technique. Acrylic acid monomer stabilized around the gelatin macromolecules in each droplet was polymerized using ammonium persulfate and tetramethylethylene diamine and crosslinked with *N*,*N*′-methylenebisacrylamide to form semi-IPN nanogels, which were sequentially crosslinked using glutaraldehyde to form IPNs (Fig. 7.3).

7.4.3 Electrospray

A relatively new technique called electrospray has been used in the preparation of nanoparticles. This method is dependent upon the surface tension force that arises due to the application of high voltage current in a polymer solution. Aerosolized

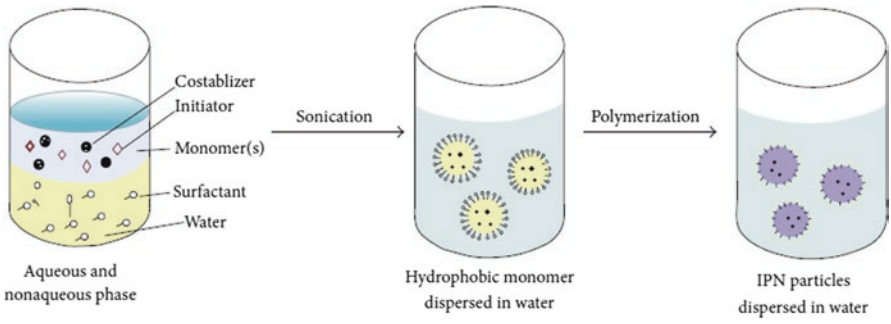

Fig. 7.3 Synthesis of IPN particles by miniemulsion polymerization (Lohani et al. 2014)

liquid droplets are formed after applying high voltage followed by passing through an emitter. The aerosolized droplets contain protein nanoparticles of colloidal size which are collected. Many researches have been done with this method to produce nanofiber, nanoparticles, and spray coating. Size of the particle formed through this method depends on many variables such as applied potential, working distance, nozzle diameter, polymer type, etc. (Zhou et al. 2007; Fathollahipour et al. 2015). It has been used largely for the preparation of gliadin and elastin-like peptide nanoparticles (Wu et al. 2008; Gulfam et al. 2012). Therapeutic drugs and nucleic acids can be well loaded by the protein nanoparticles with high efficacy through this pathway.

7.4.4 Irradiation Technique

This process is based on exposing the polymer under the radiation to facilitate the crosslinking of polymers in IPNs. Crosslinking of protein polymer such as gelatin by gamma irradiation has been reported in the literature (Kojima et al. 2004). IPNs based on *N*-vinyl pyrrolidone (NVP):gelatin, and a copolymer of NVP – acrylic acid:gelatin were prepared and studied (Singh et al. 2007). *N*,*N*′-methylenebisacrylamide (BIS) for NVP and/or acrylic acid and glutaraldehyde for gelatin were used as crosslinkers, respectively. BIS crosslinker was placed in NVP or copolymeric solution of NVP and acrylic acid before gamma irradiation and glutaraldehyde was applied after the irradiation for crosslinking the gelatin polymer. Irradiation was found beneficial in a sense that higher amount of crosslinker results in brittle and very longer degradation time product (Anderson et al. 1979) which is minimized in irradiation. Dose, composition, time, and pH of immersion medium played important role in controlling the swelling degree of prepared IPNs. Incorporation of acrylic acid leads to form IPNs with rigid as well as elastic structures that may find applications in wound dressing and drug delivery systems.

7.5 Conclusions

Interpenetrating polymer network nanoparticles can be realized for controlled delivery of anticancer drugs. The controlled delivery of anticancer drug in proper place is prime consideration in designing anticancer drug delivery system. IPN offers various advantages like specificity, excellent swelling capacity and mechanical strength, which play an important role in targeted drug delivery. Bioprotein based IPN nanoparticles are mainly designed to deliver drugs to a specific site of action with minimum fluctuation at a predetermined rate for maximizing drug availability and minimizing the dose related side effects and thus the effects of pharmacotherapy can be optimized. IPN based nanoparticle system using various polymers has the opportunity of obtaining desire materials with a range of properties that will improve drug carrier and eliminate the disadvantages of individual polymer system.

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Semi-IPN Systems for Drug Delivery

8

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Abstract

Polymers are the most valuable excipients exhibiting outstanding structural properties and are capable of offering advanced and sophisticated functions such as controlled drug release and drug targeting. Progresses in polymer science have led to the development of several novel drug delivery systems. Semi-interpenetrating polymer network (semi-IPN) system is one of them that shows much better performance over the conventional individual polymers. Consequently, the ranges of applications for such class of materials have grown rapidly day-by-day. These biocompatible, nontoxic, and biodegradable polymer networks have attracted considerable attention in biomedical and pharmaceutical field in delivering bioactive molecules particularly in controlled and targeted drug delivery applications. In the past few years, many researchers reported semi-IPN-based delivery system as a novel carrier in controlled drug delivery. This chapter emphasizes recent research on semi-IPN systems that allow acquiring a better understanding of potential drug delivery applications of polysaccharide-based semi-IPN systems.

Keywords

Interpenetrating polymer network · Drug delivery · Polysaccharide · Hydrogel · Biocompatible

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8.1 Introduction

The ever-increasing improvements of medical and pharmaceutical formulations have been obtained by means of using a number of specialized materials that allow a controlled and targeted delivery of drugs or other bioactive compounds. At present, polymers are the most valuable chemical products in the biosphere for controlled drug delivery applications. The demand of polymer products is growing rapidly in the global market. Polymers have shown excellent performance as valuable excipients in tablet and capsule formulations (Ravi Kumar and Kumar 2001) as well as into the parenteral arena as blood circulation time enhancers (Blume and Cevc 1990). In the recent decades, a new class of polymers with improved properties has been developed by blending of either natural or synthetic polymers alone or in combination. These polymers are capable of offering advanced and sophisticated functions such as controlled drug release and drug targeting (Liechty et al. 2010).

An interpenetrating polymer network (IPN) is a combination of two polymers in a physically cross-linked network where chains of one polymer are entangled with or penetrate the network of another polymer (Sperling and Hu 2014). Among various types of IPNs, semi-interpenetrating polymer networks (semi-IPNs) (Fig. 8.1) are the new system used in drug delivery applications. Two types of semi-IPNs are available: (1) *covalent semi-IPN*, when two separate polymer systems are cross-linked to form a single polymer network, and (2) *noncovalent semi-IPN*, when only one of the polymer systems is cross-linked. Therefore, semi-IPN technique is able to combine the advantage of natural as well as synthetic polymers. Among natural polymers, polysaccharides have emerged as innovative biomaterials for drug delivery due to their biocompatibility, biodegradability, hydrophilicity, and nontoxicity (Hu et al. 2014b; Matricardi et al. 2013).

In recent years, semi-IPN systems have been developed as innovative biomaterials for potential therapeutic and diagnostic applications (Liu and Chan-Park 2009). A quite large number of polysaccharides have been investigated for the design of semi-IPNs for drug delivery applications. Rokhade et al. (2009) reported a novel

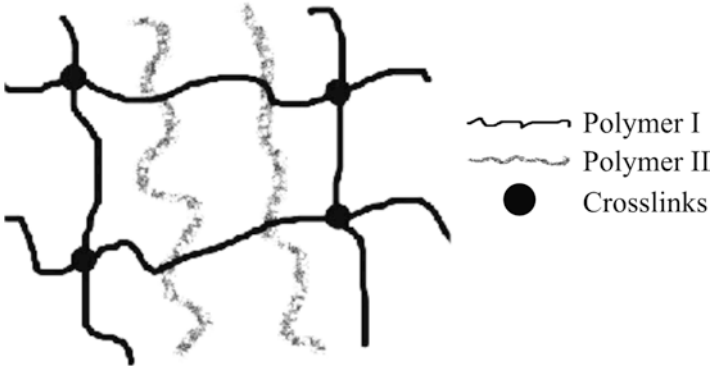

Fig. 8.1 Semi-IPN system

semi-interpenetrating polymer network of hydrogel microspheres of chitosan and hydroxypropyl cellulose (HPC). The hydrogel microspheres were prepared by emulsion-cross-linking method where glutaraldehyde (GA) was used as a cross-linker. Their study showed that the prepared hydrogel microspheres were used for controlled release of chlorothiazide drug. A series of pH/temperature-sensitive semi-IPN hydrogel beads were prepared by Shi et al. (2006) using calcium alginate and poly(*N*-isopropylacrylamide) for drug delivery applications. In addition, smart macroporous salecan/poly(*N*,*N*-diethylacrylamide) semi-IPN hydrogel was reported for anti-inflammatory drug delivery.

This chapter mainly focuses on the most studied polysaccharide-based semi-IPNs, namely, cellulose, alginate, hyaluronic acid, scleroglucan, salecan, dextran, etc. and will also give more detailed information on the potential and wide range of applications of these polysaccharide-based systems.

8.2 Preparation Methods of Semi-IPN Materials

The term "interpenetrating polymer network (IPN)" was first introduced by Miller (1960). Among IPNs, semi-IPNs are the new system where only one component is cross-linked. Recently, a large number of polysaccharides have been used for the preparation of semi-IPN materials. Many studies have been focused on the synthesis of semi-IPN materials (Matricardi et al. 2013) that are discussed in the following.

8.2.1 Casting Evaporation

Casting evaporation is one of the most common methods to prepare cross-linked polymer network. In this method each polymer constituent is heated until it is dissolved, and after that cross-linker is added to the solution (Kosmala et al. 2000). In case of sequential formation of semi-IPNs, solution of one polymer is added to the cross-linker solution and subsequently the monomers or the second polymer are loaded into the swollen network. In both cases the solution is heated and mixed and then casted and dried. This technique is used to prepare polysaccharide-based semi-IPN gels which can be used in drug delivery applications.

For example, B. Guo reported the preparation of thermo- and pH-responsive semi-IPN hydrogels (CM-CS/PNIPAm) by using carboxymethyl chitosan and poly(*N*-isopropylacrylamide) (Guo and Gao 2007).

8.2.2 Emulsification Cross-Linking

Emulsification cross-linking method is mainly based on phase separation. Generally, w/o emulsion method is used for single-emulsion cross-linking technique. In w/o emulsification method, first, the water-soluble materials are dissolved in aqueous phase and then the aqueous phase is added to oil phase to prepare w/o emulsion.

However, recently w/w emulsion method has also been developed to form semi-IPN and is known as the better method than the former one. The main advantage of w/w emulsion method is that there is no use of organic solvents which ensures less toxic semi-IPN biomaterials. Tabata and his co-workers reported the preparation of semi-IPN hydrogel microspheres by using chitosan and hydroxypropyl cellulose for controlled release of chlorothiazide drug (Tabata and Ikada 1989).

8.2.3 Miniemulsion Technique

The idea of miniemulsion technique is to initiate the creation of small stable droplets in a continuous phase by applying high shear stress (Landfester 2006). A surfactant is added to minimize the coagulation that prevents the degradation of miniemulsion through coalescence. The process of semi-IPN formation can be divided into two steps. In the first step, sonication is done to obtain constituent polymers by using specific initiator. And finally, one of the constituent polymers is polymerized and cross-linked using a cross-linking agent resulting a semi-IPN.

8.2.4 Inverse Miniemulsion Technique

In inverse miniemulsion technique, monomer solution is miniemulsified in a continuous hydrophobic phase. Hydrophilic monomers are used in inverse miniemulsion (water-in-oil) technique to prepare semi-IPN materials. The polymerization process can be initiated either from the continuous phase or from the droplet. For example, novel semi-IPN nanogels were synthesized by Koul et al. using poly(acrylic acid) and gelatin through inverse miniemulsion technique. *N*,*N*-Methylene-bis-acrylamide (BIS) was used as a cross-linker.

8.3 Comparison Between IPN and Semi-IPN Systems

Interpenetrating networks of polymers are like different distinct networks of polymers that coexist which may incorporate characteristics of the constituting polymers or show different characteristics. Semi-interpenetrating polymer networks (semi-IPNs) and interpenetrating polymeric networks (IPNs) are similar polymer networks which have gained much attention due to their potential as a drug delivery medium and being biocompatible and nontoxic. IPNs and semi-IPNs differ in their polymeric network structure as well as characteristics such as different shift in transition temperature.

Semi-IPNs and IPNs have functional groups in polymer chains and thus provide the opportunity to have tailor-made desired characteristics in the network. Furthermore, these networks are often highly regarded due to their biocompatibility.

Hence such networks hold a great future in pharmaceutical fields. In this section, a brief discussion on the comparison between semi-IPN and IPN is given.

IPNs are the network where two or more polymer networks are combined and at least one network is cross-linked, while the other polymer is present (Suthar et al. 1996). According to International Union of Pure and Applied Chemistry (IUPAC), an IPN is

> A polymer comprising two or more networks which are at least partially interlaced on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken. A mixture of two or more pre-formed polymer networks is not an IPN (Jenkins et al. 1996).

Thus a molecular-level interlaced network of two or more polymers which are chemically bonded is known as IPN, and unless those chemical bonds are broken, the polymers are theoretically not possible to separate. There are different types of IPNs depending on their method of synthesis or types of chemical bonding. Former types include sequential, simultaneous, latex, thermoplastic, and gradient IPNs. In sequential IPN, one monomer is made to form a network by means of cross-linker and initiator. Then in the presence of the other monomer and cross-linker, the network is swollen and polymerized to get an IPN. In simultaneous IPN, the mixing is done simultaneously in a single step. In latex IPN, the mixing occurs in latex particle giving the IPN a core and shell structure; hence the molding of IPN is easier in this process. In thermoplastic IPN, physical cross-linkers are used instead of chemical ones. In gradient IPNs, a gradient of one monomeric network over other is observed (Sperling and Hu 2014).

Semi-IPNs are different from IPNs because in this case, there is a network of one polymer only and the other polymer is simply dispersed into that network. The IUPAC has defined semi-IPNs as, "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." When comparing with the IPN, it stated that, "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" (Jenkins et al. 1996).

Thus the semi-IPNs are, in easier terms, linear polymers dispersed in another polymeric network. A Fig. 8.2 is given below to get a clearer picture.

In most cases, IPNs go through phase separation in the formation period. This separation is due to the difference in components that have been used to form the network. Although such phase separation occurs very slowly because of the high viscosity, it results in a heterogeneous mixture. The bonds and interlacing make it even a tardier process. As thermal properties depend on the phase condition, the transition temperature shift is rather slow in IPNs. However, semi-IPNs show a faster shift in transition temperature unlike full IPNs (Lipatov and Alekseeva 2007). Semi-IPNs are often used to synthesize a self-healing polymer network. Mechanical

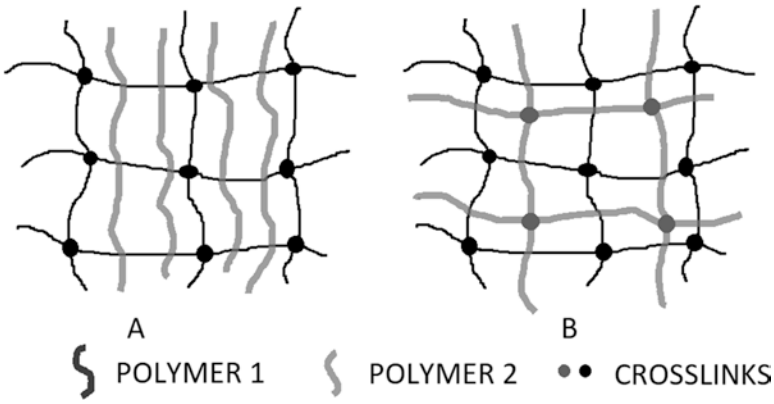

Fig. 8.2 Illustration of semi-IPN (**a**) and full IPN (**b**)

or thermal stress or cracks are often restored with the linear chains diffusing into the cracks (Penhasi and Reuveni 2018).

8.4 Existing Semi-IPN Systems

8.4.1 Hydrogel

Tuning of mechanical and thermal properties of the semi-IPNs gives a wide range of potential application fields. Addition of natural and synthetic polymers has helped further to combine different necessary properties to be incorporated in the network. One of the means to incorporate such properties is to synthesize hydrogels by means of semi-IPN. Not only the ability to have modified characteristics but also synthesizing hydrogels using semi-IPNs improved common weaknesses that are often visible when hydrogels are synthesized by other means.

Hydrogel is a three-dimensional physically or chemically cross-linked super sorbent polymer network. They retain water but do not dissolve. They are biocompatible and less toxic and can be tuned to respond to external stimuli. Hence, hydrogels have found their uses in pharmaceuticals as well as biomedical fields (Hoffman 2012). But hydrogels can suffer from some weaknesses such as fast disintegration or lower strength under wet conditions (Yoshida and Okano 2010). Preparation of semi-IPN hydrogels is a practicable way of preparing hydrogels where such shortcomings can be overcome. Semi-IPN network in hydrogels has helped to increase the water absorption and resistance to salinity and enhance strength (Pulat et al. 2011).

An example of synthesizing hydrogels from semi-IPN is described by Ross et al. In the process, semi-IPN hydrogel is synthesized based on silk sericin and poly(*N*-hydroxyethyl acrylamide) (PHEA), and the resulting hydrogel is a highly porous scaffold that can be used for dermal reconstruction (Ross et al. 2017). The silk

fiber's outer layer is known as silk sericin which has moisturizing, anti-bacterial, and bioadhesive properties and the ability to release bioactive molecules (Brown and Badylak 2013). The use of silk sericin was also to enhance the structural flexibility. The researchers stated that the reason why they have used semi-IPN structure was that it was able to make the scaffold soft and the mechanical properties was better and the swelling was comparable to human skin. Hydrogel made from the sericin and hydroxyethyl acrylamide (HEA) followed the free radical polymerization procedure where the sericin was dissolved in warm water and HEA was added and mixed. *N′N*-Methylene-bis-acrylamide (*N′N*-MBAAm) was used as the cross-linker and *N*,*N*,*N′*,*N′*-tetramethylethylenediamine was used as the catalyst. Ammonium persulfate was used as the initiator. The mixture was left to react to form the semi-IPN hydrogel network. Further washing and lyophilization produced a porous scaffold which shows good proliferation, bioadhesion, and flexibility.

Another porous semi-IPN hydrogel was produced using pullulan as the base material. Pullulan is a polysaccharide derived from bacteria. The reason for using pullulan is that it has good water absorption capability and strong fiber forming capability. The hydrogel was synthesized by putting the pullulan solution in an argon gas covered stirrer and mixing potassium persulfate as an initiator. Calcium carbonate was added as a porogen. Then acrylamide and *N*,*N*-methylene-bis-acrylamide were added and the hydrogel was formed. Then after further treatment, the porous hydrogel was achieved. Its application as a mercury removal adsorbent was tested, and it was found that the hydrogel could remove mercury at a rapid rate (Saber-Samandari and Gazi 2015).

Stimuli responsive hydrogels are also known as smart hydrogels and they are attracting much attention in drug delivery related researches. A pH-sensitive protein-based semi-IPN hydrogel has been synthesized by Park et al. The L-lysine polymer-based semi-IPN cationic hydrogel was tested for the streptokinase drug delivery and showed potential in modulating the release of drug. The hydrogel swelled when the pH is low and reversed when the pH was high. Since the hydrogel is cationic, the ionic interaction with the drug further helped to modulate its release. Free radical polymerization method and polyethylene glycol cross-linking were used to synthesize the semi-IPN hydrogel (Park et al. 2001).

Liu et al. have prepared a thermo-sensitive semi-IPN hydrogel where the hydrogel showed characteristics such as high elasticity and fast reaction to external stimuli. The hydrogel that was prepared showed a core-shell morphology with diameter of about 100 μm. According to the researchers, core layers can be filled with cargos to carry out desired functions (Liu et al. 2017).

A green semi-IPN hydrogel was prepared using biomass in a facile way. Using free radical polymerization method, modified carboxymethyl cellulose was cross-linked while the wood hydrolysate was present in the solution, and hence a semi-IPN hydrogel was formed. Wood hydrolysates are derived from hydrothermally treated biomass of wood. FTIR study showed that the wood hydrolysate is present, interlaced with the carboxymethyl cellulose network (Maleki et al. 2016).

8.4.2 Membrane

Semi-IPN networks are interlaced polymeric network and can be used for selectively permeable membrane. In this section, semi-IPN-based membranes are discussed.

A sub-micrometer porous membrane has been prepared from polyethylene glycol (PEG) and chitosan semi-IPN network. The PEG was extracted from the network to form a micrometer membrane. The membrane prepared by this process is pH responsive and has fast swelling characteristics. With the pH from 3.2 to 11, the swelling index varied from 120 to 220. The mechanical stability and strength of the membrane are high due to chitosan cross-linking. The pore size is dependable on the PEG composition and cross-linking agent (Zeng and Fang 2004).

Semi-IPN-derived membranes are often used to separate gases from gas mixtures. A common application is the removal of greenhouse gases using such types of membranes as they are often regarded as a greener and cheaper way of selectively removing greenhouse gases. Amine systems that are able to capture 90% of CO_2 in flue gas could increase the cost of electricity generation by 50–90% which is quite high (Merkel et al. 2010). However, membranes can be used instead of other regular separators because no regeneration or phase change is required (Roussanaly et al. 2016). A semi-IPN based on polyimides and cross-linked poly(ethylene oxide) (PEO) has been synthesized and tested as CO_2-selective membranes. The study shows that it has higher strength and more ductile than the pure PEO membranes alone and has the potential to work as a CO_2 separator (Kline et al. 2017). Another membrane from the semi-IPN with similar application has been studied by Kurdi and Kumar (2007). In the process a thermosetting and a thermoplastic material were polymerized to synthesize a membrane for gas separation. Polyetherimide, a thermoplastic, and bismaleimide, a thermosetting polymer, were in situ polymerized where the thermoset one physically interlaced with thermoplastic one. The resulting membrane could separate gases better than the polyetherimide membranes alone. The results also suggest that the membrane is efficient to capture CO_2 from flue gas and can be used where oxygen rich air is required.

Semi-IPN can be used to fabricate ion exchange membrane as well. One study shows that quaternized chitosan and polystyrene-based semi-IPN membrane are durable and have a hydroxyl ion conductivity of 2.80×10^{-2}siemens per centimeter. The membrane was fabricated in the presence of quaternized chitosan emulsion where the styrene was polymerized under inert (nitrogen) atmosphere and acetic acid solution.

8.4.3 Electrolytes

Semi-IPN as electrolyte has seen the most applications in lithium ion batteries. Rechargeable lithium batteries are highly regarded nowadays because of them being the power house for many electric vehicles, electronic devices and as means of large scale energy storage (Tarascon and Armand 2001). Conventional lithium batteries

had porous membranes with liquid electrolytes which posed many issues. The solvents used were volatile and flammable and they often leaked and posed fire hazard (Lu et al. 2015). In addition, lithium metal is regarded as unstable in liquid electrolytes in cycles and dendrites (whisker or needle like growth of lithium metal) usually grow on the metal anodes which may cause a short circuit when in connection with the cathode (Zhihu et al. 2011). Electrolytes in solid polymer have then come in to use where the liquid electrolyte problems can be prevented. However, they had limitations in conductivities at lower than room temperature and preventing growth of the dendrites. To improve such conditions, semi-IPN system of electrolytes is being studied and showed good potentials to use them in lithium batteries.

Liu et al. have prepared a flexible semi-IPN as electrolyte which can prevent the dendrite growth and has higher conductivity at room temperature. The semi-IPN network was prepared using the UV treated ethoxylated trimethylolpropane triacrylate (ETPTA) with alumina nanoparticles in liquid electrolyte and linear poly(ethylene oxide) (PEO). PEO is used to provide the bridge for interaction between lithium ions and electrolyte solvents (Liu et al. 2018; Kim et al. 2013). Alumina is used as the filler and for cell stability.

Another solid polymer electrolyte semi-IPN membrane has been prepared using thermal-induced free radical polymerization. In this process an oligomer (dimethacrylate) is polymerized in presence of linear PEO and supporting lithium salt. The resulting semi-IPN electrolyte is robust and flexible and usable with an aging-resistant lithium battery. According to the research, this system demonstrates a high retention even after more than 2000 charging and discharging cycles (Nair et al. 2016).

A different approach to solid state polymer electrolyte is the gel electrolyte which can also be prepared by semi-IPN network of polymers. A gel electrolyte prevents the liquid leakage and volatilization of the solvent and often regarded as good alternative to solid polymer electrolyte (Dias et al. 2000). An UV-cured semi-IPN matrix has been fabricated by cross-linking poly(ethylene glycol) diacrylate-co-poly(vinylene carbonate) in the presence of linear poly(vinylidene fluoride-co-hexafluoropropylene) polymer (Lu et al. 2015). The linear polymer helps the membrane to be flexible, while the cross-linked polymer provides thermal stability and rigidity. This network can hold a lot of electrolytes and hence has high conductivity. The semi-IPN polymer electrolytes show good characteristics that help them to be applied in the manufacturing of safer and stable lithium ion batteries.

8.5 Polysaccharide-Based Semi-IPN Systems for Drug Delivery

Polysaccharide-based semi-IPNs have emerged as innovative biomaterials nowadays. A quite large number of polysaccharides such as chitosan, alginate, hyaluronic acid, scleroglucan, salecan, dextran, etc. have been investigated for the design of semi-IPNs for drug delivery applications. They are discussed in the following sections.

8.5.1 Chitosan

Chitosan is a versatile biopolymer which has been used in different fields but extensively in biomedical applications including drug delivery, wound healing, tissue engineering (Islam et al. 2017), etc. Recently chitosan has been utilized to form semi-IPN systems with other natural and synthetic polymers, and their ability of loading and release of different drugs have been investigated. The $–NH_2$ groups of chitosan make this polymer soluble in acidic medium which favors cross-linking and the formation of interpenetrating network with other polymers.

Chitosan and polyvinyl pyrrolidone (PVP) can make semi-IPN system in the form hydrogel which is pH sensitive (Patel 2011). This hydrogel is reported to release an antibiotic, clarithromycin, by non-Fickian diffusion mechanism in the acidic environment. Preparation method of such semi-IPN hydrogel involves the mixing of solution of chitosan and PVP followed by addition of cross-linking agent like glutaraldehyde. Drugs are usually added into the system before cross-linking. This hydrogel is characterized by high mucoadhesion and swelling in acidic condition which is due to protonation of amino group. Such semi-IPN systems are useful to release drugs in acidic condition of stomach.

In case of delivery of anticancer drug, semi-IPN hydrogel prepared from acrylamide (AAm) and/or N-hydroxymethyl acrylamide (HMA) monomers with chitosan can be a potential carrier (Özbaş and Gürdağ 2015). This type of semi-IPN hydrogel can be prepared by free radical chain polymerization and the properties of this hydrogel can be tailored by varying monomer and chitosan ratio. Swelling behavior which is important for drug delivery depends on the chitosan content of the hydrogel and increase in chitosan content enhances swelling capacity of the gel. Drug is usually added before polymerization, and the extent of cross-linking in the network and the percentage of drug loading determine the release behavior of the drug.

Semi-IPN hydrogel prepared from polyacrylamide (PAAm) and chitosan can be used as potential wound dressing material as it is possible to design this hydrogel to provide burst release of drug in combination with sustained release of growth factor delivery (Kahraman et al. 2013). Free radical polymerization is used to synthesize such hydrogel, while ethylene glycol dimethacrylate can be utilized to cross-link PAAm. This hydrogel shows stable swelling characteristics in the pH range of the wound media. Both antibiotic drug and growth factor can be loaded by soaking method and the release kinetics of drug depends on the swelling kinetics of hydrogel as the diffusion of drug molecules occurs through the large pores of swollen hydrogel. Such biocompatible semi-IPN hydrogel with properties of high swelling ability, antimicrobial and bioactive properties, and sequential release profile for antibiotic and growth factor can be an ideal wound dressing material.

A thermo-sensitive semi-IPN hydrogel was prepared from chitosan and poly(*N*-isopropyl acrylamide-co-vinyl pyrrolidone) by Li at al. (2012) which showed improved loading capacity and sustained release of anionic drugs. This hydrogel was prepared by free radical polymerization using *N*,*N*-methylene-bis-acrylamide as cross-linker. An anionic and potent nonsteroidal anti-inflammatory drug Naproxen was loaded into the hydrogel by electrostatic interactions and hydrogen bonding

between carboxyl groups of Naproxen with amino and hydroxy groups of CS. The combination of chitosan and poly(*N*-isopropyl acrylamide-co-vinyl pyrrolidone) brought temperature and pH sensitivity to hydrogel. The use of chitosan in semi-IPN networks improved the swelling behavior and provided a high affinity for anionic drug because of strong interactions between drug molecules and amino groups of chitosan. This type of semi-IPN hydrogel is particularly useful for the development of stimuli-responsive controlled drug delivery system.

It is also possible to form microspheres of semi-IPN system which are particularly useful in drug delivery system. It is easier to encapsulate drug in the microsphere and the release of drug can also be controlled. Chitosan and guar gum grafted acrylamide (GG-g-AAm) semi-IPN system is an example of such microsphere in which anticancer drugs, e.g., 5-Fluorouracil can be loaded (Sekhar et al. 2011). Spherical microsphere of chitosan and guar gum-grafted acrylamide semi-IPN can be prepared by water-in-oil (w/o) emulsion-cross-linking method using glutaraldehyde as a cross-linking agent. GG-g-AAm content of the system affects the swelling behavior of this type of semi-IPN and water uptake increases with increase in GG-g-AAm content in microsphere. This microsphere semi-IPN system shows good drug encapsulation efficiency and the release behavior of drug depends on drug content in the microsphere, degree of cross-linking and graft polymer composition.

Zhao et al. prepared semi-IPN chitosan/poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels by in situ UV-photo-cross-linking of *N*-isopropylacrylamide monomer using poly(ethylene glycol)-co-poly(ε-caprolactone) as a cross-linker in the presence of chitosan (Zhao et al. 2011). This semi-IPN hydrogels were found to respond to both temperature and pH changes and this responsiveness was reversible in nature. From the study of rheological properties, it was observed that the introduction of chitosan greatly improved the mechanical strength of the hydrogels prepared. Bovine serum albumin was loaded into the hydrogel and its release profile was also evaluated. The results of in-vitro release profiles of Bovine serum albumin from the hydrogels showed that the release rate was higher in buffer solution of pH 2.0 than in pH 7.4 at 37 °C. From these results the researchers believed that such double-sensitive semi-IPN hydrogels have the potential to be used as smart carriers for delivery of drugs (Table 8.1).

8.5.2 Alginate

Natural polysaccharides such as cellulose, chitin, chitosan, alginate, carrageenan are extensively used in different applications ranging from environmental remediation to bone tissue engineering to lifesaving drug delivery (Islam et al. n.d. ; Biswas et al. 2017). All the more so, chemical and physical modifications of such natural polysaccharides can incorporate novel properties into them making them viable for use in diverse fields.

Alginate is one such natural polysaccharide commonly extracted from brown algae. It is anionic in nature and consists of repeating units of β-D-mannuronic acid and α-L-guluronic acid. It possesses vital properties like biocompatibility,

Table 8.1 Different chitosan based semi-IPN systems investigated in recent years for drug delivery application

Sl.	Semi-IPN system	Form	Stimuli response	Loaded drug	References
1.	Chitosan and poly(*N*-isopropylacrylamide-co-2-acrylamido-2-methyl-1-propanesulfonic acid)	Hydrogel	pH responsive	5-Fluorouracil	Varaprasad et al. (2012)
2.	Chitosan/polyacrylamide	Hydrogel	pH responsive	Piperacillin–tazobactam	Kahraman et al. (2013)
3.	Hemicellulose/chitosan	Hydrogel	pH responsive	Riboflavin	Karaaslan et al. (2010)
4.	Chitosan/poly(acrylic acid-co-citraconic acid)	Hydrogel	pH responsive	Fluconazole	Pulat and Asıl (2009)
5.	Carboxymethyl chitosan/P(2-(dimethylamino)ethyl methacrylate)	Hydrogel	pH and temperature responsive	Coenzyme A	Guo et al. (2007)
6.	Chitosan/(dextran-g-acrylamide)	Microspheres	pH responsive	Theophylline	Al-Kahtani and Sherigara (2009)
7.	Poly(*N*-isopropylacrylamide)/chitosan	Hydrogel	pH/thermo responsive	Pilocarpine hydrochloride	Verestiuc et al. (2004)
8.	Chitosan–poly(ethylene glycol)	Beads	pH responsive	Isoniazid	Gupta and Kumar (2001)

biodegradability, nontoxicity, transparency, and ease of gelation, which enables alginate to be widely used in biomedical applications such as wound healing, drug delivery, cell encapsulation, 3D bioprinting, and tissue engineering. A modified version of alginate is sodium alginate which is a salt of alginic acid. Sodium alginate can make ionic gel quickly in presence of divalent cations (e.g., calcium ions) through ionic interaction (Peppas et al. 2006).

A semi-IPN based on sodium alginate and polyacrylamide was developed to study drug loading and release capacity of such a promising material. The drug used was acetaminophen, a very common analgesic (Samanta and Ray 2014). Polyacrylamide, a synthetic polymer, is toxic, stable, nonresorbable, and sterile. It can form network gel structure easily by free radical copolymerization. The polymerization was carried out with a cross-linker *N*,*N*-methylene-bis-acrylamide (MBA) and redox initiator pair, i.e., ammonium persulfate and sodium metabisulfite. The essential feature of this polymer is that it's biocompatible and not inimical to human body. Moreover, its porous structure facilitates drug loading making it a very attractive carrier for drug delivery applications (Risbud and Bhonde 2000). The study reported that the swelling ratio increased with increase in initiator concentration. As drug release properties was dependent on the swelling ratio, increasing initiator concentration led to more uptake of drug. This could be attributed to reduction in molecular weight causing gel imperfection and more chain ends. The optimum concentration of initiator was found to be 1.0 wt%. The drug loading or entrapment capacity also increased with increase in alginate wt% and decrease in cross-linker concentration. pH of the media also affected this capacity to great extent. The semi-IPN hydrogel exhibited initial burst release followed by a sustained release of drug. This could be explained with the fact that at first the drug associated to the surface was released. Then the drug absorbed inside the structure could come out. The drug release was governed by concentration gradient between hydrogel and release medium. The cumulative drug release was 95% after 5 h (Samanta and Ray 2014).

Another semi-IPN based on alginate and poly((2-dimethylamino) ethyl methacrylate-glycidyl methacrylate) P(DMAEMA-GMA) was fabricated by radical polymerization without using any catalyst and cross-linker (Gao et al. 2012). This method was facilitated by polymerization of the double bonds and ring opening of the epoxy groups present in glycidyl methacrylate (GMA). GMA is a bifunctional monomer which contains an epoxy group and a double bond (Bayramoğlu et al. 2005). These structural features of GMA is utilized to synthesize hydrogels. DMAEMA is a water-soluble monomer. It possesses a tertiary amino group that can be protonated in acidic medium (Şen and Sarı 2005). This hydrogel was used to study loading and release capacity of Aminophylline. It is a drug used for relaxation of bronchial smooth muscle. It is basically a mixture of theophylline and ethylenediamine in a 2:1 ratio. Theophylline confers pharmacological effects on the formulation, while ethylenediamine provides solubility improvement of theophylline. The permissible concentration of theophylline in blood is 10–20 μg/ml. The patient risks danger of toxic effect of the drug if the concentration is higher than 20 μg/ml. Patients can suffer from nausea, vomiting, insomnia, convulsions, and coma embolism. So, it is very important that the hydrogel regulates the drug release. The release

experiment was carried out in a buffer solution of pH 1.2 to reflect the environment of stomach. The environment similar to intestine medium was created by using a buffer solution of pH 7.4. It was observed that alginate–P(DMAEMA-GMA) hydrogels exhibited an initial burst release of aminophylline at pH 1.2 and pH 7.4. This could be attributed to aminophylline molecules adhered to the hydrogel surface. This hydrogel showed total aminophylline release up to 96.38% in 420 min at pH 7.4. This work revealed that the alginate–P(DMAEMA-GMA) semi-IPN hydrogels showed promising pH and temperature sensitivity. Moreover, the swelling ratio decreased as pH value and temperature increased. All the more so, incorporation of alginate chain helped to improve the mechanical strength of semi-IPN hydrogels. Additionally, results obtained from model drug aminophylline implied that the hydrogel could be suitable for colon targeted drug delivery (Gao et al. 2012).

Few more alginate based semi-IPN drug delivery systems were also reported. They include a biomaterial based semi-IPN hybrid hydrogels composed of bacterial cellulose nanofiber and sodium alginate. This semi-IPN was a dual-stimuli responsive drug release system. The system exhibited pH and electric field stimulus-responsive swelling properties and the stimulus-responsive drug release behaviors. The sodium alginate–bacterial cellulose showed increased swelling ratio from less than 8 times at acidic conditions (pH 1.5) to more than 13 times when the pH value was 11.8. Moreover, the swelling ratio was also affected by electric field. When the electric field changed from 0 to 0.5V, the semi-IPN system exhibited an increasing swelling ratio from 8 times its dry state to 14 times its dry state. The loading and release of Ibuprofen (IBU), a nonsteroidal anti-inflammatory drug (NSAID), was studied with this semi-IPN system. Interestingly, the release of the drug could be controlled by the action of deprotonation or protonation of calcium alginate in the hydrogels under different pH conditions. The system showed quicker release of drug in alkaline conditions and slower in acidic conditions (Shi et al. 2014).

A semi-IPN of acrylamide grafted sodium alginate (AAm-g-NaAlg) microspheres were developed by emulsion-cross-linking method using glutaraldehyde as a cross-linking agent. The grafting of acrylamide onto sodium alginate was carried out by free radical graft polymerization where ceric ammonium nitrate was used as initiator. Diclofenac sodium is an anti-inflammatory drug. This drug was successfully encapsulated with an efficiency varying between 83% and 95% (Al-Kahtani and Sherigara 2014).

A dual stimuli responsive semi-IPN hydrogels were fabricated by using alginate and poly(*N*-isopropylacrylamide) (PNIPAAm). It was a comb-type graft semi-IPN hydrogels sensitive to both temperature and pH. It was prepared by cross-linking alginate network and grafting with PNIPAAm. The attractive feature of this semi-IPN system was that it exhibited comparatively quicker pH and thermal responses due to free and mobile graft chains (Ju et al. 2001).

8.5.3 Hyaluronic Acid

Hyaluronic acid (HA) is a biodegradable natural polysaccharide. It is a linear glycosaminoglycan composed of repeating disaccharide unit of D-glucuronic acid and *N*-acetyl-D-glucosamine. These components are arranged in a chain where they are connected through alternating β-1,4 and β-1,3 glycosidic bonds. HA is highly biocompatible, nonthrombogenic, nonimmunogenic and does not induce chronic inflammation (Prestwich 2008; Ibrahim and Ramamurthi 2008). HA is one of the major components present in extracellular matrix of most animal tissue. Moreover, HA is able to interact with specific cell receptors that can recognize and bind it selectively. Though HA have quite a few advantageous properties, there are some pesky drawbacks associated with HA which include its degradation catalyzed by human hyaluronidases and its poor mechanical properties. To tackle these problems and to make it viable for different applications chemical derivatives of HA are prepared. Three functional groups in the structure of HA are generally chemically modified. They are the glucuronic acid group, the primary and secondary hydroxyl groups, and the amine group (after deacetylation of *N*-acetyl group). These alterations in the properties have made HA suitable for tissue engineering and drug delivery applications (Allison and Grande-Allen 2006; Wei and Cuie 2006) (Fig. 8.3).

A semi-IPN based on hyaluronic acid and poly(*N*-isopropylacrylamide) (PNIPAAm) was prepared by combining cross-linked PNIPAAm with HA (Santos et al. 2010). The target of this work was to develop a drug delivery system responsive to external stimuli. Generally, hydrogels that respond to external stimuli have become a major attraction for controlled release of therapeutic reagents. These hydrogels usually respond to environmental stimuli by bringing changes in their physical or chemical behavior which includes a volume phase transition at specific stimulus that causes sudden changes in the solubility of the polymeric network resulting in the release of an entrapped drug in a controlled manner. PNIPAAm is a popular polymer which has shown to be responsive to temperature changes. PNIPAAm based hydrogels showed low cytotoxicity (Hsiue et al. 2002). Moreover, it has physicochemical properties required for many biomedical and biotechnological applications. However, PNIPAAm based hydrogels exhibits very slow swelling/deswelling transition too (Coughlan et al. 2004). This is due to the formation of a hydrophobic skin that inhibits the release of encapsulated molecules and limits the

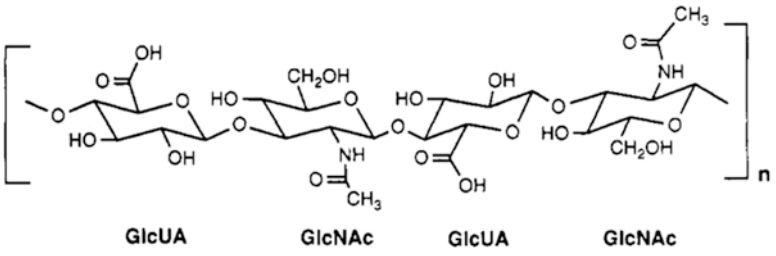

Fig. 8.3 Structure of hyaluronic acid. (Pouyani and Prestwich 1994)

deswelling of the hydrogel. This is a major hindrance while designing efficient drug delivery system. Among different strategies deployed to tackle this problem, the one that utilized grafted PNIPAAm cross-linked to its chain and combined with HA showed impressive results. Gentamicin, aminoglycoside antibiotics, was loaded as a model drug. As HA was incorporated into the semi-IPN hydrogel, complete release of gentamicin from the hydrogel was recorded. Even a very small amount of HA brought about substantial improvement in drug release capacity. Furthermore, these HA–PNIPAAm semi-IPN hydrogels respond to both changes in temperature and pH making it a promising delivery system for therapeutics. At pH 7.4, HA–PNIPAAm semi-IPN hydrogels revealed to have considerably greater and faster swelling at 25 °C and a more complete deswelling at 37 °C compared to pure PNIPAAm hydrogels. All the more so, with the incorporation of HA, deswelling process of the semi-IPN exhibited marked improvement at 37 °C. A noticeable increase in both water uptake capability and swelling kinetics of the hydrogels was also recorded at 25 °C. This study showed the potential of HA in designing smart external stimuli responsive drug delivery system (Santos et al. 2010).

Another similar biosynthetic semi-IPN hydrogel showed great potential to be useful in drug delivery system. This semi-IPN hydrogel was prepared from synthetic 2-ethyl-(2-pyrrolidone)methacrylate (EPM) and hyaluronic acid (HA) by radical-induced polymerization using *N*,*N*′-methylene-bis-acrylamide or triethylene glycol dimethacrylate as cross-linker (Magalhães et al. 2013). A massive drawback of poly(vinyl pyrrolidone) (PVP) and the monomer vinyl pyrrolidone (VP) was that it had poor reactivity in free radical polymerization reactions. Fortunately, it was possible to improve the reactivity of pyrrolidone ring based systems by introducing a methacrylate group, which led to fabrication of a material with properties similar to poly(2-hydroxyethyl methacrylate). 2-Ethyl-(2-pyrrolidone)methacrylate was one such hydrophilic polymer showing characteristics required for biomedical applications (Chen et al. 2000). As a result, this type of synthetic polymer found utilization in different biomedical applications due to their high water uptake capacity, soft consistency, and low superficial tension (Langer and Vacanti 1993). Combining 2-ethyl-(2-pyrrolidone)methacrylate (EPM) and hyaluronic acid (HA) a highly flexible and stable semi-IPN hydrogel was prepared at low or moderate temperature followed by lyophilization. The addition of HA in this semi-IPN system with the synthetic and hydrolytically degradable EPM enhanced the swelling capacity of these hydrogels. All the more so, this addition also resulted in the improvement of mechanical properties of the hydrogels. Not only that, addition of a cross-linking agent, triethylene glycol dimethacrylate (TEGDMA), which is nontoxic, led to hydrogels exhibiting maximum swelling ratios and stable viscoelastic behavior. But temperature sensitivity of this material was befuddled with the addition of HA as the phase transition temperature of the hydrogels was affected by the physically entangled hyaluronic acid. On the other hand, the addition of HA in the system resulted in noticeable pH sensitivity, which led to the swelling of the hydrogels over a range of pH. This change in pH sensitivity was due to HA having ionizable character, in addition to the amphiphilic character of the poly[2-ethyl-(2-pyrrolidone)methacrylate] (PEPM). The semi-IPN hydrogel also exhibited

noncytotoxic property. As a result, this semi-IPN system could be a very promising drug delivery vehicle with pH sensitivity (Magalhães et al. 2013).

A semi-IPN hydrogel based on entrapped poly(aspartic acid) into hyaluronic acid (HA) cross-linked network was developed by M. T. Nistor et al. The addition of poly(aspartic acid) in the HA gel network induced acidic pH responsiveness to this semi-IPN system. All the more so, the semi-IPN exhibited higher swelling capacity which could lead to potential use in drug delivery systems (Nistor et al. 2013). More hyaluronic based semi-IPN hydrogels having potential to be applicable in drug delivery systems were reported by Bae et al. (2015) and Tsaryk et al. (2015).

8.5.4 Cellulose and Its Derivatives

Cellulose, the most abundant polysaccharide in the world, is the major constituent of plant cell wall. Cellulose is also prevalent in some bacteria, marine algae, and other biomass. Even some animals (such as tunicates) have cellulose in their structure. As a result of this sheer abundance, the total annual amount of cellulose is a few billion tons, so the economic value of this material is not ignorable. Indeed, cellulose has attracted attention of science fraternity as a go-to material in new innovative applications (Islam et al. 2018). Naturally abundant carbohydrate polymers like cellulose possesses some intrinsic limitations in their reactivity and processability. Chemical and physical modifications are carried out to alter the structure of the cellulose to overcome the deficiencies. Carboxymethyl cellulose (CMC) is one such modified version of natural polymer – cellulose, which is extensively used in applications like food, pharmaceutical, paper, and other industries. It is generally used as sodium carboxymethyl cellulose (NaCMC). NaCMC is an anionic, water soluble, polyelectrolyte ether of cellulose. Carboxymethyl groups replaces hydroxyl groups present on 2-glucopyranose residue of cellulose (Lopez et al. 2015). A semi-interpenetrating network (semi-IPN) based on cellulose derivative sodium carboxymethyl cellulose (NaCMC) was fabricated by combining with poly(acylamide-co-2-acrylamido-2-methy-l-propanesulfonic-acid). The hydrogel was prepared by free radical polymerization using redox initiator ammonium persulfate and activator *N*,*N*,*N*,*N*′-tetramethylethylenediamine. This semi-IPN hydrogel was used to study drug loading and release capacity of ranitidine hydrochloride, an antiulcer drug. Due to the presence of more hydrophilic components, NaCMC and poly(acylamide-co-2-acrylamido-2-methy-l propanesulfonic acid) semi-IPN hydrogels showed super absorbing properties. This could be attributed to the chains of the network becoming solvated when semi-IPN hydrogel was immersed in the media by absorbing the solvent molecules. But importantly, the cross-linking present in the semi-IPN system thwarted the dissolution of polymer chains in the swelling media but facilitates the expansion of the network system. All the more so, the opening and disruption of NaCMC structure enabled the absorption and diffusion of the swelling media molecules. It was found that the absorption and diffusion capacity of semi-IPN hydrogel depended on NaCMC and ammonium persulfate ratio in the hydrogel and also depended on the cross-linker, initiator, and activator

concentration. In this study it was revealed that the semi-IPN hydrogel containing 0.25 g of NaCMC exhibited maximum swelling ratio, which can be explained as a result of the rise in hydrophilic nature of semi-IPN hydrogel which is due to increase in the number of –OH groups. The semi-IPN hydrogels exhibited up to 99.95% cumulative drug release capacity in 24 h (Vimala et al. 2011).

Another work utilized sodium carboxymethyl cellulose by combining with gelatin to prepare a semi-interpenetrating polymer network (IPN) microspheres for controlled release of drug Ketorolac tromethamine (KT). Gelatin is a natural protein which constitutes the skin, bones, and connective tissues of animals. More importantly, gelatin is biocompatible, biodegradable and also exhibits solubility at the body temperature. These are attractive and ideal properties for application in pharmaceuticals (Tabata and Ikada 1998). Ketorolac tromethamine (KT) is an anti-inflammatory and analgesic agent and a member of the pyrrolo-pyrrole group of nonsteroidal anti-inflammatory drug (NSAID). This drug was encapsulated into Sodium carboxymethyl cellulose and gelatin microspheres, which exhibited about 67% loading capacity. The extent of cross-linking and the amount of NaCMC used to fabricate the semi-IPN system determined the drug release capacity which was up to 10 h in in-vitro analysis.

Similar semi-IPN hydrogels type drug delivery systems based on cellulose derivative were reported. A semi-IPN hydrogel blend microspheres were fabricated from hydroxyethyl cellulose and gelatin by a water-in-oil (w/o) emulsion technique. This semi-IPN system was studied for the controlled release of theophylline (THP), an antiasthmatic drug. This semi-IPN system exhibited about 74% encapsulation of THP. Moreover, the system displayed a prolonged drug release capacity up to 24 h (Kajjari et al. 2011). Chitosan and hydroxypropyl cellulose based semi-IPN was developed by emulsion-cross-linking method using glutaraldehyde (GA) as a cross-linker. This system was used to study drug loading and release profile of Chlorothiazide (CT) which is a diuretic and antihypertensive drug with limited water solubility. This drug was successfully encapsulated into this semi-IPN drug delivery system. The system exhibited encapsulation of drug up to, the system was also able to show slow release capability which could be up to 12 h (Kulkarni et al. 2009). Synthetic polymers such as poly(*N*-vinyl-pyrrolidone) (PVP) hydrogels garnered interest as a novel thermo-sensitive material. But an unfortunate drawback of temperature sensitivity exhibiting PVP hydrogel is that it does not exhibit thermo-sensitivity under typical conditions. In this work, semi-interpenetrating polymer network (semi-IPN) hydrogels based on PVP and carboxymethyl cellulose (CMC) were prepared (Lü et al. 2010). This semi-IPN system was studied with a model drug Bovine serum albumin (BSA). In the semi-IPN, good swelling properties and the swelling ratio of hydrogels increased with increasing CMC content, this could be attributed to the hydrophilicity of CMC. The system also exhibited pH sensitivity and possessed the highest equilibrium swelling ratio at pH 3.92. It could be inferred from the results of this study that this PVP/CMC semi-IPN hydrogels could serve as potential candidates for protein drug delivery in the intestine.

8.5.5 Scleroglucan

Scleroglucan is a biopolymer which has been investigated extensively in recent years in semi-IPN technology to form three-dimensional networks capable of loading and release drugs. It is a nonionic polysaccharide consisting of a backbone made up of (1→3)-β-linked glucopyranosyl residues substituted with a single (1→6)-β-glucose residue every third backbone units (Matricardi et al. 2007). Several approaches have been made to form semi-IPN system of Scleroglucan, both of its native and chemically modified form.

Matricardi et al. prepared a semi-IPN system by interspersing sodium alginate chains into a scleroglucan/borax hydrogel network intended to be used as a drug delivery matrix (Matricardi et al. 2006). The resulted hydrogel was freeze-dried and compressed to form tablets with controlled porosity in which a protein Myoglobin was loaded. The release behavior of Myoglobin (MG) from the matrix was observed in distilled water, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF). In distilled water, MG release was greatly dependent on alginate content of the matrix. With increase in alginate content, a corresponding increase of MGB release rate was observed. The presence of alginate chain leads to a disaggregation of the matrix and contributed to a complete release of the MG in 24 h in distilled water. However, the release rate of MG from the matrix in SIF is slightly lower than in distilled water because the salts present in solution were able to screen the carboxylate groups of alginate thus reducing the water uptake of the system. But the release behavior changed dramatically when SGF was used as release medium. In SGF, MG was not released at all from the matrix which was accounted for poor dissolution of alginate in acidic media and ionic interaction between carboxylate ions carried on alginate chains and the protonated form of the protein. The results of such research work indicated a semi-IPN drug delivery matrix which can protect protein drugs in acidic media but able to release them in neutral environment.

In another research, semi-IPN system in the form of injectable hydrogel was obtained from dextran methacrylate (DEX-MA) and carboxymethyl derivative of scleroglucan (Scl-CM) (Corrente et al. 2013). The system was designed in a way to have enough viscosity to be injected and the hydrogel showed pseudo-plastic behavior, with the viscosity value that reduced as the shear stress increased. Three model drugs, theophylline (THP), myoglobin (MGB) and vitamin B12 (VitB12) were used to investigate the loading and release behavior of the prepared semi-IPN system. Because of smaller size of THP, the system could not maintain it inside and as a result it quickly diffused out from the matrix. In case of other two model drugs, about 80% VitB12 was released in 24 h and MGB was released slowly (40% in 2 weeks) from the hydrogel in a sustained manner.

8.5.6 Salecan

Salecan, an linear extracellular water-soluble microbial polysaccharide produced by salt-tolerant strain of *Agrobacterium* sp. ZX09 has been investigated extensively in recent times for its ability to form semi-IPN systems (Hu et al. 2014a). It contains high density of hydroxyl groups, which provide sites for chemical modification and greater flexibility in the preparation of semi-IPN hydrogels. It also shows excellent biological activities like antioxidation and nontoxicity which is useful in applications like drug delivery.

A thermo-responsive, macroporous semi-IPN hydrogel was synthesized from salecan and poly(*N*,*N*-diethylacrylamide) by free radical polymerization which was capable of loading and releasing anionic drugs (Wei et al. 2016). The presence of salecan in the hydrogel improves hydrophilicity and enhances the release rate of this system. The prepared hydrogel showed macroporous structure, and it was found that the pore sizes increased with increase in salecan concentration. As a result, hydrogels containing higher amount of salecan showed higher swelling ratios and were more sensitive to temperature. Diclofenac sodium, an anionic anti-inflammatory drug was used as a model drug to test loading and release behavior of the system. Drug loading was performed by absorbing drug molecules into hydrogel and from results it was found that salecan content has direct effect on loading efficiency as loading efficiency of the system increased with increase of salecan content. The release profile of the drug from the hydrogel was temperature dependent; the release rate was increased with the increase of temperature from 25 °C up to 37 °C. Such semi-IPN hydrogels with the ability to load and release drugs upon changing the temperature are very promising to be used in controlled drug delivery systems.

Another semi-IPN hydrogel prepared from salecan and poly(*N*,*N*-diethylacrylamide-co-methacrylic acid) (PDM) was investigated for its ability to load and release an anticancer drug, doxorubicin (DOX) (Wei et al. 2015). This hydrogel was synthesized by copolymerizing *N*,*N*-diethylacrylamide (DEA) and methacrylic acid (MAA) in the presence of cross-linker *N*,*N*′-methylene-bis-(acrylamide) and salecan. A schematic representation of synthesis route of this semi-IPN hydrogel and application as drug carrier is shown in Fig. 8.4. The prepared hydrogel showed both pH and thermal response which was effective in controlled drug delivery. The model drug DOX was loaded into hydrogels by equilibrium partitioning method, and then the release behavior was observed in two pH values, 4.0 and 7.4, respectively. From the results, it was found that the release rate of DOX increased significantly at high temperature (37 °C) and low pH value (4.0). Moreover, the salecan content and cross-linker content had direct effect on release rate. Increasing salecan content and decreasing cross-linker content led to a rise in swelling ratio which ultimately resulted in faster release rate and higher release quantity of the drug.

Derivatives of salecan can also be utilized to prepare semi-IPN systems. Hu et al. synthesized a redox/pH dual-stimuli-responsive degradable semi-IPN hydrogel from itaconic acid (IA)-grafted salecan and 2-hydroxyethyl methacrylate (HEMA), and *N*,*N*-bis(acryloyl)cystamine (BAC) was used as cross-linker (Hu et al. 2017). BAC was a disulfide-functionalized cross-linker and its addition into hydrogel

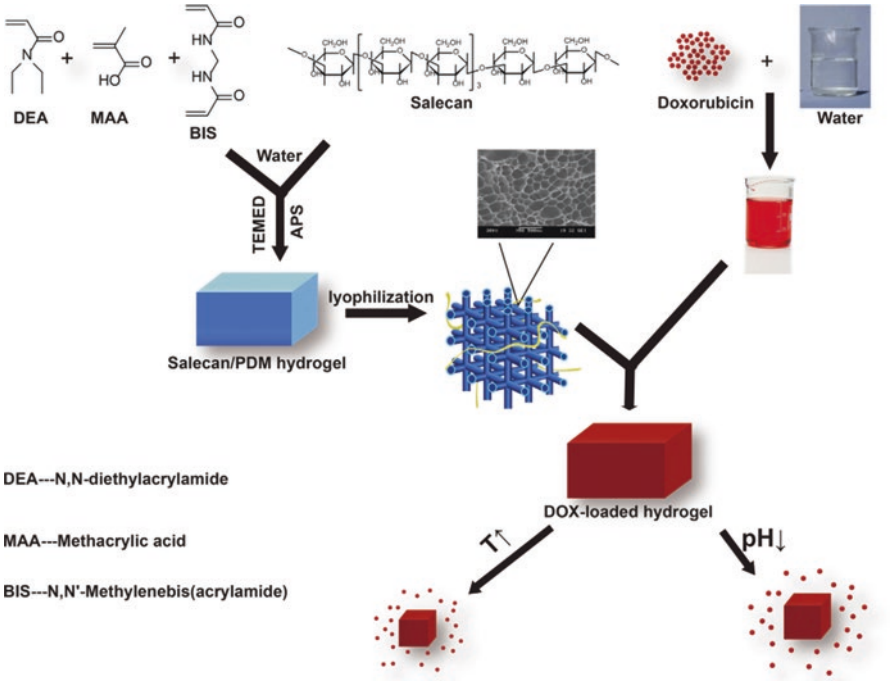

Fig. 8.4 Schematic representation of synthesis route of salecan/PDM hydrogel and its application as drug carrier. Reprinted with permission from (Wei et al. 2015)

brought reduction-sensitivity to the system. The swelling behavior of this hydrogel was dependent mainly on the salecan and BAC content. The water uptake capacity of the hydrogel was found to increase with increase in salecan content. However, with increase of BAC content, the cross-linking density of the hydrogel also increased which reduced the mobility of macromolecular chain, hence lowering water uptake capacity of the system. Positively charged DOX was efficiently loaded into negatively charged hydrogel through electrostatic interaction, and a controlled release of the drug from the system was obtained via pH control and swelling–shrinking mechanism. All these results indicate that this semi-IPN hydrogel system has potential to be used as a carrier in anticancer drug delivery (Table 8.2).

8.5.7 Dextran

Dextran is a polysaccharide having some properties useful for drug delivery like good compatibility with human body, biodegradability, stability under mild acidic and basic conditions (Lima et al. 2011), etc. It also contains large number of hydroxyl groups for conjugation which makes it an excellent candidate in semi-IPN technology. Both dextran and dextran based derivatives are used to fabricate semi-IPN systems which find applications in drug delivery.

Table 8.2 Different salecan based semi-IPN systems investigated in recent years for drug delivery application

Sl.	Semi-IPN system	Form	Stimuli response	Loaded drug	References
1.	Salecan/poly(*N*,*N*-diethylacrylamide-co-methacrylic acid)	Hydrogel	pH/thermo responsive	Doxorubicin	Wei et al. (2015)
2.	Salecan/poly(*N*,*N*-diethylacrylamide)	Hydrogel	Thermo responsive	Diclofenac sodium	Wei et al. (2016)
3.	Salecan/poly(2-acrylamido-2-methylpropanosulfonic acid-co-[2-(methacryloxy)ethyl] trimethylammonium chloride)	Hydrogel	pH responsive	Insulin	Qi et al. (2017a)
4.	Salecan/2-acrylamido-2-methyl-1-propanesulfonic acid	Hydrogel	pH responsive	Insulin	Qi et al. 2017c)
5.	Salecan/poly(*N*-(3-dimethylaminopropyl) acrylamide-co-acrylamide)	Hydrogel	pH responsive	Amoxicillin	Qi et al. (2017b)
6.	Salecan/poly(*N*-isopropylacrylamide-co-methacrylic acid)	Hydrogel	pH/thermo responsive	Doxorubicin	Qi et al. 2016)
7.	Salecan/poly(methacrylic acid)	Hydrogel	pH responsive	Doxorubicin	Qi et al. 2015)
8.	Salecan-g-SS/poly(IA-co-HEMA)	Hydrogel	Redox/pH responsive	Doxorubicin	Hu et al. 2017)

In order to design a novel drug delivery system capable of loading and releasing anti-HIV agent in a controlled manner, Sullad et al. prepared semi-IPN microspheres from dextran-grafted-acrylamide (Dex-g-AAm) and poly(vinyl alcohol) (PVA) by emulsion-cross-linking method (Sullad et al. 2011). An anti-HIV agent, abacavir sulfate was encapsulated into the microsphere by dissolving it in polymer blend solution and emulsified slowly into light liquid paraffin. Drug release from the microsphere was investigated in buffer media of pH 1.2 and 7.4 which mimic stomach and intestinal conditions, respectively. From the results, it was found that the release of drug followed diffusion-controlled non-Fickian transport mechanism and the degree of cross-linking as well as polymer blend ratio affected the release behavior.

Another derivative of dextran, dextran-methacrylated (dextran-MA), was used in the preparation of semi-IPN system with poly(*N*-isopropylacrylamide) (PNIPAAm) and investigated for its ability to be used in controlled delivery of drug (Lima et al. 2011). These semi-IPN hydrogel particles were prepared by mixing photo-cross-linked dextran-MA, PNIPAAm, and a model drug (insulin or bovine serum albumin) and then dropped on superhydrophobic surfaces like polystyrene, aluminum, and copper followed by drying under UV light. Spherical particles with porous structure were obtained by this method, and these particles showed temperature-sensitive swelling behavior which could further be tuned by the weight ratio of dextran-MA/PNIPAAm. The encapsulation yield of insulin and bovine serum

albumin (BSA) obtained with these particles was almost 100%. The release profile of both insulin and BSA showed an initial rapid release in the first 5 h followed by a sustained release during several days.

Semi-IPN microspheres of acrylamide-grafted dextran and chitosan were investigated for its application as a carrier of antiviral drug, acyclovir (Rokhade et al. 2007). Emulsion-cross-linking method was used to prepare these microspheres and glutaraldehyde was used as cross-linker. The model drug, acyclovir, was encapsulated successfully into the semi-IPN microspheres with good encapsulation efficiency of up to 79.6%. However, polymer composition, extent of cross-linking, and percentage of drug loading were found to affect encapsulation efficiency. The release of drug from the matrix followed non-Fickian diffusion mechanism with initial burst release of up to 40% which later extended up to about 80% in 12 h.

8.6 Future Scope of Semi-IPN for Drug Delivery Application

The purpose of drug delivery research is mainly to develop such a system in which the delivery approach can serve just adequate amount of drug to only specific target site in a controlled manner without decreasing the efficacy of therapeutic drugs and not imparting toxicity in the process. The research in this arena is focused on development of suitable drug carriers to overcome the limitations of inaccurate dosage supply and nonspecific distribution by conventional drug dosage forms. A number of materials in different physical forms have so far been utilized as drug carriers, and some researchers called it "smart materials" for drug delivery. Polymers, lipids, and inorganic materials with different dimensions such as microparticles, nanoparticles, membranes, films, scaffolds, etc. are commonly studied in this category. In this emerging field of drug delivery system, IPN-based carriers of polymers have taken much attraction among scientists. Biomedical applications of IPN-based drug carriers can be classified into two major groups; one is tissue-engineering application and another is therapeutic application. Among the various types of IPN, semi-IPN has the uniqueness of self-healing capacity of its polymeric structure (Roland 2015). For that reason, semi-IPN has some scope of mechanical strength recovery capacity along with the controlled drug release ability. Table 8.3 shows a list of biomedical applications of semi-IPN.

Biomedical applications of semi-IPN have been reported in a number of areas such as repair and regeneration of living organs, protein delivery, medical implant, tissue scaffold, wound healing, infectious diseases, ophthalmic applications, cancer therapy, obesity control, chronic pain control, immunotherapy, cardiac disease, asthma diseases, etc. Hectic modern life is causing some chronic medical problems to modern people like arthritis, obesity, cardiac diseases, hormone imbalance, diabetes, etc. These types of medical problems require a prolong treatment with regular drug intake. Semi-IPN drug carriers in such cases can provide proper pharmacokinetics and pharmacodynamics for the drugs. Semi-IPN-based products in drug delivery are approaching toward various stimuli-responsive behavior. The controlled release of drugs is very much influenced by different environment factors,

Table 8.3 Biomedical applications of semi-IPN

Name of polymers	Formulation	Biomedical applications	References
Prevulcanized natural rubber latex and chitosan	Semi-IPN film	Medical products	Lu et al. (2012)
Polydimethylacrylamide, hyaluronic acid, and glucose oxidase	Semi-IPN film	Biosensors	Zhang et al. (2014)
Chitosan and hypromellose	Semi-IPN film	Wound dressing	Mayet et al. (2014)
Polyurethane urea, *N*-isopropylacrylamide, and acrylic acid	Semi-IPN film	Wound dressing	Thimma Reddy and Takahara (2009)
Hemicellulose and chitosan	Semi-IPN hydrogel	Wound dressing	Karaaslan et al. (2012)
Chitosan and polyvinyl pyrrolidone	Semi-IPN hydrogel	Antibiotic drug delivery	Vaghani and Patel (2011)
Polydimethylsiloxane, polyethylene glycol, and chitosan	Semi-IPN hydrogel	Bioadhesives	Rodkate et al. (2010)
Chitosan and polyaniline	Semi-IPN hydrogel	Biosensors	Kim et al. (2005)
Chitosan and polyacrylamide	Semi-IPN hydrogel	Oral hypoglycemic drug delivery	Kim et al. (2005)
Gellan gum and poly(*N*-isopropylacrylamide)	Semi-IPN microspheres	Cardiac disease drug delivery	Mundargi et al. (2010)
Gelatin and sodium carboxymethyl, cellulose	Semi-IPN microspheres	Anti-inflammatory drug delivery	Kassem et al. (2013)
Acrylamide-grafted dextran and chitosan	Semi-IPN microsphere	Antiviral drug delivery	Rokhade et al. (2007)
Chitosan and guar gum-grafted acrylamide	Semi-IPN microspheres	Anti-inflammatory drug delivery	Sekhar et al. (2011)
Chitosan + poloxamer	Semi-IPN sponges	Wound dressing	Kim et al. (2007)
Chitosan, PVA, and sodium alginate	Semi-IPN membranes	Antiasthmatic drug delivery	Somya et al. (2015)
Gelatin and PEO	Hydrogels	Oral drug delivery	
PVA and polymethacrylic acid	Hydrogels	Insulin delivery	
Gelatin and acrylic acid	Nanogels	Cancer targeting drug delivery	
Gellan gum and PEO	Xerogels	Anti-psychotic drug delivery	

such as pH, temperature, ionic strength, light, ultrasound, chemicals, etc. Compared to full IPN, semi-IPN-based products can control the drug delivery better by recovering the integrity of its own network structure to some extent. In the future, the stimuli-responsive drug carriers will be self-regulating; they would be able to trigger off potential amount of drug sensing enzyme interaction, various ionic concentration, body temperature, antigen or antibody interaction, etc. Semi-IPN has

already been used for drug delivery in some infectious diseases. They have shown a potential for cancer therapy; however, their application in immunity response (like AIDS, cold sores, contagious diseases, etc.) is yet to be determined. In tissue engineering fields, semi-IPN will probably be focused on its manufacturing techniques. Bone and cartilage substitutes entail 3D distribution of substituent materials for regeneration of tissues without stress management problem. Mechanical behavior of these substitutes should have a good match with their degradation pattern. A controlled mechanism of action of this type biomedical implants greatly depends on its dimension, porosity, density, surface properties, etc. For precise manufacture of semi-IPN-based tissue substitutes, bioprinting technology is already in progress. Customized 3D structured semi-IPN-based tissue substitutes will govern the future tissue implant materials.

8.7 Conclusions

At present, semi-IPN plays a very important role in controlled and targeted drug delivery applications. Due to their excellent and improved properties, one has the opportunity to develop semi-IPN systems using various natural as well as synthetic polymers that will overcome the disadvantages of individual polymer network. Many polysaccharide-based semi-IPNs have been successfully developed so far, however, there is still possibility to develop their physico-chemical and biological properties that fulfill unmet medical and pharmaceutical needs. In conclusion, it can be clearly demonstrated that the semi-IPN system opens up new possibilities and has the potential to be used as a drug carrier based on drug loading and drug release performance.

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IPN Systems for Cancer Therapy

9

J. Jeslin, B. S. Dhanya, and M. Chamundeeswari

Abstract

Interpenetrating polymer network (IPN) is the innovative biomaterial that forms a breakthrough in the polymeric science. It is an intelligent polymeric multicomponent system which is biocompatible and biodegradable as well known for its specific drug-releasing tendency with response to a stimuli. It also possesses a dual-phase continuity other than interpenetrating at the molecular level. As a result, the IPN system is widely preferred in the field of cancer therapy with zero-order drug delivery method that retains minimized fluctuations. The potentiality of this IPN system makes it a vast research area to diagnose and treat cancer and related diseases. The complexity and synergistic nature of this IPN system overcome the drawbacks of the individual polymeric carriers for cancer diagnosis and treatment, thus making it as a unique drug delivery vehicle. The comprehensive view of IPN classification, methods of preparation, their applications in cancer treatment and mechanism of drug release and action are explicitly focussed on in this chapter.

Keywords

Interpenetrating polymer network · Biocompatible · Synergistic · Carrier molecule

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9.1 Introduction

Interpenetrating polymer network ensembles the alloys of cross-linked polymers that are formed by synthesizing and/or cross-linking one polymer on the other without a covalent bond formation which can be detached only by breaking the chemical bonds between them (Myung et al. 2008; Sperling 2005). The synergistic nature of this polymeric multicomponent system will possess a unique drug release profile (Kim et al. 2004). The IPN system containing sulphur, natural rubber, with partially reacted resins of phenol formaldehyde is first found by Aylsworth in 1914 and is first termed in the 1960s by Miller while studying polystyrene network. The IPN system is known as a polymer alloy due to its peculiar nature that deviates from the other system such as (Lohani et al. 2014):

- IPN swells, but not dissolves in solvent as a polymer blend.
- IPN involves both chemical linkage and cross-linking, while polymer complex or graft copolymers involve either one of them.
- IPN has a bi-continuous structure with improved properties in terms of its toughness, morphology and strength.

This IPN system is nontoxic, biodegradable as well as biocompatible that predominantly employs them in the drug delivery applications. For targeted and sustained drug release applications, the attractive physicochemical properties of IPN find a distinct position relating to the preparation of capsules, tablets, hydrogels, micro- and nanoparticles, etc. The tremendous involvement of IPN in the biomedical field encouraged the researchers in developing smart drug release strategies on the target site (i.e.) stimuli-responsive drug release approach at the desired site (Reddy et al. 2008; Alsuraifi et al. 2018). This stimulus signal can be either external (induced artificially) or internal (physiological condition) depending upon the target site. This in turn permits it to be applied as a self-regulated mechanism for an effective as well as for a secure targeted drug delivery.

The major advantages of this IPN system are given below:

- The synergistic properties of natural and synthetic polymers expose an improved mechanical strength and a high-phase stability of the desired final product with high biocompatibility (Jain et al. 2013; Wu et al. 2007).
- The incessant zero viscosity of the hydrogel prevents the phase separation-blended polymers in the IPN system (Isiklan 2006).
- The stable interlocked networks of the polymers with the reacting components prevent the thermodynamic incompatibilities, and on the applied stress, the phase detached remains to be together (Bhardwaj et al. 2012; Margaret et al. 2013).

Cancer being a dreadful disease needs a suitable perception for its proper diagnosis and treatment. The conventional treatment results in the burst release and inverse reaction of drug molecules (Chamundeeswari et al. 2018). The ability of IPN to encapsulate or incorporate drug molecules within themselves enables an

advanced targeted drug delivery for the cancer treatment at a predetermined rate. This facilitates a zero-order drug delivery pattern with reduced fluctuations. The greater drug payload, nontoxicity, stability and sustainability, increased half-life of systemic circulation and therapeutic index, site-targeted action avoiding non-specific reactions and greater biodistribution and biodegradability make the IPN system to serve as a promising source for the cancer treatment (Raj et al. 2018; Soman et al. 2014).

9.2 Classification of IPN Systems (Fig. 9.1)

9.2.1 Based on the Chemical Bonding

Based on the bond that cross-links the polymers to get entangled, IPN system is classified into two types (Murugesh and Mandal 2012):

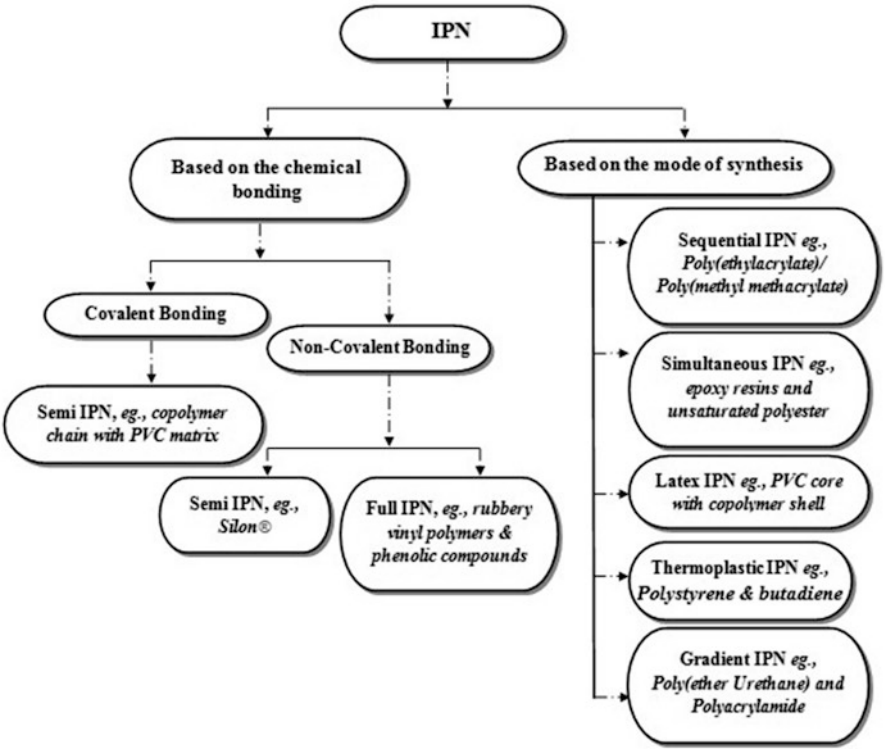

Fig. 9.1 Schematic classification of IPN system. The properties of the IPN system vary according to the individual property of the polymeric system used. This makes the formation of different types of IPN system that can be differentiated based on their chemical bonding and their mode of synthesis

9.2.1.1 Covalent Bonding

Semi IPN: Single IPN system forms by cross-linking two discrete polymers.

9.2.1.2 Non-covalent Bonding

Semi IPN: Only one polymer molecule will get cross-linked to the other polymer system to form a single IPN system.

Full IPN: Independent cross-linking of two or more polymer networks occurs to produce completely entangled IPN system.

9.2.2 Based on the Mode of Synthesis

Sequential IPN: Sequence of reaction occurs to produce an IPN system. Firstly, monomer I get polymerize with the cross-linkers I to produce a first polymer network; sequentially, the monomer II swells with the cross-linkers II and polymerizes with the first polymer network to form an IPN system (Sperling and Hu 2003).

Simultaneous IPN: In simultaneous IPN formation, simultaneous reaction of polymerization and cross-linking of monomer I and II occur. The one-step reaction without any interfering routes will produce a complete intercalated IPN system (Kiguchi et al. 2004).

Latex IPN: Latex IPN possesses a core and shell structure. It is prepared by polymerizing the monomer II on the latex seed of cross-linked polymer network I along with the activators and cross-linkers. The morphology of the produced IPN system depends on the path of polymerization (Chikh et al. 2011).

Thermoplastic IPN: The thermoplastic IPN flows at an elevated temperature (similar to thermoplastic elastomer), and the cross-linking of polymers occurs through physical bonding than the chemical bonding (Ignat and Stanciu 2003).

Gradient IPN: For the formation of gradient IPN, the monomer I is allowed to swell monomer II network surface following the polymerization of both the networks. The monomer II concentration varies against the monomer I (Karabanova et al. 2005) (Fig. 9.2).

9.3 Methods for IPN Synthesis

The preparation of an IPN system by any method employs three major steps such as:

- Three-chemical immiscible-phase formation
- Coating agent deposition
- Rigidization of coating agent

Some of the methods used for preparing IPN system are given below:

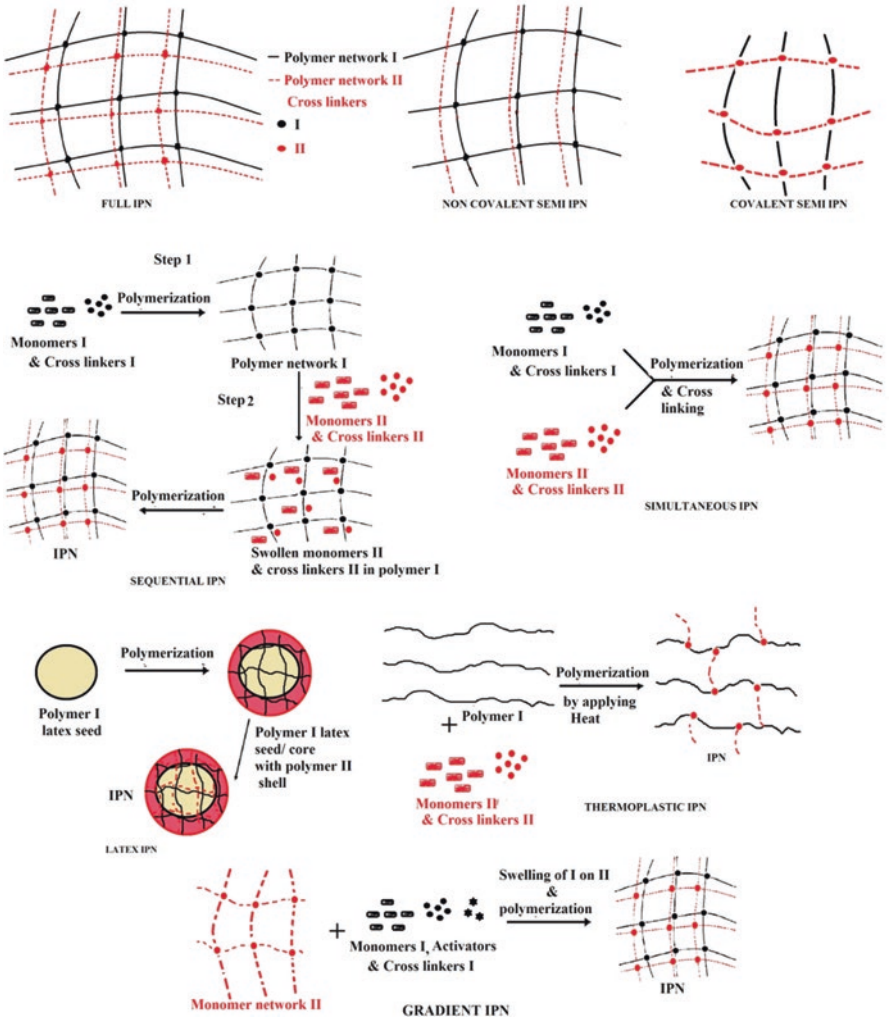

Fig. 9.2 Pictorial representation of different IPN system. The morphology of different IPN varies according to method adopted for its synthesis. This can enable sufficient encapsulation of drug molecules with the advantage of using it in the targeted drug delivery for cancer treatment

9.3.1 Casting Evaporation

The casting evaporation technique relies on the heating of polymers and casting it to produce an intercalated polymeric structure. To produce a sequential IPN gel, the polymer I and the cross-linkers I are heated to dissolve the polymer, and subsequently, polymer II and the cross-linkers II are added, heated and then cast. This will produce a cross-linked IPN system (Kosmala et al. 2000).

9.3.2 Emulsification Cross-Linking

This technique utilizes a phase separation technique, in which emulsions (w/o or o/w) are cross-linked together to form an intercalated IPN system. The w/o emulsion is produced by dispersing water-soluble polymers in aqueous solution and then mixing with an oil phase. In case of o/w emulsion, aqueous polymeric phase is mixed with the aqueous phase containing another polymer. Then they are cross-linked to produce an IPN system (Banerjee et al. 2010; Bhattacharya et al. 2013). The emulsion polymerization technique is usually preferred for preparing nano-IPN system.

9.3.3 Mini-Emulsion/Inverse Mini-Emulsion Technique

In mini-emulsion technique, hydrophobic monomeric droplets are dispersed in water (oil in water). A semi-IPN system is obtained by polymerizing and cross-linking the hydrophobic monomeric droplets (obtained by sonicating polymers with specific initiators) using cross-linkers I and by adding cross-linkers II. The second polymer is polymerized and cross-linked to form a full-IPN system. The hydrophilic monomers can be polymerized and cross-linked by inverse mini-emulsion technique. This technique follows mini-emulsification of hydrophilic monomeric droplets in hydrophobic phase (water in oil), i.e., inversion of mini-emulsification technique. Both these techniques use a high shear stress (Banerjee et al. 2010; Landfester 2006).

9.3.4 Coacervation Phase Separation

Coacervation is one of the physicochemical methods of microencapsulation technique. They can be simple or complex process. The simple coacervation process is the partial desolvation (by adding solvents or by changing temperature) of polymer in a ternary or a binary system (Gander et al. 2002). While in complex process, two oppositely charged polymers react to form a complex system (Lazko et al. 2004).

9.3.5 Multiorifice-Centrifugal Process

This method is a physicomechanical process that depends on the impact of centrifugal force. The apparatus consists of a rotating cylinder with three grooves in which the lower and the upper grooves are meant to carry the polymer molecules. The intermediate groove is the aperture through which the final film is formed by the rotation of the cylinder (Venkatesan et al. 2009).

9.3.6 Pan Coating

Pan coating is also a physicomechanical process in which the polymer solution is sprayed over the tumbled mass on the mixer. This method is suitable for making micro-IPN system (Burgess and Hickey 2007).

9.3.7 Air Suspension Coating

Air suspension coating, a physicomechanical process, consists of an air distribution plate, a nozzle, a coating chamber and a control panel. The polymer solution is sprayed from the coating chamber over the core/seed material (Das et al. 2011).

9.3.8 Spray Drying and Spray Congealing

The spray dryer consists of an atomizer, air heater, cyclone, blower, spray chamber and product collector. In this technique, the polymeric solution or the coating material is mixed with core material, and a hot stream is flown from the atomizer following solidification (Takahashi et al. 2005; Vyas and Khar 2002). In spray congealing, the polymeric solution is used as a melt.

9.4 Strategies for Drug Release Using IPN Systems for Cancer Treatment

The drug molecules bind either physically or chemically to the IPN system. They can also bind through electrostatic or through hydrophobic interactions. The drug release mechanism from the IPN system depends on the binding force. The drug release majorly occurs through:

9.4.1 Degradation-Controlled Monolithic System

The degradation-controlled drug release involves the degradation of the polymeric matrix for the controlled release of the drug molecules. In this case, the drug molecules are finely distributed on the polymeric matrix, such that there will be a slow degradation of matrix occurring for the controlled release of drug molecules. The dissolution of the matrix over time is the rate-limiting step for degradation-controlled drug release system (Von Burkersroda et al. 2002). It occurs through surface or bulk erosion. For a homogeneous bulk degradation mechanism, the drug release rate will be initially slow following a rapid release due to the bulk degradation. The system geometry does not affect the release rate and it highly depends on the degradation rate. The surface gets degraded inwardly in the surface erosion mechanism (Saralidze et al. 2010; Grassi and Grassi 2005).

The drug release rate can be given by the following equation:

$$\mathrm{Mt} / \mathrm{M}\infty = 1 - \left[\left(1 - t / t\infty\right)\right]^3$$

where

M_t = amount of drug released at time t
M_∞= amount of drug released at time of complete degradation

9.4.2 Diffusion-Controlled Monolithic System

The diffusion is the ability of a drug molecule to affect the external environment when exposed to a stimulus. It can be either a reservoir-type or a matrix-type diffusion system. The permeation of water in the polymer causes swelling of the polymeric matrix, thereby increasing the pore size of the matrix and diffusion of the drug molecules (Stevenson et al. 2012). The diffusion-controlled monolithic system involves diffusion of drug molecules with polymeric matrix degradation. The rate of drug release depends on the heterogeneous or homogeneous polymeric degradation mechanism. The reservoir type involves degradation of polymeric matrix only after the drug is diffused out (Singh et al. 2011). The diffusion rate depends on Fick's law of diffusion.

9.4.3 Erodible Poly-Agent System

In this type of mechanism, the drug molecules are bonded chemically to the matrix such that the drug molecule-polymer hydrolysis will be rapid compared to the polymer degradation. The rate-limiting step depends on the hydrolysis of drug-polymer matrix (Singh et al. 2011).

9.5 Stimuli-Responsive IPN System

The non-specific drug release can be achieved by the local drug release by diffusion and through degradation of the IPN system. In order to make it target-specific and finely tuned controlled drug release, stimuli-responsive smart drug delivery system will increase their applicability in the cancer therapy. This triggered drug release will control the rate of consistent drug release at the target site. The major stimuli that trigger the drug release include:

9.5.1 pH-Responsive Drug Release

Oral drug delivery is the most preferred non-invasive drug delivery route. However, it possesses a series of demerits such as premature drug release due to the acidic condition in the GI tract and the enzymatic degradation of the drug molecules. Therefore, pH-responsive smart drug delivery system will overcome the drawbacks to serve as an efficient cancer therapy system (Dai et al. 2008). The anionic and neutral polymers are found to possess less response to the acidic pH. The ampholyte and electrolyte polymers are found to response to the external pH changes. The cationic polymers like chitosan can exhibit a higher response to the lower acidic pH. Depending upon the isoelectric point, swelling and dissolution of the polymers, it readily responds to the acidic pH of the GI tract (Ahmed et al. 2009). Many anti-cancer drugs like doxorubicin and 5-fluorouracil are successfully synthesized as a pH-responsive smart drug delivery system for tumour cells (Qi et al. 2015).

9.5.2 Enzyme-Responsive Drug Release

The drug release can also be effectively achieved by fabricating an external enzyme-responsive smart system. The hydrogels synthesized from the polysaccharides can be used in case of any colon cancer treatment due to the active enzymes present in the GI tract. However, the polymer degradation can also cause pH fluctuations henceforth affecting the drug release activity (Jain et al. 2007; Sinha and Kumria 2001).

9.5.3 Temperature-Responsive Drug Release

The thermal-sensitive natural and synthetic polymers are found to be a valuable source for the synthesis of a smart temperature-responsive IPN drug delivery system. They involve in the transition to a more hydrophobic component from a hydrophilic one due to the temperature trigger that is also a reversible process. The IPN hydrogels can be two types based on its thermal sensitivity: upper critical solution temperature and lower critical solution temperature hydrogels (Klouda and Mikos 2008; Zhang et al. 2004a, b). Different polymers like alginate and chitosan are utilized for the thermal-responsive drug delivery system for cancer treatment (Guilherme et al. 2005). There are also dual- or multi-responsive drug release system fabricated for targeting tumour cells.

9.5.4 Electro-sensitive Drug Release

External stimuli like electrical signal can be used to trigger the drug delivery system for the target-specific action. Polyelectrolytes are widely used hydrogels for electro-sensitive drug release (Murdan 2003). On applying electric signal, the hydrogels

release active agents by the deformation of the system. The moieties that respond in terms of applied electric field are formulated within the IPN-drug complex. The complex system is implanted subcutaneously and is directed using an external electric field. The electric potential conduction patch is externally applied on the gel over the skin. In response to the applied stimulus, the carrier system delivers the active agent at the appropriate site. The major mechanism of drug release on the applied electric pulse includes diffusion, erosion due to electric pulse, de-swelling and electrophoresis of active drug molecules. The drug release rate at varying physiological states is found to be challenging for the electrostimulated drug release system (Kim et al. 2002; Liu et al. 2008).

9.6 Targeting Mechanism of IPNs on Tumour Cells

9.6.1 Active Targeting

The active targeting by the micro- or nano-IPN system involves the binding of the specific ligands to the overexpressed protein receptors on the surface of the non-dividing cells or tumour cells (Nacev et al. 2010). This targeting mechanism along with the stimuli-responsive drug release will enhance the cellular uptake of the active agents to the target site. The stabilized carrier molecules by PEGylation along with the small-molecule ligands like transferrin, growth factors, antibodies, peptides, etc. help in the active binding of the ligand molecules to the target-specific receptors (Danhier et al. 2010; Pérez-Herrero and Fernández-Medarde 2015). The direct binding of ligands to the target site prevents the off-site drug delivery such that reducing the adverse impact of the drug release to the healthy cells (Sun et al. 2014). The mechanism of active targeting of carrier molecule is shown in Fig. 9.3.

9.6.2 Passive Targeting

The passive targeting mechanism of IPN system on the tumour cells is primarily based upon the enhanced permeability and retention effect. The cancer or tumour tissues possess a leaky vasculature with irregular angiogenesis (Chrastina et al. 2011). The drug-IPN complex enters the endothelial interstitial space and accumulates on the specific cancer site, while small drug molecules can diffuse away from the accumulated target site, and the EPR effect is found to be size dependent varying with different patients (Acharya and Sahoo 2011; Lee et al. 2010).

9.7 IPN-Based Dosages for Drug Delivery Systems for Cancer Therapy

IPN, a complex of two or multiple polymers in network form (Sperling 1977, Mundargi et al. 2008), is highly an efficient carrier for delivering the cancer-curing drugs in a controlled manner that delivers patient compliance and reduces adverse

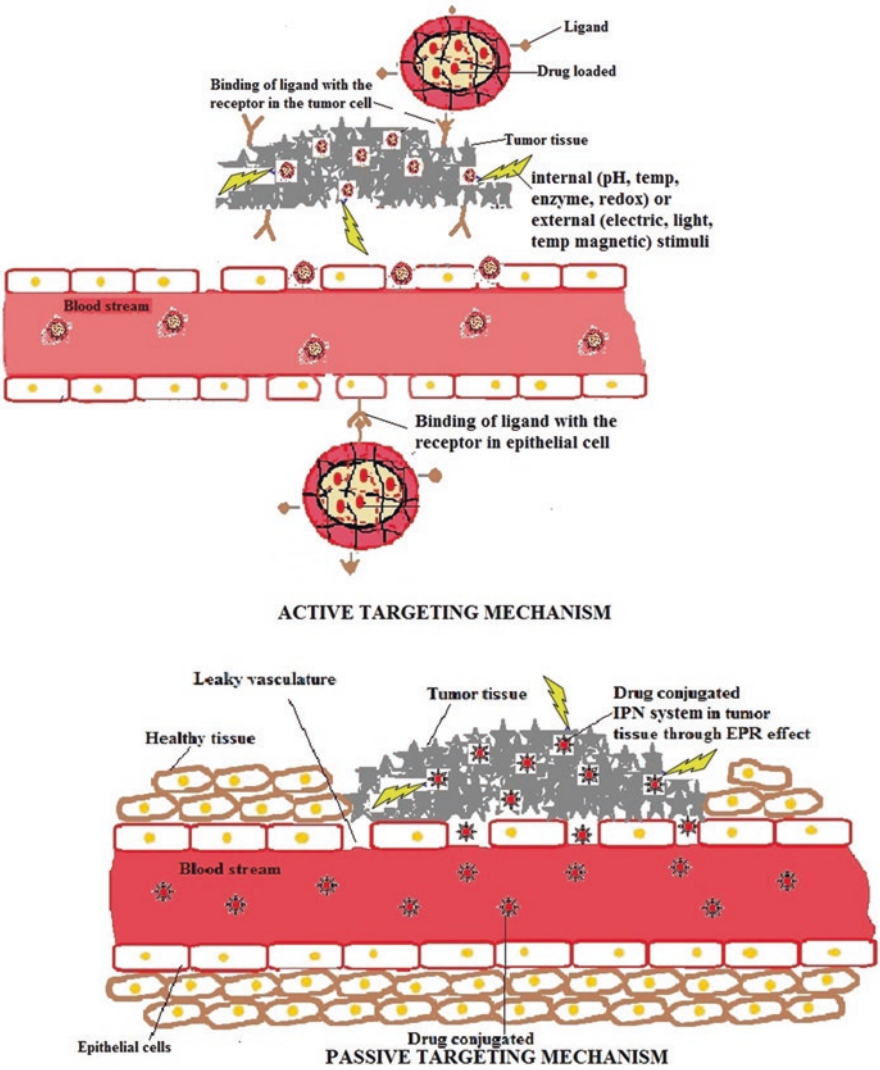

Fig. 9.3 Targeting mechanism on cancer cells. Targeting can be by either by active or passive mechanism. The active mechanism occurs by the direct binding of the ligand molecules to the specific protein in the cancer cell surface, and passive mechanism occurs by the efficient accumulation of IPN system in the tumour site by EPR effect

reactions (Liu et al. 2007). The amount of drug to be distributed at the target site is important for the successful implementation of cancer therapy. The basic designing of the IPN drug delivery system is in the form of zero-order pattern with reduced fluctuation (Jain et al. 2011).

9.7.1 Microspheres

Microspheres are a type of colloidal drug delivery system with less than 200 μm particle size. The major advantage of the microsphere-based drug delivery system is its minimized toxicity in the non-targeted cells and its augmented effectiveness (Suzuki 1994). For tumour vascular endothelial cells, microsphere is one of the selective drug delivery systems (Rajput and Agrawal 2010). It is widely used as carrier during liver transplantation or surgery (Sharma et al. 2007). By using the process of embolization, the microspheres can be incorporated into the peritumoural vessels, and 95% of the doses can be targeted. Cytotoxin-loaded microspheres for breast cancer, enteric-coated pancreatin microsphere for pancreatic cancer, radioactive microsphere, magnetic microspheres, microsphere as cancer vaccine delivery system and drug-eluting microspheres are some examples of microspherical drug delivery system (Rajput and Agrawal 2010). Chitosan-polyethylene oxide-g-acrylamide intermolecular rigid network, an IPN-based microspherical drug delivery system, is developed for the delivery of capecitabine, the antineoplastic drug for enhancing the time of drug delivery by forming a network (Agnihotri and Aminabhavi 2006).

9.7.2 Transdermal Membranes

Transdermal technology is one of the promising drug delivery system which is applied in breast cancer treatment. For the effective use of this drug delivery system, transdermal patches are engineered for targeted delivery of drugs to the cancer cells, thus avoiding the side effects of the drugs to the non-cancerous cells and minimizing the dosage of drugs. The drug is provided through the skin. The major advantages of using transdermal patches include site-specific drug delivery to the target site, providing maximum bioavailability. Transdermal patch includes a linear, compartment, adhesive, permeable membrane and a backing (Tan 2010).

9.7.3 Tablets

IPN matrix tablets can be prepared by wet granulation method or covalent cross-linking method (Mandal et al. 2010) and compressed to tablets for the sustained release of anticancer drugs. The release of drug is maintained by the swelling capacity of the IPN matrix, and the drug release is prolonged for a long time (Vineet et al. 2012).

9.7.4 Capsules

IPN-reinforced capsules can be prepared using the micron-sized colloidosomes of polymethyl methacrylate-co-divinyl benzene microgels which leads to the

formation of structures with a raspberry core-shell morphology (Kweon et al. 2008). It was found that the cell proliferation was greatly decreased when the anticancer drugs such as 5-Fu and paclitaxel are encapsulated with additional advantage of sustainable release and free drugs causing side effects are not available (Iqbal et al. 2017). Also, a study indicates that the nucleolin aptamer-capped fluorescein-loaded mesoporous silica nanocapsules specifically target the cancerous cells (Frank et al. 2013).

9.7.5 Nanoparticles

Huge-potential anticancer drugs can be released in a controlled manner by using the IPN device-thermally active nanoshells (Mayet et al. 2014). Drugs that find difficulty to cross the blood-brain barriers can be crossed easily with the help of nanoparticles and can deliver the anticancer drugs in tumour sites (Koziara et al. 2004). In a study, it was found that the lipid cationic nanoparticle coupled with integrin-targeted ligand induces apoptosis in the tumour cells and shows reversion of the metastatic tumours (Hood et al. 2002). The most important features of nanoparticles-mediated IPN drug delivery system are its bioavailability and drug-targeting capacity (Dubin 2004; Dimendra et al. 2012).

9.7.6 Hydrogels

There exists a lot of disadvantages with the synthetic polymers, and so natural polymers such as hyaluronic acid and collagen are widely applied for the sustained release of anticancer drugs (Mayet et al. 2014). In a study, anticancer drugs such as 5-fluorouracil, bleomycin A2 and mitomycin C are incorporated into the hydrogel matrix (collagen-poly (HEMA) hydrogel) for controlled-release formulation (Jeyanthi and Rao 1990). Also, hydrogel-based drug delivery system increases the gene therapy and chemotherapy efficacy by maximizing the half-life of the anticancer drug and inducing sustained release of drugs. In addition to this, hydrogels are used as a substitute for tissues in tumour microenvironment reconstruction (Mohammadmajid et al. 2017).

9.7.7 Sheet

Sheeting is one of the potential method for the IPN-based drug delivery system (Jain et al. 2011). For enhanced anticancer drug delivery, ruthenium complex-loaded monolayer-layered double-hydroxide ultra-thin nanosheets are used which act as theranostic agent for light-switchable cancer imaging and increase the luminescence lifetime for photodynamic therapy (Shanyue et al. 2018).

9.7.8 Sponges

Nanosponges are porous, colloidal, 1 micrometer-sized tiny meshlike carriers that can be used to incorporate both hydrophilic and lipophilic drugs with improved anticancer drug bioavailability and solubility at the targeted site. Various factors such as temperature, degree of substitution, polymer used, type of drug and the method of preparation affect the formulation of nanosponges. The methods used to prepare nanosponges include ultrasound-assisted synthesis, emulsion solvent diffusion method and solvent method. Cyclodextrin-based nanosponge is one of the best available anticancer drug delivery methods (Tukaram et al. 2017). In a study, sponges are developed using freeze-drying alginate-oxidized nanocellulose with a cross-linker in which the addition of novel carboxyl group leads to the formation of mechanically stable alginate-based sponge structure. Further, mechanical strength was induced by oxidized cellulose nanocrystals. The advantages of sponges include increased compression strength, ultrahigh porosity, concomitant water absorption and retention (Babu et al. 2013).

9.7.9 Films

Anticancer drug delivery using nanofilm structures can hold proteins, dendrimers or nanoparticles with accurate controlling capacity. Nanofilm is one of the promising techniques in which the anticancer drug with DNA is synthesized and arranged with peptide by layer-by-layer deposition method (Younghyun et al. 2014). Radical solution polymerization is another technique used to prepare full-IPN film. The concentration of polymeric material and extent of cross-linking are the two factors to be considered while preparing a film (Rodkate et al. 2010).

9.7.10 Calcifiable Matrix System

Elastin and collagen are the basic components of the connective tissues. These basic components along with IPN matrix can be used as a calcifiable matrix system for effective drug delivery system in bone cancerous tissues and tissue calcification (Meaney 1995; Stolzoff and Webster 2016). The clinical fate and therapeutic efficiency of the biomaterials are affected by the calcification (Schoen 1992) which depends on various chemical factors related to the cells (Wada et al. 1999).

9.8 IPN-Induced Cancer Therapeutics

Different anticancer drugs complexed with the IPN are widely investigated for the targeted and controlled drug delivery system. Some of the anticancer drugs are given below:

9.8.1 5-Fluorouracil

5-Fluorouracil is the broadly studied anticancer drug for pancreatic, breast and gastric cancer treatment (Longley et al. 2003; Dickson and Cunningham 2004). For example, the microsphere of IPN hydrogel (pH and temperature sensitive) containing N-isopropylacrylamide and sodium alginate is analyzed for the drug release rate and gives 90% 5-fluorouracil release within 12 h (Reddy et al. 2008). The hybrid multifunctional and photothermal-responsive Au in IPN-PNIPAAm nanosystem is found to possess biocompatibility even in the low concentration employed for the cellular imaging purposes (Zhao et al. 2011). It is also found that the natural polymeric system possesses higher swelling and drug-releasing properties with higher pH-responsive drug release (PAsp and starch containing semi-IPN system in colon 5-FU delivery) (Liu et al. 2011).

9.8.2 DOX Hydrochloride

DOX hydrochloride generally acts by the intercalation of DNA. Hence, it is used majorly in soft tissue sarcoma, hematological malignancy and carcinoma treatment. There are only fewer reports found in the DOX delivery by the IPN system with reduced toxic effect. One of the examples for IPN-based DOX release is the hydrogel containing hydrophilic gelatin, and hydrophobic divinyl ester is found to exhibit 85% drug release rate in 6–10 days with a lesser burst release rate (Brayfield 2014; Mohamed et al. 2011) (Table 9.1).

Table 9.1 IPN-based therapeutic drugs for cancer treatment. Different anticancer drugs are selectively bound to the IPN systems like semi and full IPN that produced sufficient drug release and action at the target site

Drug	IPN system	Synthesis method
5-Fluorouracil	IPN nanogels	In situ polymerization and cross-linking (Zhao et al. 2011)
	Semi-IPN hydrogels	Free radical polymerization (Rao et al. 2008)
	IPN hydrogels	Sequential polymerization (Zhang et al. 2004a, b)
	Semi-IPN microspheres	w/o emulsification (Sekhar et al. 2011)
Doxorubicin hydrochloride	IPN pluronic P105 micelles	Ultrasonic (Husseini et al. 2002)
	IPN hydrogels	Emulsion cross-linking (Mohamed et al. 2011)
	Semi-IPN hydrogels	Free radical polymerization (Jaiswal et al. 2013)
Capecitabine	Semi-IPN microspheres	Emulsion cross-linking and free radical (Agnihotri and Aminabhavi 2006)
Oxaliplatin	Semi-IPN microspheres	Direct polymerization (Chen et al. 2008)

9.8.3 Capecitabine

Capecitabine is a prodrug which on administration will get converted into 5-FU. This drug is widely utilized for cancer treatments such as breast and colorectal metastatic cancer. This drug possess serious side effects like nausea, cardiac damage, dermatitis, vomiting, etc. Hence, only 2.5 g/m^2 is recommended per day. An IPN system of 82–168 μm hydrogel microsphere is prepared using glutaraldehyde cross-linked poly(ethylene oxide-g-acrylamide). Chitosan gives 74% drug release at the intestinal pH and at 1.2 pH (Agnihotri and Aminabhavi 2004; Agnihotri and Aminabhavi 2006).

9.8.4 Oxaliplatin

The hydrophilic oxaliplatin antitumour drug with half-life approximately 10–25 mins is used for the colorectal cancer treatment in the advanced state. A higher antitumour activity is found by a pH and thermal-sensitive semi-IPN nanosystem with 70% within 48 h of administration (Chen et al. 2008).

9.9 Biomedical Applications of IPN-Based Drug Delivery System

IPN, a widely used biomaterial, is used in the controlled-release drug delivery system. IPN-based drug delivery system is basically designed to transport drugs with minimum fluctuation, with specific time period and at a predetermined rate. The existence of various biological and physical characteristics such as biodegradability, excellent swelling capacity, enhanced solubility of hydrophobic drugs, weak antigenicity, imparting drug stability in the formulations, biocompatibility and drug targeting increases the use of IPN drug delivery system for various biomedical applications that include bioengineered tissues, cartilage scaffolds, bone substitutes, cancer therapy, etc. (Jain et al. 2011).

9.9.1 Bioengineered Tissue

Replacement of tissues which are impaired can be executed with the principles of tissue engineering, an emerging field. IPN-related materials which are purely natural, biodegradable, mechanically stable and biocompatible (George et al. 2008) are used as biomaterials for the development of bioengineered tissues (Chen et al. 2002). Such type of biomaterials favours cell proliferation and cell adherence. Collagen is a widely used biomaterial, and so it is used in generating bioengineered tissues such as blood vessels, heart valves and ligaments (Auger et al. 1998). The hemostatic feature of collagen enhances the blood-clotting mechanism and in turn can be used in tissue repairing process. In bone tissue-engineering process,

collagen-hydroxyapatite composites which are highly porous scaffolds are used immensely. The collagen-hydroxyapatite composites are biocompatible in nature, and the scaffold's histocompatibility is not affected by hydroxyapatite (Liu et al. 2003). For load-bearing artificial tissues, hydrogels synthesized using double-network techniques are used (Yasuda et al. 2005) which exhibit the property of low-friction resistance and biocompatibility and one of the best scaffolds for the cells cultured (Azuma et al. 2007). A tough scaffold is necessary for the cell culture for implanting the engineered tissues within the body that enhances the repairing and regeneration process in living organs (Yasuda et al. 2005). While introducing poly (N,N0-dimethylacrylamide) [PDMAAm] to the body, it leads to the automatic repairing process in cartilage with signs of no inflammation (Hago and Li 2013). The anisotropic mechanical property exhibited by the liquid crystalline DN gel and cellulose-based DN gel is essential for the anisotropic functioning in the human system (Imabuchi et al. 2011). The calcification of implantable bioprosthetic heart valve can be induced by using collagen and elastin as controlled cardiovascular drug delivery device (Park et al. 2000).

9.9.2 Bone Substitutes

In human system, bone is one of the most powerful regenerating tissue that gains wide importance in the field of tissue engineering. Most of the orthopaedic problems can be treated using the composite form of collagen with other polymers. Acquired and congenital orthopaedic defects can be cured with combined bone graft material made up of demineralized bone collagen and porous hydroxyapatite (Takaoka et al. 1988). The resultant osteoinductive material, the combined form of grafted demineralized bone collagen and hydroxyapatite, is used as a bone substitute and also for expressing biological activity by using the combined IPN as a carrier of bone morphogenetic protein (BMP). The bone is also regenerated using the enzymatically degradable IPN (edIPN), which is non-fouling in nature and made up of poly(AAm-co-EG/AAC) that helps in introducing the cell signalling domain (Ho et al. 2007). Peptide-modified IPNs of poly(acrylamide-co-ethylene glycol/acrylic acid) functionalized with an Arg-Gly-Asp (RGD) containing 15 amino acid peptides are used in the regeneration of bone in the peri-implant region (Barber et al. 2007). Water-swellable IPN having both ionic polymer and thermoplastic polymer is used for repairing natural cartilage in the joints (Gupta and Ravi-Kumar 2000).

9.9.3 Cartilage Scaffolds

Using freeze-drying process, semi-IPN scaffolds made up of alginate and chitosan are prepared with the cross-linking agent $CaCl_2$, and this scaffold plays an important role in the cartilage tissue engineering (Tigli and Gumusderelioglu 2009). The IPN PVA/GE hydrogels, prepared by freeze-thawing method with varied mechanical and physical properties such as the swelling property and absorbing capacity, are

used for exudative wounds, and it shows the importance of scaffolds in tissue engineering (Rani et al. 2010).

9.9.4 Cancer Treatment

IPN-based nanoparticles are used in treating various stages of cancer; more specifically, IPN nanoshells are introduced for the leaky vasculature of cancer. For example, PEG polymeric nanoparticle is used for treating neoplasms since the PEGylated nanoparticles get stacked in the tumour cells (Auger et al. 1998). IPN in the form of chitosan and collagen as biodegradable polymer scaffold is used as an alternative substrate for in vitro culture of human epidermoid carcinoma cells (HEp-2). Here, glutaraldehyde acts as the cross-linking agent. The cultured HEp-2 cell is used for testing anticancerous drugs (Shanmugasundaram et al. 2001; Atyabi et al. 2008).

9.10 IPN System for Different Tumour Cells

Conventional chemotherapy is not a promising field in destroying the tumour cells since it has side effects. Drug targeting can be achieved using different types of IPN systems for varied tumour cells.

9.10.1 Lung Cancer

Paclitaxel-loaded PLGA microspheres, camptothecin-loaded PEGylated microspheres and nanosponges are some examples of IPN-based drug delivery system for curing lung cancer. No toxicity is associated with the delivery of paclitaxel using the PLGA microspheres (Rajput and Agrawal 2010). It is found that human serum albumin and gelatin constitute the biodegradable nanoparticle that acts as a good pulmonary drug carrier (Dimendra et al. 2012). Also, nanosponges are applied in delivering the anticancer drug to the targeted site in the lungs (Tukaram 2017).

9.10.2 Breast Cancer

The occurrence statistics of breast cancer is high among women. Adriamycin-loaded albumin microspheres, cytotoxin-loaded microspheres, transdermal patches, mitoxantrone-loaded albumin microspheres, oligonucleotides combined with nanoliposomes and polylactic-acid-encapsulated microspheres with IL-12, TNF-alpha and granulocyte macrophage-colony stimulating factor are some examples of IPN-based drug delivery system for breast tumour regression. When cytotoxin-loaded microspheres are administered intra-arterially, the conjugate is carried to the capillary bed and the drug is delivered at the targeted site. Mitoxantrone-loaded albumin microspheres greatly minimize the toxic effect of the drug (Rajput and Agrawal

2010). Additionally, transdermal patches deliver the drug to the cancerous sites by undergoing some modifications for drug penetration with lesser side effects (Tan 2010). Recently, it is found that oligonucleotides combined with nanoliposomes target the cancer cells and deliver the nucleic acids, thus stopping the generation of alpha folate receptor (Dimendra et al. 2012).

9.10.3 Liver Cancer

Phosphorus-32 microspheres, poly-lactide-co-glycolide microspheres, Y-90 microsphere, degradable starch microspheres with iodized oil and SIR-Spheres ^{99m}Tc-labelled microspheres can be cited as examples of IPN-based drug delivery system for targeting hepatocellular carcinoma cells. Y-90 microspheres loaded with oxaliplatin, fluorouracil and leucovorin are studied in hepatocellular carcinoma cells. ^{99m}Tc-labelled microspheres are widely applied in the field of neoplastic lesions imaging, and SIR-Spheres are used for treating metastatic liver tumours (Rajput and Agrawal 2010).

9.10.4 Pancreatic Cancer

Enteric-coated pancreatin microspheres, pH-sensitive histidylated oligolysine with the drug liposome complex and antisense technology are some of the IPN-based drug delivery system for pancreatic cancer treatment. Enteric-coated pancreatin microspheres are used to treat the pancreatic cancer that reduce the occlusion of the pancreatic duct and weight loss (Rajput and Agrawal 2010). Antisense technology (use of oligonucleotides) is used for treating pancreatic cancer, and the pH-sensitive histidylated oligolysine with the drug liposome complex enhances the drug delivery to pancreatic cells without inducing the toxic effect (Dimendra et al. 2012).

9.10.5 Bladder Cancer

Chlorin e6 loaded in polystyrene microspheres and poly paclitaxel microspheres are two examples for the IPN-based drug delivery system for bladder cancer therapy. Chlorin e6 loaded in polystyrene microspheres provides the intracellular localization site to destroy the carcinoma cells by photodynamic process, and poly paclitaxel microsphere is a potential therapy for superficial bladder cancer (Rajput and Agrawal 2010).

9.10.6 Brain Cancer

Brain consists of a blood-brain barrier that stacks the entry of foreign particles to the brain cells, and this phenomenon acts as a blocking process for the entry of

anticancer drugs. To overcome this blood-brain barrier, drugs are loaded with IPN system. Poly(methylidene malonate) loaded with 5-fluorouracil microsphere shows a sustained drug delivery system, and so it is widely used to treat malignant tumour (Rajput and Agrawal 2010). Poly(valerolactone-allylvalerolactone) loaded with temozolomide acts as a nanosponge drug delivery system (Tukaram 2017).

9.10.7 Colorectal Cancer

Methotrexate-loaded guar gum microspheres, Eudragit P-4135F-based microspheres and poly(D,L-lactic-co-glycolic acid) microspheres with interleukin-12 gene are IPN-based drug delivery system for targeting the colorectal cancerous cells. Guar gum microspheres have the ability to carry the drug and control its release when it reaches the colon, and Eudragit P-4135F-based microsphere is a pH-dependent colon drug delivery system (Rajput and Agrawal 2010).

9.11 Challenges in Cancer Treatment Using IPN System

A novel drug delivery system is highly applicable in the cancer diagnosis and treatment. Cancer treatment lays many difficulties in the proper fabrication of the effective drug delivery system. The cancer cells are found to have a defense mechanism through altered targets, elevated drug metabolism, efflux pump overexpression or self-repairing capacity (Gottesman 2002). The challenges associated with the IPN system in cancer treatment include inability of the polymeric matrix to effectively release the active components at the target site, absence of cohesiveness, reduced-phase molecular interactions (due to less efficient interfaces), heterogeneity, kinetic profiles of interpenetrated polymers and various other parameters in process like operating condition, reactor type and the mechanism behind the IPN formation (Somya et al. 2015).

9.12 Conclusion and Future Perspectives

Thus, the propensity of the IPN system has made it to be an effective agent for the controlled drug delivery system for cancer diagnosis and treatment. Though it has attained a remarkable position in the biomedical field, advanced work has to be carried out in order to bring them to the large-scale in vivo cancer treatment from preclinical and in vitro tests. The standardization of stimuli-responsive smart drug delivery IPN system is necessary to enhance the properties to readily serve as promising drug carrier for the cancer treatment. The production of novel polymers and the cross-linkers with Good Manufacturing Practice are also essential to pave an everlasting position for the IPN system in the drug delivery field.

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Semi-interpenetrating Networks Based on (Meth)acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) Hydrogels for Biomedical Applications

10

Marija M. Babić and Simonida Lj. Tomić

Abstract

In this study, three series of semi-interpenetrating networks were synthesized based on 2-hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), itaconic acid (IA), and poly(vinyl pyrrolidone) (PVP) as interpenetrating polymer. Syntheses were performed by free radical cross-linking/polymerization reaction. The first series represented hydrogels based on 2-hydroxyethyl methacrylate, poly(vinyl pyrrolidone), and itaconic acid, varying of poly(vinyl pyrrolidone) content. The second series of samples were hydrogels based on 2-hydroxyethyl acrylate, poly(vinyl pyrrolidone), and itaconic acid, varying of itaconic acid content. The third series of synthesized samples were based on 2-hydroxyethyl acrylate, poly(vinyl pyrrolidone), and itaconic acid, varying of poly(vinyl pyrrolidone) content. The content of component was varied in order to examine the influence on the structure, pH- and temperature-sensitive swelling-"intelligent" behavior, mechanical properties of hydrogels, as well as antimicrobial and biocompatible potential of hydrogels. Poly(vinyl pyrrolidone) is a linear polymer, which shows satisfactory biocompatibility and hydrophilicity. Itaconic acid gives pH-sensitive-"intelligent" behavior and better hydrophilicity. Hydrogels based on HEMA and HEA show excellent biocompatibility and satisfactory hydrophilicity.

All three series of samples showed satisfactory cytocompatibility, as well as the antimicrobial potential tested against most common microbes. The results obtained and presented in this research can contribute to the development of new efficient polymeric biomaterials for biomedical applications.

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Hydrogels · 2-hydroxyethyl (meth)acrylate · Poly(N-vinylpyrrolidone) · Itaconic acid · Biomedical applications

10.1 Introduction

Hydrogels as polymeric biomaterials due to their properties and potential applications in medicine and pharmacy are examined for a period of 50 years (Peppas et al. 2000). Hydrogels are cross-linked polymeric materials that can swell in water and biological fluids, but do not dissolve in them. Because of the high content of water, they have soft consistency and a favorable degree of flexibility that is very similar to living tissues. The ability of hydrogels to absorb water derives from hydrophilic functional groups bound to the polymer chain, while their dissolution resistance arises from the cross-linking of the chain's network. Hydrogels can absorb a large amount of water, making them very similar to living tissues, which results in increased biocompatibility, resulting in ever more extensive use in biomedicine. In the swollen state, they can reach a mass of 10–20% to up to tens of thousands more compared to the mass of dry gel, xerogel. Water retention capacity and permeability are very important properties of hydrogels. Cross-linking between different polymer chains results in viscoelastic, sometimes purely elastic, behavior and contributes to the gel having the appropriate hardness and elasticity. The choice of the starting components and the fraction in the synthesis provide the possibility of adjusting the structure of hydrogels; mechanical, thermal, and morphological properties; as well as swelling and sensitivity to external stimuli (Gulrez et al. 2011; Himi and Maurya 2013; Hoffman 2012; Chai et al. 2017; Pal et al. 2009).

Hydrogels are three-dimensional, physically or chemically cross-linked polymers, which have the ability to absorb a large amount of water or physiological fluids while preserving their dimensional and structural stability. High content of water, soft and elastic consistency, and minimal tendency of adsorption of proteins to the surface of hydrogels are basic properties that make hydrogels very similar to living tissues. A special group of hydrogels as biomaterials is made up of so-called "intelligent" hydrogels that have the ability to recognize the effect of the dominant stimulus in the body, giving it a response in the form of changing their physical and/or chemical properties. In 1960, Wichterle and Lim synthesized the first hydrogel based on 2-hydroxyethyl methacrylate (PHEMA), which was intended to produce contact lenses. Since then, interest in hydrogels has started to grow. Many studies have been carried out to modify the structure and properties using different monomers and polymers. For hydrogels based on HEMA and HEA, they have been shown to exhibit exceptional biocompatibility and high hydrophilicity and are used for the synthesis of hydrogels that can be found in biomedicine and pharmacy. Poly(vinyl pyrrolidone) (PVP) is a linear polymer widely used for the synthesis of interpenetrating grid (IPN) hydrogels, due to its impact on the properties of hydrogels such as morphology, swelling, and release of the drug. Poly(vinyl pyrrolidone)

has antimicrobial properties to some extent. It can be used to produce medical devices such as surgical threads, prostheses, coatings, and implants. Its application can prevent the appearance of an infection, which may be due to the application of these devices. It is one of the most used polymers for the synthesis of semi-IPN networks. Itaconic acid gives the pH a delicate, "intelligent" behavior and contributes to hydrophilicity.

A group of hydrogels showing changes in swelling, in response to the action of stimuli from the environment, such as pH, temperature, ionic strength of the solution, intensity of light, and magnetic and electric field strength, belong to "intelligent" materials. Due to its specific properties, the application of hydrogels in the field of medicine and pharmacy has an increasing importance and scope. For application it is important that hydrogels during the swelling remain mechanically strong enough, i.e., to retain their geometry and shape, to be flexible, and to be able to release the active agent molecules that are loaded by fluid absorption in the polymer network at controlled rate. For certain bioengineering applications, the properties of "mimicry" are important, i.e., imitating the properties of living tissues.

A significant area where hydrogels are applied are controlled-release systems for active substances, as matrices in which active substances that are controlled release into the body fluids after loading are released; synthetic skin coatings in the treatment of wounds and burns that are also a barrier to bacteria; and contact lenses for use in ophthalmology, which may include drug (Okay et al. 2009; Singh et al. 2014; Jiao et al. 2006).

In order for hydrogels to be used in medicine and pharmacy, they must be biocompatible. This implies the interaction between the material and the organism in which the material is applied. In order to be used for making medical implants, which are used in contact with the cells, tissues, or body fluids of a human organism, they must satisfy certain criteria. An important feature of the material that it must possess is harmlessness, which means that the material does not cause cytotoxicity. They should not cause unwanted reactions to the body, irritation or allergy. Also, they must not be mutagens or carcinogens. If the tissue cannot accept the "foreign body," then it is unacceptable for making the implants.

Biocompatibility is one of the most significant characteristics of newly synthesized hydrogels. In a preliminary biocompatibility assessment, in vitro cytotoxicity tests are most often used. If the newly synthesized materials would not be biocompatible, i.e., if they were to show a cytotoxic effect on normal cells, they could induce necrosis (accidental cell death) or apoptosis (programmed cell death) of these cells and thus could not be used for human use. This is the first phase in the research and development of new potential pharmaceutical products. Most of the toxicity problems in hydrogels are related to unreacted components during synthesis. Therefore, the understanding of the toxicity of various monomers used as constituents of hydrogels is very important. The relationship between chemical structures and the cytotoxicity of acrylate and methacrylate monomers has been studied. Some of the measures taken to solve this problem include modifying the polymerization kinetics to achieve greater conversion and rinsing of the hydrogel obtained. The formation of hydrogels without the initiator supplements was

examined to eliminate the problem of a lagging initiator. The most commonly used technique is gamma radiation (Ristić et al. 2011).

Hydrogels have significant applications in medicine and pharmacy, such as contact lenses and artificial skin, in controlled drug release. Infections associated with the application of medical devices made of polymeric biomaterials represent a significant clinical problem in medical practice, and there are still serious complications in the application of these products. When there is already an infection, there are difficulties in the treatment of existing antibiotic agents. More recently, new biomaterials based on polymers develop, which have specific antimicrobial properties over a longer period of time. An additional advantage is that some antimicrobial agents can be incorporated into such biomaterials to ensure infection control at the target site where the application of the given biomaterials is carried out. Synthesis of polymeric biomaterials for use in medicine and pharmacy, showing specific antimicrobial properties, is one of the important goals.

Infection is the most common complication associated with the use of medical devices (catheters, prostheses, surgical sutures, implants). One of the most well-known polymers, which in itself possesses to some extent antimicrobial properties, is poly(vinyl pyrrolidone) (PVP). One of the more important testing of polymers for their application in medicine is testing their antimicrobial activity. The most common causes of infection are known, and these microorganisms are used to test the antimicrobial potential of new materials (Rodriguez-Hernandez 2016; Salome and Schneider 2013).

Poly (N-vinyl-2-pyrrolidone) (PVP) is a synthetic, linear polymer. Based on the chemical structure, PVP is a polymeric lactam with internal amide linkages. It contains a very polar amide group that gives hydrophilic properties and nonpolar methylene groups that provide hydrophobic properties. This polymer possesses good biocompatibility and can be used as the main component in the synthesis of materials used for temporary skin dressings and bandages (Erizal et al. 2013; Ajji et al. 2005). Its use as biomaterials in artificial blood plasma dominated the Second World War. Lately, polymers based on PVP have been tested quite a lot. There are other applications, such as controlled drug delivery systems, tissue regeneration, and implant replacement (Barros et al. 2011).

PVP is one of the most used polymers for the synthesis of semi-interpenetrating networks. Semi-interpenetrating polymer network (semi-IPN) hydrogel polyacrylamide grafted with poly(vinyl alcohol)/poly(vinyl pyrrolidone) (PAM-g-PVA/PVP) was synthesized by free radical polymerization initiated by PVA $(NH_4)_2Ce\ (NO_3)_6$. It has been shown on the basis of the results that the morphology and swelling degree of PAM-g-PVA/PVP hydrogels can be changed and controlled using a different content of PVP. Also, it has been shown that these hydrogels are temperature-sensitive; the highest swelling degree is observed at a temperature of about 42 °C. The vicinity of the physiological temperature opens the possibility of the use of these copolymers in controlled drug release systems (Wei et al. 2014).

The pectin-PVP hydrogel membranes based on pectin and poly(vinyl pyrrolidone) (PVP) (PEVP) were synthesized. The properties of these samples and the influence of increased PVP fraction were examined. The DSC study shows that T_g

rises after mixing with PVP. It has been found that mechanical strength increases with increasing PVP content in hydrogel membranes. It has also been found that the synthesized samples are biocompatible with the melanoma B16 cell line. It has been shown that these samples are pH-sensitive. As a model for controlled release of the drug, salicylic acid was incorporated into the hydrogel membrane by the diffusion method. Based on the results of this study, it can be concluded that these hydrogels may be candidates for various biomedical applications, such as in controlled drug release (Mishra et al. 2008).

Hydrogel based on poly(vinyl pyrrolidone) (PVP) and κ-carrageenan (CK) is synthesized using radiation. The aim of this research is the synthesis of biomaterials that can be used in medicine. The characterization of these samples has been performed. Properties such as gel fractions, water absorption, water evaporation, elongation at break, and strength were tested to assess for the usefulness of wound healing hydrogels (Erizal et al. 2013).

pH-sensitive hydrogels based on poly(vinyl pyrrolidone) and acrylic acid (PVP/AA) are synthesized by free radicals polymerization. The content of monomers, polymers, and a cross-linking agent varies, and their influence on the swelling and release properties is tested. N,N-methylenebisacrylamide (MBA) was used as a cross-linker and tramadol HCl as a drug model. Grafting of PVP with AA improves the mechanical properties of the hydrogels and also makes them pH-sensitive. pH sensitivity of PVP/AA hydrogels can be modified by changing the composition and degree of cross-linking to be used for optimal drug release to the colon (Sohail et al. 2014).

Interpenetrating nets (IPN) of hydrogels based on chitosan (Ch), poly(vinyl pyrrolidone) (PVP), and poly(acrylic acids) (PAAc), cross-linked with glutaraldehyde (GA) and *N,N*-methylenebisacrylamide (MBA), were synthesized and their properties tested. The possibility of potential application as a controlled drug release system to the gastrointestinal tract was investigated. Amoxicillin was used as a drug model. Based on the results, it can be concluded that these pH-sensitive hydrogels can serve as a potential material for the drug release to the stomach or small intestine (Ekici and Saraydin 2007).

Three different formulations of PVP hydrogels have been synthesized, characterized, and used as a carrier for various drugs. Theophylline is incorporated into a PVP hydrogel with different concentrations. The mechanism of the theophylline release from the tablet, as well as the time of its release, was tested. Based on these results, it can be concluded that PVP hydrogels can be used as controlled drug release system (Ahmad et al. 2013).

The research was carried out on hydrogels as decubitus dressings, which were synthesized on the basis of poly(vinyl pyrrolidone) (PVP), polyethylene glycol (PEG), and agar. The study examined the biocompatibility of these samples. An in vitro biocompatibility test included cytotoxicity, antifungal, and antibacterial tests, which are most important for the application of samples as decubitus dressings. Based on the results, it can be concluded that these hydrogels show good biocompatibility (Biazar et al. 2012).

Wound healing is a dynamic process. The condition of the wound may further affect the progress of the healing process. It is widely accepted that a warm, humid environment encourages rapid healing. The state-of-the-art wound care products are designed to provide these conditions. Hydrogels are used for skin dressings based on poly(vinyl pyrrolidone) (PVP), κ-carrageenan, and polyethylene glycol (PEG) using ^{60}Co gamma radiation. These materials are suitable for use in tropical conditions and have a relatively long lifetime. Based on the results, it can be noted that the properties of the synthesized hydrogel are similar to commercial hydrogels and that they can be applied as wound dressings. The results of the study confirm that hydrogels based on PVP/κ-carrageenan/PEG can continue with clinical trials (De Silva et al. 2011).

Semi-interpenetrating polymer networks (semi-IPNs) and interpenetrating polymeric networks (IPNs) have emerged as innovative materials for biomedical and pharmaceutical applications. The interest in these structures is due to the possibility of combining the favorable properties of each polymeric component of the IPNs or semi-IPNs leading to a new system with properties that often differ from those of the two single components (Bhardwaj et al. 2012; Rana et al. 2015; Bajpai et al. 2008; Singh et al. 2012; Sperling 1981; Banerjee et al. 2010). All these overall properties allow tailoring new materials, thus designing desired properties and preparing new hydrogels useful in the biomedical field (Das 2013; Roland 2013; Wang et al. 2011).

Poly(2-hydroxyethyl (meth)acrylate) (PHE(M)A)s are commonly used polymers for the synthesis of hydrogels, due to its biocompatibility and high hydrophilicity. PHE(M)A hydrogels found a lot of applications in pharmaceutical and biomedical fields. It can be used for biomaterials as coatings, intraocular lenses, tissue scaffolds, and devices for controlled drug delivery. To improve mechanical stability or swelling properties of hydrogels based on PHE(M)A, they can be combined with hydrophilic polymers into blends or semi-IPNs (Prasitsilp et al. 2003; Barrett et al. 1986; Menapace et al. 1989; Tomić et al. 2006; Babić et al. 2015; Baino 2010; Sanna et al. 2012; Chen et al. 2007).

Poly(vinyl pyrrolidone) (PVP), a water-soluble synthetic polymer, is widely used in medical applications, such as a blood plasma extender and a carrier for drug delivery. PVP has low toxicity, and it is used in medicine, food, and cosmetics and as a film-forming agent. Because of its special molecular structure, PVP has many outstanding properties. PVP has satisfied biocompatibility and hydrophilic properties, which have been used for composite tissue engineering matrices. It is one of the most frequently used interpenetrating polymers because it can be expected to influence hydrogel's morphological, swelling, and drug release characteristics (Abdelrazek et al. 2013; Chadha et al. 2006; Domingues et al. 2013; Erizal et al. 2013; Giri et al. 2011; Marsano et al. 2005; Naghdeali and Adimi 2015; Tomar and Sharma 2013; Wang and Wang 2010; Wei et al. 2014; Yanpeng et al. 2006).

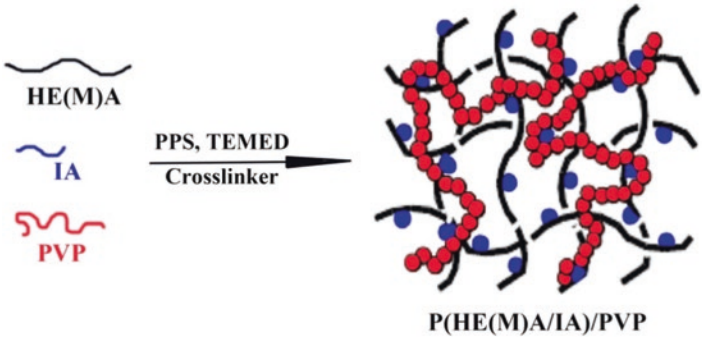
Itaconic acid (IA) can be produced from renewable sources by fermentation. IA is very hydrophilic and is expected to show good biocompatibility because of its

natural source. Small amounts of IA render good pH sensitivity and increased degree of hydrogel swelling (Bera et al. 2015; Gils et al. 2011; Okabe et al. 2009; Petruccioli et al. 1999; Rashid et al. 2016; Sakthivel et al. 2014; Sariri and Jafarian 2002; Sudarkodi et al. 2012).

2-Hydroxyethyl methacrylate (HEMA), 2-hydroxyethyl acrylate (HEA), itaconic acid (IA), and poly(vinyl pyrrolidone) (PVP) have specific properties and are therefore selected as components for samples syntheses to achieve biomedical applications. Three series of samples were synthesized. The fraction of components is varied to examine the effect on structure, pH- and temperature-sensitive swelling-"intelligent" behavior, and mechanical properties of gels. Their effect on the antimicrobial properties and cytotoxicity of the hydrogels was also examined.

10.2 Semi-interpenetrating Networks Based on (Meth) acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) Hydrogels

Semi-IPNs were obtained by a simultaneous method where a single cross-linker which has no possibility of any interaction with the second polymer is used. HE(M)A and IA monomers were polymerized simultaneously in the presence of PVP polymer, in such a way that the HE(M)A/IA copolymer is cross-linked and intermingled with PVP linear polymer. TEMED is used as an activator in order to activate polymerization process, and EGDMA is used as cross-linker in order to create a three-dimensional polymeric network (Scheme 10.1).



Scheme 10.1 Schematic representation of the formation of semi-IPN hydrogels composed of HE(M)A, IA, and PVP

10.3 Hydrogels Based on 2-Hydroxyethyl Methacrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied Poly(vinyl Pyrrolidone) Fraction

10.3.1 Structural Properties of P(HEMA/IA)/PVP Gels

FTIR spectra were recorded to identify the most important functional groups containing the synthesized copolymer gels recorded. Structural analysis of gels with different PVP content shows the difference in all three spectra through the appearance of the shoulders at 1664 cm^{-1}. As the proportion of PVP increases (indicated by the peak shoulder size), so the smallest is for the P(HEMA/IA)/2PVP sample, and the largest for P(HEMA/IA)/10PVP. Characteristic OH peaks at 3450 cm^{-1} (C–O stretching), peak characteristic for esters at 1730 cm^{-1} (C=O stretching), and aliphatic peaks in the range of 2900–3000 cm^{-1} (C–H stretching) (Boran and Hitit 2015; Podkoscielna et al. 2012; Tu et al. 2008).

10.3.2 pH- and Temperature-Sensitive Swelling of P(HEMA/IA)/PVP Hydrogels

In order to test the pH sensitivity of P(HEMA/IA)/2PVP, P(HEMA/IA)/5PVP, and P(HEMA/IA)/10PVP hydrogels, the samples were immersed at 37 °C solutions of different pH values (2.20, 3.85, 4.50, 5.45, 6.25, 6.80, 7.40, 8.00). It can be noticed that all samples exhibit a similar trend in the behavior of the equilibrium degree of swelling (q_e) with a change in the pH value. The lowest q_e values have samples at a low pH of 2.20. The lowest q_e values are below pK_a of the values of IA groups, when the carboxylic groups of itaconic acid are non-ionized. Then, intramolecular hydrogen bonds are formed, resulting in greater network compactness. The ionization occurs when the pH of the medium rises above the pK_a values of both carboxyl groups (pK_{a1} = 3.85 and pK_{a2} = 5.45). With an increase in ionization, the amount of permanent charge increases, causing an increased electrostatic repulsion between ionizing groups and therefore chains, the network's hydrophilicity, and the degree of swelling. With increasing IA content in hydrogels, q_e values increase. The maximum q_e values for all samples are at pH 6.25. The highest values were obtained for samples with 10 mol% IA as expected. Above this pH, the values of q_e slightly decrease, and above pH 7.40, there is a slight increase.

In order to investigate the behavior of hydrogels P(HEMA/IA)/PVP in buffer solutions, having pH values similar to biological fluids, the samples were swollen to an equilibrium state in buffer pH 7.40 at temperatures of 25, 37, 45, and 55 °C.

The dependence of the equilibrium degree of swelling on temperature in the buffer pH 7.40 P(HEMA/IA)/PVP hydrogels shows that all samples exhibit temperature sensitivity. The value of q_e drastically increases above 41 °C, which represents the temperature at which there is a change in volume (VPTT) of hydrogels P(HEMA/IA)/PVP. P(HEMA/IA)/PVP hydrogels have a VPTT at a physiologically important temperature of about 41 °C. When the temperature is below VPTT, the hydrogels

exhibit hydrophilic nature, because the interactions of polymer chains with water molecules dominate, and because of the hydration of the polymer chain groups, hydrogen bonds and hydrogel swells are established. However, when the temperature rises above the VPTT, there is a discontinuance of the established hydrogen bonds, the polymer chains are shrinking, and water is pushed out so that the gel is contracted into the hydrophobic state. This phase transformation is more pronounced for samples with a higher PVP content.

Based on these values, we see that the equilibrium swelling (q_e) decreases in the function of PVP and temperature. The equilibrium degree of swelling (q_e) for P(HEMA/IA)/PVP hydrogels is in the range of 2.56–3.78. If the influence of PVP content in gels on the equilibrium swelling degree (q_e) is observed, it can be concluded that as the PVP increases from 2 to 10 mol% at all temperatures, the equilibrium swelling degree decreases. This behavior of gels can be explained by the fact that with the increase in the PVP content, there are chains of the linear hydrophilic polymer of extremely high molecular weight (360000 g/mol) that affect the density of cross-linking within the polymer network and that, depending on the temperature, quantity of absorbed fluids decreases.

When considering the influence of temperature on the equilibrium degree of swelling, it can be observed that q_e decreases as the temperature rises in the range of 25–55 °C, for all gel samples, where this decrease is most pronounced for the sample with the highest PVP. This behavior of gels can be explained by the occurrence of long PVP chains difficult to move at higher temperatures and to increase q_e with increasing PVP content. It can be concluded that the loading of high-molecular-weight PVP within the gel polymer network reduces the degree of swelling of gels compared to gels of similar composition, but without PVP.

10.3.3 Morphology of (HEMA/IA)/PVP Hydrogels

The requirement for hydrogels to attach cells is to easily deliver oxygen, nutrients, water-soluble metabolites, and waste products between inside and outside of hydrogel. In order to fulfill these requirements, hydrogel must exhibit a large number of interconnected pores. In order to examine the morphology of synthesized gels, a scanning electron microscopy was performed. P(HEMA/IA)/10PVP xerogel showed porous structure, with texture of the honeycomb model. There is no significant difference in the pore size of the sample, which is consistent with the swelling of the samples. These hydrogels are suitable for biomedical applications.

10.3.4 Mechanical Properties of P(HEMA/IA)/PVP Hydrogels

Mechanical properties are expressed through the measurement of the shear modulus of the semi-IPN hydrogels. The value of the modulus ranges from 290 to 440 kPa. It can be seen that P(HEMA/IA)/PVP hydrogel possesses good mechanical properties, which are affected by long-chain PVPs within the gel network. A small amount

of PVP incorporated in semi-IPN hydrogels improves their mechanical properties (Shi et al. 2016). The value of *G* increases as the PVP fraction increases. The highest value is for the sample with 10 mol% PVP. P(HEMA/IA)/2PVP hydrogel shows the smallest *G* value. This behavior can be explained by the ability of PVP to improve mechanical properties due to the formation of hydrogen bonds. The addition of PVP to hydrogels significantly improves the mechanical properties of the gels, with the best properties being obtained for the sample with the highest content of PVP.

10.3.5 Biocompatibility of P(HEMA/IA)/PVP Hydrogels

Biocompatibility of semi-IPN P(HEMA/IA)/PVP hydrogels was examined in a cytotoxicity test on human cervix carcinoma (HeLa) epithelial cells. The results of this study for P(HEMA/IA)/PVP hydrogels with different PVP content are shown in Fig. 10.1. The obtained values show that there is no significant change in cell viability with the change of PVP content and confirm that P(HEMA/IA)/PVP hydrogels show a high level of cytocompatibility and therefore suitable for use as biomaterials. Cell viability is over 90% for all samples, for all concentrations of the extract.

10.3.6 Antimicrobial Properties of Hydrogels

Determination of antimicrobial activity is of great importance for biomedical application. Based on the results shown in Fig. 10.2, it can be noticed that the

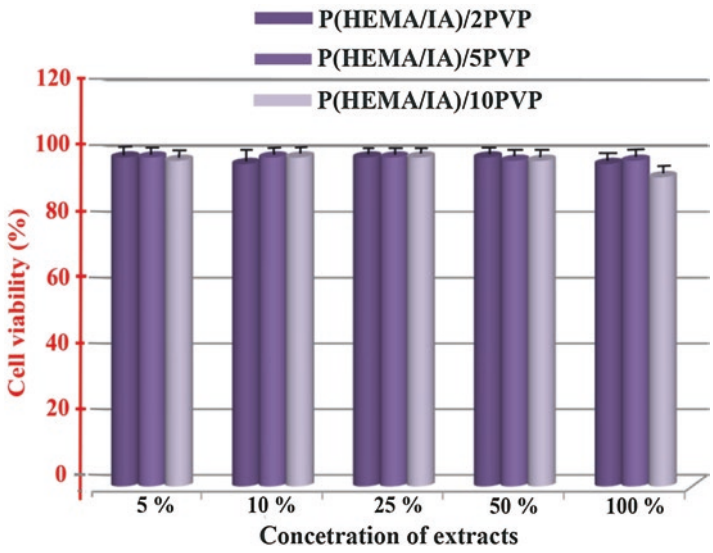


Fig. 10.1 Cell viability of P(HEMA/IA)/PVP hydrogels

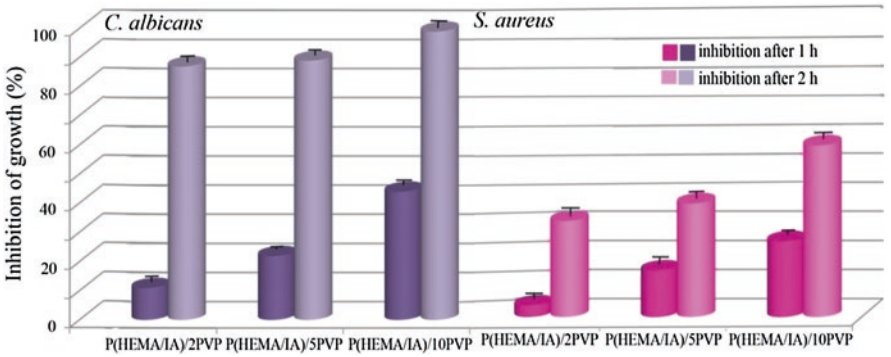


Fig. 10.2 Antimicrobial activity of P(HEMA/IA)/PVP hydrogels against *C. albicans* and *S. aureus*

antimicrobial effect depends on the fraction of PVP in the gels, and how the PVP fraction increases, thus increasing the efficiency. The highest sensitivity to the tested gels was obtained for *C. albicans* pathogenic yeast cells where the percentage reduction was achieved by about 85–95%. A slightly lower sensitivity to the antimicrobial activity of PVP hydrogels was shown by the Gram-positive *S. aureus* bacterium, with a percentage of the cell number reduction of about 60 % after the second hour of exposure for the sample with the highest proportion of PVP. Considering the influence of the exposure time of PVP hydrogels to the indicator strains, an increase in the percentage of cell number reduction with the duration of exposure is observed. This trend is present in all samples and refers to microbial cultures used.

10.4 Characterization of Hydrogels Based on 2-Hydroxyethyl Acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied Fraction of Itaconic Acid

10.4.1 Structural Properties of P(HEA/IA)/PVP Gels

The FTIR spectra P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels showed peak characteristic of PHEA, OH peaks at 3450 cm^{-1} (C–O Stretch), a peak characteristic of esters at 1730 cm^{-1} (C=O stretching), and aliphatic peaks in the range of 2900–3000 cm^{-1} (C–H stretching). The increase in the intensity of the peak C=O groups of about 1730 cm^{-1} in the spectrum indicates an increased number of C=O groups of IA for samples P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP. The absorption peaks about 1650 cm^{-1}, characteristic of the stretch vibration C=O, and those at 1290 cm^{-1} and 1020 cm^{-1} characteristic of C-N vibration, show that the PVP polymer is embedded in the HEA/IA network. The spectra of all the samples also show a wider peak of about 3400 cm^{-1} which originates from OH stretching vibration of the carboxyl groups IA overlapping with the O–H stretch vibration of PVP (Arndt et al. 1999).

10.4.2 Morphology of P(HEA /IA)/PVP Gels

Morphology of P(HEA/5IA)/PVP and P(HEA/10IA)/PVP hydrogels shows that hydrogels have a wavy surface, resembling a "coral" texture with microchannels. Hydrogels exhibit a large number of interconnected pores, which meets the requirements to be used in biomedicine.

10.4.3 Mechanical Properties of P(HEA/IA)/PVP Hydrogels

The evaluation of mechanical properties is important for the biomedical application of hydrogels. It is necessary to test the behavior under the influence of the appropriate stress. The shear modulus, G, was measured to examine whether P(HEA/IA)/PVP hydrogels are suitable biomaterials for biomedical applications. The values of the shear modules are in the range from 7.86 to 13.04 kPa. It can be concluded that the modulus of P(HEA/IA)/PVP hydrogels increases with an increase in IA content of 2–5 mol%, but for a sample with 10 mol% IA, there is a reduction in the modulus due to the higher hydrophilicity of that sample, which leads to a higher-degree swelling (Quitana et al. 2002).

10.4.4 pH- and Temperature-Sensitive Swelling of P(HEA/IA)/PVP Hydrogels

"Intelligent" materials exhibit significant changes in the swelling degree, depending on the change in the external stimulus pH, ionic strength, temperature, and others. pH-sensitive hydrogels show a change in the properties due to a change in the pH of the surrounding medium. In order to examine the pH sensitivity of P(HEA/IA)/PVP hydrogels, the samples were characterized by measuring the equilibrium degree of swelling depending on the pH value (2.20–8.00) at a temperature of 37 °C. It may be noted that the lowest q_e values have samples at a low pH of 2.20. The lowest q_e values are below pK_a of the group IA values, when the carboxylic groups of itaconic acid are unionized and form intramolecular hydrogen bonds, resulting in greater network compactness. q_e values increase around pK_a values of IA carboxyl groups (pK_{a1} = 3.85 and pK_{a2} = 5.45). The ionization occurs when the pH of the medium rises above the pK_a value of both carboxyl groups. With an increase in ionization, the amount of permanent charge increases, causing an increased electrostatic repulsion between the ionizing group and therefore the chains. As a result, the network's hydrophilicity, as well as the degree of swelling, is increasing. Hydrogels containing IA have a higher degree of swelling with a rise in pH. With increasing IA content in hydrogels, q_e values increase. The dependence of the pH of the surrounding medium shows a similar trend for all samples. The maximum q_e values for all samples are at pH 6.80. The highest values were obtained for samples with 10 mol% IA as expected. Above this pH, the values of q_e decrease slightly, and above pH 7.40, they increase again.

The temperature sensitivity of P(HEA/IA)/PVP hydrogels in the temperature ranges from 10 to 60 °C, in the buffer pH 7.40, which represents the pH of the physiological fluid, and was also tested. All samples show a similar trend. The swelling degree increases with an increase in the molar ratio of itaconic acid. The highest values of q_e are samples with 10 mol% of itaconic acid. The maximum q_e values for all samples are at 25 °C. All samples show a VPTT temperature of about 47 °C. Temperature-sensitive samples of hydrogels shrink due to the increase in temperature above VPTT and the swelling under VPTT. When the temperature is below the VPTT value, hydrogen bonds between hydrophilic groups and water molecules dominate, so q_e values are higher. When the temperature is above VPTT, hydrogen bonds are broken; hydrophobic interactions in hydrogels become dominant, which leads to the shrinking of hydrogels; and q_e values are reduced.

10.4.5 In Vitro Controlled Release of Vitamin B_3 from P(HEA/IA)/PVP Hydrogels

In this study, the controlled release of vitamin B_3, in the form of nicotinamide from P(HEA/IA)/PVP hydrogels, was studied to evaluate the influence of the structure and properties of the active substances on the release kinetics of the hydrogels, which contain a different fraction of IA. The kinetics of the release of active substance molecules from the hydrogels is shown in Fig. 10.3. It depends on several

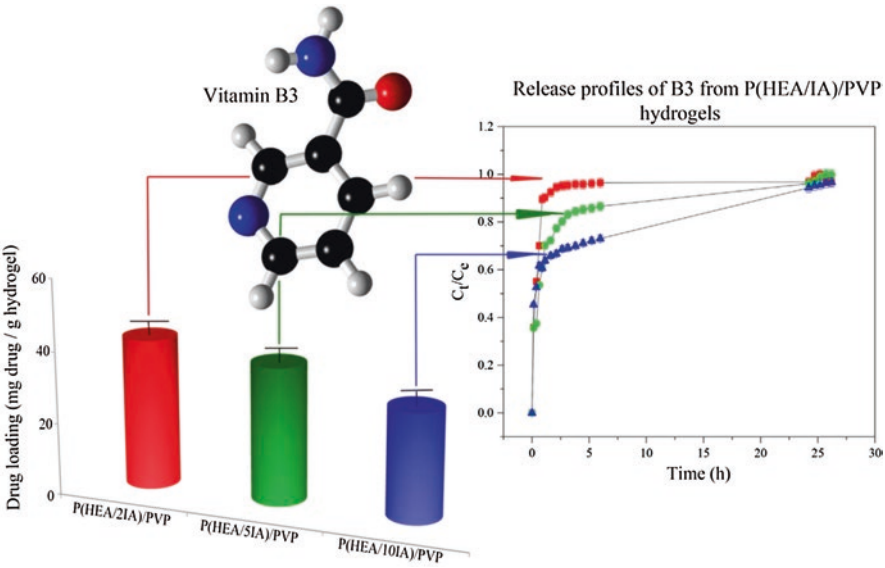


Fig. 10.3 The drug loading of vitamin B_3 into P(HEA/IA)/PVP hydrogels. Vitamin B_3 release profiles from P(HEA/IA)/PVP hydrogels

factors, such as the chemical structure of the polymer, the network structure, the release conditions, and other factors.

The values of drug loading (DL) of P(HEA/IA)/PVP hydrogels are shown in Fig. 10.3. Hydrogels with 2 mol% of IA are able to load highest amount of vitamin B_3 (DL 47.35 mg drug/g hydrogel). Increasing mole content of IA, DL is reduced, so that the smallest value was for sample with 10 mol% of IA (DL 40.00). At the same time, there are two parallel processes: diffusion of the drug into hydrogel and swelling of hydrogel. Degree of swelling increases with increasing of itaconic acid content, i.e., hydrophilicity increases. The highest water uptake is obtained for sample of 10 mol% IA. It is obvious that more water is absorbed within the hydrogel, which affects the reduction of DL.

Controlled drug release process from hydrogels shows initially a quick release of the drug, i.e. the initial burst effect (the so-called burst effect) appears, followed by the second phase-slower drug release. At the beginning of the release process, the release of the drug molecules that are attached to the surface and layers is closer to the surface of the hydrogel disk. The slow release phase takes place by the diffusion mechanism in the pores of the hydrogel. Sample with 2 mol% IA releases the highest amount of vitamin, and sample with 10 mol% at least. It was found that the release rate of the active substance from the samples P(HEA IA)/PVP depends on the content of IA in the gels.

Different release models were applied to the experimentally obtained release data of the active substance from synthesized hydrogels to calculate the characteristic parameters (Barakat et al. 2009; Canan et al. 2011; Peppas and Sahlin 1989; Serra et al. 2006). Akaike Information Criterion (AIC) is calculated for all samples, and it determines which equation best describes the polymer system in the controlled-release process. Based on the AIC criteria, it can be concluded that P(HEA 2IA)/PVP hydrogel best describes the release of the Peppas-Sahlin equation. The drug release from P(HEA/5IA)/PVP and P(HEA/10IA)/PVP hydrogels best describe by Ritger-Peppas equation. The exponent values, n, calculated using the Ritger-Peppas model are less than 0.5, indicating Fick's transport mechanism. On the basis of the obtained values for the coefficient R^2, one can determine which model best describes the mechanism of release of vitamin B_3. It can be noted that the Ritger-Peppas model very well describes the release of vitamin B_3 from hydrogels P(HEA/IA)/PVP.

10.4.6 Biocompatibility of P(HEA/IA)/PVP Hydrogels

Biocompatibility of materials is a significant feature of the material for their further use in medicine and pharmacy. Biocompatibility of P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels was tested using the MTT test, most commonly used as a fast method for determining the cytotoxicity of the test materials against different cells. Different concentrations of extracts (5, 10, 25, 50, and 100%) were used to examine the effect on cellular viability. Based on the results shown in Fig. 10.4, it can be noticed that all the tested hydrogels in all the investigated

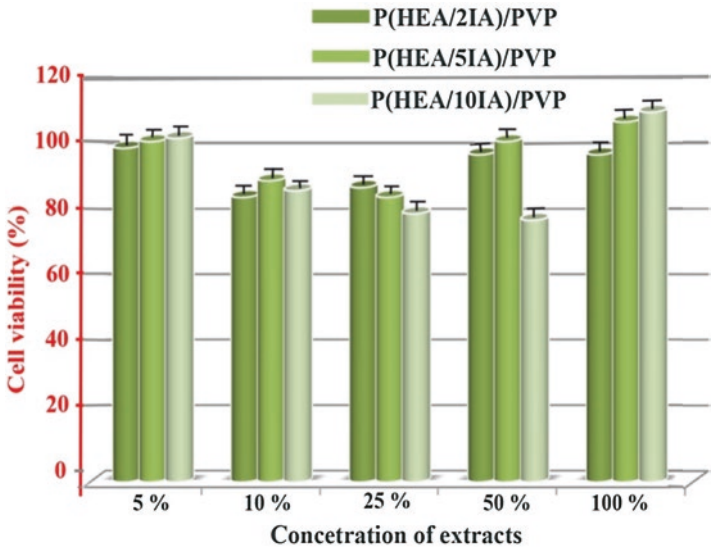


Fig. 10.4 The viability of L929 cells after cultivation with extracts of P(HEA/IA)/PVP hydrogels

concentrations of their extracts exhibited an acceptable level of cytotoxicity to normal mouse fibroblasts, as the percentage of viable cells in no case was less than 70% compared to control-treated cells. Cell viability depends on the molar fraction of itaconic acid. For values of 5 and 100% of the extract, with an increase of molar fraction of itaconic acid, cellular viability increases. So the best values are for the sample of IA 10 mol%. For 10 and 50%, extracts of the highest value are for the sample with 5 mol% of IA.

10.4.7 Antimicrobial Activity of P(HEA/IA)/PVP Hydrogels

The ability to inhibit the growth of microorganisms (antimicrobial activity) is important for the biomedical application of hydrogels. Antimicrobial activity of P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels was examined according to Gram-positive bacterium *Staphylococcus aureus* and yeast *Candida albicans*, which are the most common causes of infection. The study was carried out after one- and two-hour treatment of the cultures of the aforementioned pathogens. The results are shown in Fig. 10.5. It turned out that 1-hour treatment has shown to have a better effect compared to a 2-hour treatment.

The type of pathogen and the proportion of itaconic acid also affect antimicrobial activity. The results of the study show that samples with 2 mol% IA according to the pathogens of *Staphylococcus aureus* and *Candida albicans* have the best antimicrobial activity, while the lowest value for the sample is 5 mol% of IA. The highest

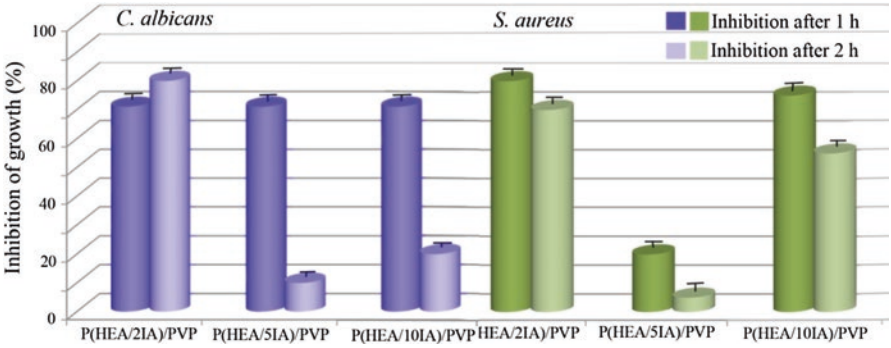


Fig. 10.5 Antimicrobial activity of P(HEA/IA)/PVP hydrogels according to *C. albicans* and *S. aureus*

percentage of inhibition of pathogen growth showed a sample with 2 mol% of IA, according to *Staphylococcus aureus*.

10.5 Characterization of Hydrogels Based on 2-Hydroxyethyl Acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied Poly(vinyl Pyrrolidone) Fraction

10.5.1 pH- and Temperature-Sensitive Swelling of P(HEA/IA)/PVP Hydrogels

In order to test the pH sensitivity of P(HEA/IA)/PVP, P(HEA/IA)/PVP, and P(HEA/IA)/10PVP hydrogels, the dry samples were immersed until the equilibrium state in the buffers of different pH values at a temperature of 45 °C. Based on the results shown, it can be noticed that the samples show the smallest swelling at low pH values (pH 2.20). This was expected because the pH values below the pK_a of IA gels are in the non-ionized state, and the swelling is the smallest. With the rise in pH, the swelling degree is also increasing, which is expected due to the presence of IA. Itaconic acid has two carboxyl groups that ionize above the pK_a value ($pK_{a1} = 3.85$, $pK_{a2} = 5.45$). The degree of ionization of lateral acidic groups affects the swelling of the samples. With the rise in ionization, the amount of constant charge increases, which leads to increased electrostatic repulsion between negatively charged acid groups and chains. As a result, the network's hydrophilicity and also the degree of swelling increase. As the PVP content increases, the swelling degree is lower, as the long PVP chains represent additional cross-linking, prevent the mobility of polymeric chains, and lead to reduced swelling.

Temperature sensitivity of P(HEA/IA)/PVP hydrogels was carried out at temperatures from 10 to 60 °C, in buffer pH 7.40, which simulates the physiological fluid. Dry disk samples were immersed in buffer pH 7.40 at the selected temperatures. After certain time intervals, the samples were removed, filtered by a paper

filter, and weighed. After the measurement, they were returned to the buffer until an equilibrium state was established.

The temperature sensitivity of P(HEA/IA)/PVP hydrogels was performed in the temperature range from 10 to 60 °C, in a pH buffer of 7.40, which is the pH of the physiological fluid. All samples have a VPTT temperature of about 47 °C. Temperaturally sensitive samples shrink at the temperature above the VPTT value and swell below the VPTT. When the temperature is below the VPTT value, hydrogen bonds between hydrophilic groups and water molecules dominate; thus, q_e values are higher. When the temperature is above VPTT, hydrogen bonds are broken; hydrophobic interactions in hydrogels become dominant, leading to hydrogel shrinkage; and q_e values are reduced. Based on the dependence of q_e on the temperature, we see that the value of q_e depends on the fraction of PVP. For a sample with 10 mol% PVP, q_e value is lowest, at all temperatures, except at 10 °C. It can be assumed that PVP acts as an additional cross-linking, and therefore, as its content increases, swelling is reduced. The highest q_e value is at 25 °C and the lowest at 50 °C for all samples.

10.5.2 Mechanical Properties of P(HEA/IA)/PVP Hydrogels

Mechanical properties of hydrogels are one of the most important properties for biomedical applications. It is important that the samples during swelling retain shape and geometry and remain strength enough. G values are in the range of 5.36 KPa to 10.59 KPa. The values of the modulus depend on the degree of cross-linking of the samples; thus, the addition of PVP to hydrogels significantly improves the mechanical properties of the gels. With the increase of PVP content, the value of G increases. For the sample with 2 mol%, PVP is the lowest. The highest G value is for the sample with 10 mol% PVP. This behavior can be explained by the ability of PVP to improve mechanical properties due to the formation of hydrogen bonds. Hydrogen connections act as an additional, physical cross-linking.

10.5.3 Biocompatibility of P(HEA/IA)/PVP Hydrogels

Biocompatibility of P(HEA/IA)/2PVP, P(HEA/IA)/5PVP, and P(HEA/IA)/10PVP hydrogels was tested using the MTT test. Different concentrations of extracts (5, 10, 25, 50, and 100%) were used to examine the effect on cellular viability. The results are shown in Fig. 10.6. It can be seen that cell viability depends on the molar fraction of PVP. Cell viability increases with increase of PVP molar fraction (for 25, 50, and 100% of the extract). The highest cell viability is for the sample with 5% molar fraction of PVP (for 5 and 10% of the extract). For all samples, cell viability is over 60% for all concentrations of the extract. P(HEA/IA)/PVP hydrogels with varied PVP content showed satisfied cytocompatible properties for biomedical applications.

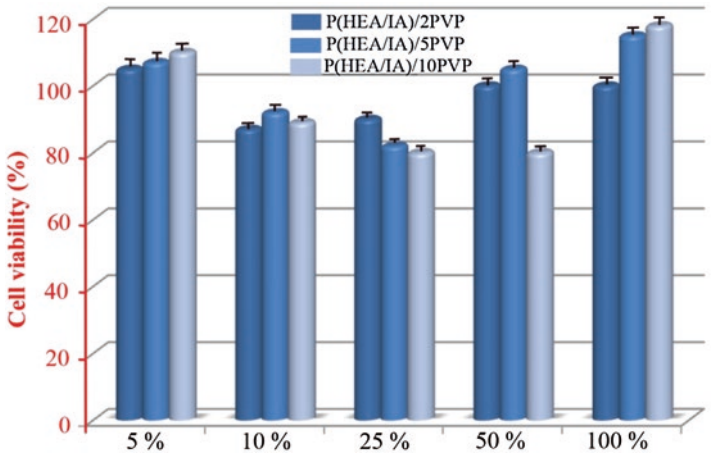


Fig. 10.6 The viability of L929 cells after cultivation with extracts of P(HEA/IA)/PVP hydrogels

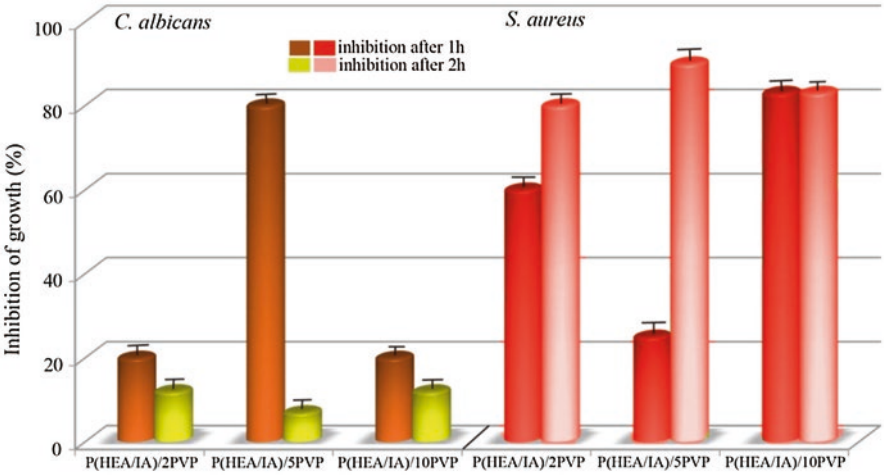


Fig. 10.7 Antimicrobial activity of P(HEA/IA)/PVP hydrogels for *C. albicans* and S. *aureus*

10.5.4 Antimicrobial Activity of P(HEA/IA)/PVP Hydrogels

The ability of the bacterial growth inhibitory of P(HEA/IA)/PVP samples is determined by performance of antimicrobial activity. The study of antimicrobial activity of P(HEA/IA)/2PVP, P(HEA/IA)/5PVP, and P(HEA/IA)/10PVP hydrogels was realized using bacterial strains *Staphylococcus aureus* and yeast *Candida albicans*. The results are shown in Fig. 10.7. The PVP content, type of pathogen, and exposure time have an effect on antimicrobial activity. For *C. albicans* after the first hour

of exposure, there is significantly better antimicrobial activity than after second hour. For the *S. aureus* strains, better results are obtained after the second hour of incubation for samples with 2 and 5 mol% PVP, while for a sample with 10 mol% of PVP, the values are the same after 2 h.

The best antimicrobial activity for *S. aureus* shows samples with 2 and 10 mol% PVP, after 2 h. These samples show the smallest percentage of cellular number reduction for *C. albicans* after 2 h of exposure. A sample of 5 mol% PVP shows the best antimicrobial activity after the first hour for the *C. albicans* strains and after the second for *S. aureus*.

10.6 Conclusions

In our work three series of semi-IPN polymeric networks, based on monomers of 2-hydroxyethyl methacrylate, 2-hydroxyethyl acrylate, and itaconic acid, with poly(N-vinylpyrrolidone) as interpenetrating agent, were synthesized by free radical copolymerization/cross-linking reaction. All samples showed "intelligent" behavior and properties that are extremely favorable for biomedical application-swelling, morphology, mechanical properties, controlled drug release, and cytocompatible and antimicrobial potential.

10.6.1 Hydrogels Based on 2-Hydroxyethyl Methacrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied Poly(vinyl Pyrrolidone) Fraction

In this study, semi-IPN hydrogels based on 2-hydroxyethyl methacrylate, itaconic acid, and poly(vinyl pyrrolidone) with different PVP (2, 5, and 10 mol%) fractions were synthesized by free radical copolymerization/cross-linking in the aqueous environment.

The spectroscopic analysis of P(HEMA/IA)/PVP hydrogels showed the presence of bands indicating that PVP interpenetrate through the P(HEMA/IA) copolymer hydrogels and loaded at different content.

"Intelligent" behavior of P(HEMA/IA)/PVP hydrogels has been examined for potential application in medicine and pharmacy. Swelling of P(HEMA/IA)/PVP hydrogels depends on the PVP fraction and temperature. The equilibrium degrees of hydrogels in buffer pH 7.40 and at temperatures 25, 37, 45, and 55 °C are in the range from 2.56 to 3.78. The lowest equilibrium degree of swelling is shown for P(HEMA/IA)/10PVP at 55 °C, and the highest for P(HEMA/IA)/10PVP at 25 °C. When we consider the effect of temperature on the equilibrium degree of swelling, it can be noted that q_e decreases as the temperature rises in the range of 25–55 °C for all hydrogel samples, whereby the decrease is most pronounced for the sample with the highest PVP fraction. The sensitivity of these samples to pH values from 2.20 to 8.00 at 37 °C was also tested.

Mechanical properties of P(HEMA/IA)/PVP hydrogels, presented by the shear modulus, show the dependence on the composition of hydrogels. The largest value of modulus has a gel with the highest content of PVP, and the smallest with the smallest content of PVP. The value of the modulus ranges from 290 to 440 kPa.

Based on the results obtained by examining the cytotoxicity, it can be seen that P(HEMA/IA)/PVP hydrogels do not show significant changes in cell viability with the change in PVP content and are extremely cytocompatible and are therefore suitable for use as polymeric biomaterials for biomedical applications.

The antimicrobial activity of these samples depends on the proportion of PVP in the gels and how the PVP fraction increases, thus increasing the efficiency. The best antimicrobial properties were shown hydrogels for *C. albicans*, so almost 100% bacterial growth inhibit was almost complete. The lower gel activity is according to the *S. aureus* strain. The largest inhibition for the sample with a maximum of PVP content is 60%. In the investigated period of antimicrobial activity, inhibition of bacterial growth increases with time, for all samples and bacterial strains.

Analyzing the behavior of synthesized P(HEMA/IA)/PVP hydrogels, it can be concluded that all have shown satisfactory properties in in vitro conditions, which candidate them as optimal polymeric biomaterials for use in medicine and pharmacy, especially in topical and transdermal systems.

10.6.2 Hydrogels Based on 2-Hydroxyethyl Acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied of Itaconic Acid Fraction

The synthesis of 2-hydroxyethyl acrylate, itaconic acid, and poly(vinyl pyrrolidone) was performed by free radical copolymerization/cross-linking. The PVP fraction was constant, while the IA (mol%, 2, 5, and 10) and HEA fraction were varied. FTIR spectra P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels were recorded. The increase in the intensity of the C=O peaks of about 1730 cm^{-1} in the spectrum indicates an increase in the number of C=O groups of IA for the samples (IA content varied). Morphology of P(HEA/5IA)/PVP and P(HEA/10IA)/PVP hydrogels was recorded. Hydrogels have a wavy surface, resembling a "coral" texture with a large number of interconnected pores, which meets the requirements to be used in biomedicine.

The shear modulus, G, was measured to examine whether P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels are suitable for biomedical applications. The shear modulus depends on the itaconic acid content. The values of the shear modules are in the range from 7.86 to 13.04 kPa.

In order to examine the pH sensitivity of P(HEA/IA)/PVP hydrogels, the samples were characterized by measuring the equilibrium rate of swelling depending on the pH value (2.20 to 8.00) at a temperature of 37 °C. The lowest q_e values have samples at a low pH of 2.20. The maximum q_e values for all samples are at pH 6.80. The highest values were obtained for the sample with 10 mol% of IA.

The temperature sensitivity of P(HEA/IA)/PVP hydrogels in the temperature range from 10 to 60 °C, in the buffer pH 7.40, was also tested. The swelling degree increases with an increase in the molar ratio of itaconic acid. The highest value of q_e has a sample of 10 mol% of itaconic acid. The maximum q_e values for all samples are at 25 °C. All samples show a VPTT temperature of about 47 °C.

The in vitro controlled release of the active substance, vitamin B_3 from P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels, was performed. The highest amount of vitamin B_3 was released from sample of 2 mol% IA, and the smallest from sample of 10 mol%. It was found that the release rate of the active substance from the samples P(HEA/IA)/PVP depends on the content of IA in hydrogels. Different release models were applied to the experimentally obtained release data of the active substance from synthesized hydrogels to calculate the characteristic parameters. Based on AIC, the P(HEA/2IA)/PVP hydrogel best describes the release of the Peppas-Sahlin equation, while for P(HEA/5IA)/PVP and P(HEA/10IA)/PVP hydrogel the Ritger-Peppas equation. The exponent values, n, are calculated using the Ritger-Peppas model and are less than 0.5 indicating Fick's transport mechanism. Based on the obtained values for coefficient R^2, the Ritger-Peppas model describes a very good release of vitamin B_3 from hydrogels P(HEA/IA)/PVP.

P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels showed satisfied cytocompatible properties. Cytotoxicity depends on the molar fraction of itaconic acid. The antimicrobial activity of P(HEA/2IA)/PVP, P(HEA/5IA)/PVP, and P(HEA/10IA)/PVP hydrogels was tested according to *Staphylococcus aureus* and *Candida albicans*. The type of pathogen, the exposure time, and the content of itaconic acid affect antimicrobial activity.

Based on the results of this study, it can be concluded that all samples have shown satisfactory properties. It can be used as polymeric biomaterials in medicine and pharmacy.

10.6.3 Hydrogels Based on 2-Hydroxyethyl Acrylate, Itaconic Acid, and Poly(vinyl Pyrrolidone) with Varied of Poly(vinyl Pyrrolidone) Fraction

The synthesis of 2-hydroxyethyl acrylate, itaconic acid, and poly(vinyl pyrrolidone) was performed by free radical copolymerization/cross-linking. The IA fraction was constant, while the PVP (mol%, 2, 5, and 10) and HEA fraction were varied. pH and temperature sensitivity were tested. In order to test the pH sensitivity of P(HEA/IA)/2PVP, P(HEA/IA)/5PVP, and P(HEA/IA)/10PVP hydrogels, the dry samples were immersed until the equilibrium state in the buffers of various pH values, at a temperature of 37 °C. With an increase in pH, the swelling degree is increased due to the presence of IA. The temperature sensitivity of P(HEA/IA)/PVP hydrogels was tested in the temperature range from 10 to 60 °C, in a buffer pH of 7.40. All samples have a VPTT temperature of about 47 °C. Based on the dependence of q_e on the temperature, it showed that the q_e value depends on the PVP content.

The lowest q_e value is for sample with 10% PVP, at all temperatures, except at 10 ° C. The highest q_e values are at 25 ° C, and the lowest q_e values are at 50 °C, for all samples.

Mechanical properties of the samples were examined as shear modulus dependence on frequency. *G* values are in the range of 5.36 KPa to 10.59 Kpa and depends on the PVP fraction.

P(HEA/IA)/2PVP, P(HEA/IA)/5PVP, and P(HEA/IA)/10PVP hydrogels showed satisfied cytocompatible properties. Cytotoxicity depends on the molar fraction of PVP. Antimicrobial activity of P(HEA/IA)/2PVP, P(HEA/IA)/5PVP, and P(HEA/IA)/10PVP hydrogels was studied for *Staphylococcus aureus* and *Candida albicans*. The PVP content, type of pathogen, and exposure time have an effect on antimicrobial activity. For pathogen *C. albicans* after the first hour of exposure, there is significantly better antimicrobial activity than after a second hour. For the *S. aureus* strains, better results are obtained after the second hour of incubation for samples with 2 and 5 mol% PVP, while for a sample with 10 mol% of PVP, the values after 2 h are the same. The best antimicrobial activity after 2 h shows samples with 2 and 10 mol% PVP for *S. aureus*. These samples show the smallest percentage of cell reduction for *C. albicans* for both hours of exposure. Based on the results of this study, it can be concluded that these samples have satisfactory properties; they could have potential application in medicine and pharmacy.

Three groups of semi-interpenetrating hydrogel networks were synthesized based on 2-hydroxyethyl methacrylate or 2-hydroxyethyl acrylate, itaconic acid, and poly(vinyl pyrrolidone) by free radical copolymerization/cross-linking. The fraction of each component were varied to examine the influence on the properties of the P(HE(M)A/IA)/PVP hydrogels. All samples exhibit satisfactory properties, which candidate them as optimal polymer biomaterials for use in medicine and pharmacy.

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significance events printed in its integrity, 47 papers presented at international significance conferences printed in the form of abstracts, 4 papers presented at national importance assemblies printed in its integrity and 26 papers presented at national importance events. Dr. Tomić has published a prominent monograph of national importance. So far, she has been mentor of 4 doctoral theses, 40 diploma works, and 5 master theses. Dr. Tomić's papers were quoted 1175 times. She was the leader of an international project. Participated or participates in 3 international and 8 national projects. Dr. Tomić was invited reviewer in 33 international journals.

Biomedical Applications of Interpenetrating Polymer Network Gels

11

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Abstract

Polymers are one of the most researched materials in different fields of study like engineering, chemistry, biomedicine, etc. Interpenetrating polymer network-based gels are highly versatile as they possess the properties of two or even more type of polymers from which they are formulated. The 3D structure of IPN hydrogels possesses the ability of holding large quantities of water. These network gels show higher strength and are safe and biocompatible which make them useful for a range of biomedical applications. The combination of natural and synthetic polymers in IPN gels further adds to their advantage. The IPN gels are now also prepared from "smart polymers" which can be easily modified in terms of shape and volume and are sensitive to selected stimuli like temperature, pH, pressure, etc. All these make the development and evaluation of IPN gels a key thrust area for research. The present chapter highlights the various biomedical applications of IPN gels.

Keywords

Hydrogels · Interpenetrating polymeric network · Drug delivery · Scaffold

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Abbreviations

AA	Acrylic acid
AN	Acrylonitrile
APS	Ammonium persulfate
APTMACI	3-(Acrylamidopropyl)-trimethylammonium chloride
BH	Buflomedil hydrochloride
CMC	Carboxymethylcellulose
CMLBG	Carboxymethylated locust bean gum
HEMA	2-Hydroxyethylmethacrylate
LBG	Locust bean gum
Ni	Nickel
NIPAM	N-Isopropylacrylamide
NP	Nanoparticle
Pd	Palladium
PDMS	Poly(dimethyl siloxane)
PEGDA	Polyethylene glycol diacrylate
PEGMA	Polyethylene glycol methacrylate
PHA	Poly(2-acrylamido 2-methylpropanesulfonic acid-co-N-hydroxymethyl acrylamide)
PNIPAM	Poly(N-isopropylacrylamide)
PVA	Polyvinyl alcohol
PVC	Polyvinyl alcohol
SBC	Sugarcane bagasse cellulose
TEMED	N,N,N′,N′-Tetramethylethylenediamine
TEMED	Tetramethylethylenediamine
TSP	Tamarind seed polysaccharide
VP	4-Vinylpyridine
ZnO	Zinc oxide

11.1 Overview

Polymers are flexible macromolecules having the ability to reorganize themselves even after huge deformations. They have always remained as essential tools in designing of dosage forms for delivery of drugs into the biological system. Apart from being used as simple formulation additives in conventional dosage forms, they have also shown potential in designing and manipulating the drug release pattern of novel drug delivery systems. Different polymers like cyclodextrin, PEG, HPMA, NIPAAM, etc. are widely used either alone or in combination in the design and development of novel drug delivery systems. With the developments and advancements in polymer sciences, the interpenetrating polymeric networks (IPNs) came into the existence toward the later period of the twentieth century. Since then, the gels based on IPN systems have gained significant attention of biomedical scientists (Roland 2013).

The IPNs are complex matrix-like systems involving two different but mutually compatible/miscible polymers which are cross-linked to modify their properties. In many instances, the properties of IPNs are unique relative to the parent polymers. Natural polymers can be combined with synthetic polymers to yield IPN with unique and desirable properties for selected biomedical application(s). The main characteristics which make IPNs suitable for biomedical applications are biocompatibility, safety, and tendency to form stable gels by swelling (Somya et al. 2015).

11.2 Polymers Used in Forming IPN Gels

A range of hydrophilic polymers or their precursors are used to synthesize hydrogels. The main classes are of natural polymers and their derivatives and synthetic polymers containing hydrophilic functional groups such as –COOH, –OH, –$CONH_2$, SO_3H, amines and R_4N^+, and ether.

11.2.1 Chitosan

This cationic, linear polysaccharide is among the most widely used polymers in design of delivery systems. It is having characteristics like biocompatibility and biodegradability. Cross-linking of chitosan polymers is obtained either chemically using cross-linkers and photopolymerization or by physical cross-linking using ionic or electrolyte-based polymerization. The IPNs can be formed between chitosan matrices or between chitosan and synthetic polymers. Due to its cationic nature, chitosan is attracted to the negatively charged biological membranes providing selective drug delivery (Ahmadi et al. 2015). Many successful chitosan-based hydrogels and cryogels have been reported to successfully deliver drugs/proteins.

11.2.2 Alginate

Sodium alginate is another sugar-based natural copolymer which easily forms cross-links with guluronic residues of other polymers and forms hydrogels. It can be combined with various temperatures, pH, and other stimuli-responsive synthetic polymers to yield a variety of biomedically useful IPN hydrogels. A combination of sodium alginate with different synthetic polymers has led to the development of IPN hydrogels with characteristics like sustained/controlled drug release, high porosity, and multiresponsiveness.

11.2.3 Starch

Structurally modified starch derivatives are used for developing IP hydrogels having biomedical relevance. Semi-IPN hydrogels as well as semi-IPN cryogels have been developed using potato starch cross-linked with synthetic polymers.

Apart from the abovementioned polymers, a number of other polysaccharides and proteins are utilized for preparing IPN hydrogels with varying and desirable properties. This includes cellulose, gelatin, silk fibroin, hyaluronic acid, guar gum, etc.

11.2.4 Synthetic Polymers

The synthetic polymers have an edge over the natural ones as they can be easily modified and even synthesized according to functional requirements. Such IPN gels are more responsive against stimuli as compared to natural polymer-based IPN gels. Polymers like poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), poly(acrylic acid), poly(acrylamide), and others have been extensively used for developing IPN hydrogels.

11.3 Physicochemical Characteristics of IPN Gels

The primary objective behind the designing of IPNs is to improve the physical/mechanical and chemical properties of polymers and make them more suitable for biomedical and other applications.

11.3.1 Mechanical Properties

In relation to individual polymers, the IPNs offer improved mechanical strength which is one of the prerequisite in designing of drug delivery systems to withhold the pH and other conditions within a biological system and protect the carried drug till it is released. The mechanical strength of IPNs can also be varied either by changing the degree of cross-linking between the two polymers or by introducing copolymerization. However, these changes should be made keeping in the mind not to make the resultant structure brittle or with reduced flexibility (Lohani et al. 2014). The concept of "double networks" has also shown marked improvement in fracture strength of polymers (Gong et al. 2003).

11.3.2 Morphology

Two notable changes while designing IPNs that take place are phase separation and gelation. The sequence of these two events affects the morphology of final product. With phase separation prior to gelation, the size of formed domains will remain relatively large and vice versa. Insufficient cross-linking leads to presence of graft copolymer, while stirring during polymerization will lead to phase inversion. An increase in cross-links also reduces domain size. The IPN particles are often spherical or cylindrical in shape.

11.3.3 Network Structures

The polymers form networks in two ways, viz., dual-phase continuity network or co-continuous networks. In the former case, both the polymeric phases can be seen through the matrix of the final product. However, in case of co-continuous networks, the network of one polymer traverses over the second polymer and is attached to it mechanically.

Phase inversion or change in the continuity of the two polymeric phases also takes place in IPNs and is an important characteristic of thermoplastic IPNS. The homogeneity of two IPN phases depends largely on the method of preparation.

11.3.4 Full/Complete IPNs

Here both the polymeric phases are present as separate cross-linked networks. Sometimes the network of one polymer is spread over the network of other polymer as a film (gradient IPN). There is hardly any bonding between the two polymers. Such polymeric networks show better properties than their specific polymers due to the enhancement of the mechanical properties with a controlled morphology.

11.3.5 Semi-IPNs

Here only one polymeric phase remains as chain, whereas the other one is either branched or linear. The linear component of such IPN structure imparts unique properties to it.

11.4 Forms of Interpenetrating Polymer Network Gels.

11.4.1 Hydrogels

IPN-based hydrogels have attracted attention of researchers dealing in drug delivery for their biomedical applications. Hydrogels are three-dimensional networks which are hydrophilic in nature and have the capability to hold substantial amounts of water or other biological fluids in them. The hydrogels based on linear and mono polymers show fragile mechanical properties. Their swelling is mostly delayed and not sufficient as per the biomedical requirements. The IPN-based hydrogels thus gained importance in recent times which offer better mechanical strength and swelling properties. The IPN hydrogels have shown potential as successfully drug delivery vehicles especially for controlled release systems. By modulating the extent of cross-linking in IPN hydrogels, their drug release pattern can be easily modified as per requirements. Researchers have preferred hydrophilic biopolymers while designing IPN hydrogels because of their higher biocompatibility, safety, and regulatory acceptance. These polymers show high degree of binding with other

polymeric molecules by cross-linking and, thus, forming IPN systems. These hydrogels offer a range of applications from drug delivery to tissue engineering. The hydrogels can be administered easily via oral route and also by parenteral route.

Recently, the term "smart polymers" was coined which are very sensitive to conditions like temperature and pH and easily modify their shape and/or volumes. These smart hydrogels undergo size/volume transformations in response to changes in biological system. Temperature-sensitive hydrogels are among the most researched ones. The swelling and de-swelling properties are the unique feature of IPN hydrogels which make them better suited for stimuli-responsive drug delivery. The drug delivery by hydrogels can also be modulated using external forces like ultrasound waves. The drugs can thus be delivered in a controlled manner without affecting neighboring tissues. The ultrasound waves initiate the drug release by their thermal and mechanical action (Parodi and Khaled 2015).

11.4.2 Cryogels

These are unique, highly porous hydrogels which are prepared below freezing point of water. The pores of cryogels vary in size from 1 to 100 μm and are connected with each other. Cryogels show high mechanical strength, rapid swelling, and stability in biological system(s). Studies have also shown high flexibility of cryogels. All these features make them ideal for successful drug delivery. The semi-IPN cryogels have shown faster swelling than IPN cryogels.

11.4.3 Nanogels

Nanosized polymeric complexes have shown tremendous potential in delivering drugs to respective target(s). Due to the size-wise similarity with biological proteins, the nano carriers offer descent penetrability. The nanosized hydrogels or the nanogels thus offer a range of advantages over their macro counterparts. The polymeric nano systems have shown higher sensitivity against various stimuli like temperature, pressure, and pH. The concept of multi-polymeric nanogels has countered many problems associated with delivery systems like drug loading, entrapment efficiency, sustained-release pattern, and stability in biological system. Moreover, incorporating specific molecules or ligands, the drug can be delivered to specific cell types actively (Sahiner et al. 2006).

For design and preparation of nanogels, the miniemulsion (ME)/inverse miniemulsion (IME) process seems to be the most efficient and reliable technique. Sufficient polymerization of monomers can be done by this method to obtain network-like structure(s).

11.5 Biomedical Applications of IP Gels

11.5.1 Drug Delivery

11.5.1.1 Hydrogels

Hydrogels are three-dimensional network of chemically or physically cross-linked polymers which can absorb a large amount of water or biological fluid and get swell. The hydrogel is made of hydrophilic polymers which retain their 3D structure in aqueous medium (Faraji et al. 2011, Chen et al. 2013). It represents a potential drug carrier system for the effective delivery of drug and biomaterial for tissue engineering, wound healing, etc. (Shen et al. 2016). The major constraint of hydrogel (either of natural or synthetic origin) is the poor mechanical strength owing to the good swelling behavior (Li et al. 2011; Lalani and Liu 2012). To improve the stability and efficiency of hydrogels, various unique approaches have been adapted nowadays. In comparison to the single cross-linked network, the IPN increases the stability and mechanical properties of hydrogel due to chain entanglement (Matricardi et al. 2013). One of such strategy is the utilization of interpenetrating polymeric network for hydrogel preparation. The works based on the application of IPN for hydrogel synthesis are discussed below:

Pan et al. (2018) developed a strategy for the IPN synthesis to produce dual-responsive, SBC-, PNIPAM-, and CMC-based hydrogel as an effective drug carrier system. The sugarcane bagasse is the leftover fibrous residue of the sugarcane stalks, produced by sugar industries after extraction of the juice (Pandey et al. 2000). Around 540 million of bagasse is annually processed by sugar and alcohol industries as by-product throughout the world (Cardona et al. 2010). This bagasse mainly consists of crystalline cellulose (40–50%); amorphous hemicellulose including galactose, mannose, arabinose, and xylose (30–35%); and a small proportion of lignin (around 20%), minerals, wax, and ash (Wang et al. 2017). The bagasse cellulose offers a smart material for the preparation of intelligent hydrogels which respond to the physiological stimulus like pH, temperature, ionic strength, light, electric field, etc. (Kabir et al. 2018; Haq et al. 2017). In his work, Pan et al. initially extracted the SBC from pretreated bagasse pulp, purified it, and further dissolved the SBC in sodium hydroxide-urea solution. The SBC solution was mixed with CMC in the presence of epichlorohydrin (cross-linking agent) under high agitation to produce a primary pH-responsive hydrogel. Further, the primary hydrogel was immersed into the PNIPAM solution for in situ polymerization in the presence of MBA as a cross-linking monomer and ammonium persulfate as an initiator, to produce dual-sensitive hydrogel (Fig. 11.1).

The SBC imparts the primary support and improves the durability as well as the mechanical strength of the hydrogel. Also, the CMC is responsible for pH responsiveness and increases the elasticity and water absorption capacity of the hydrogel, while the PNIPAM is attributed to the thermoresponsive behavior of the IPN hydrogel. Upon the stimulus of pH and temperature, the IPN hydrogel shows rapid drug releases. Overall, the study represents a unique approach which has a great scope in the future to be developed as a potential carrier system (Pan et al. 2018).

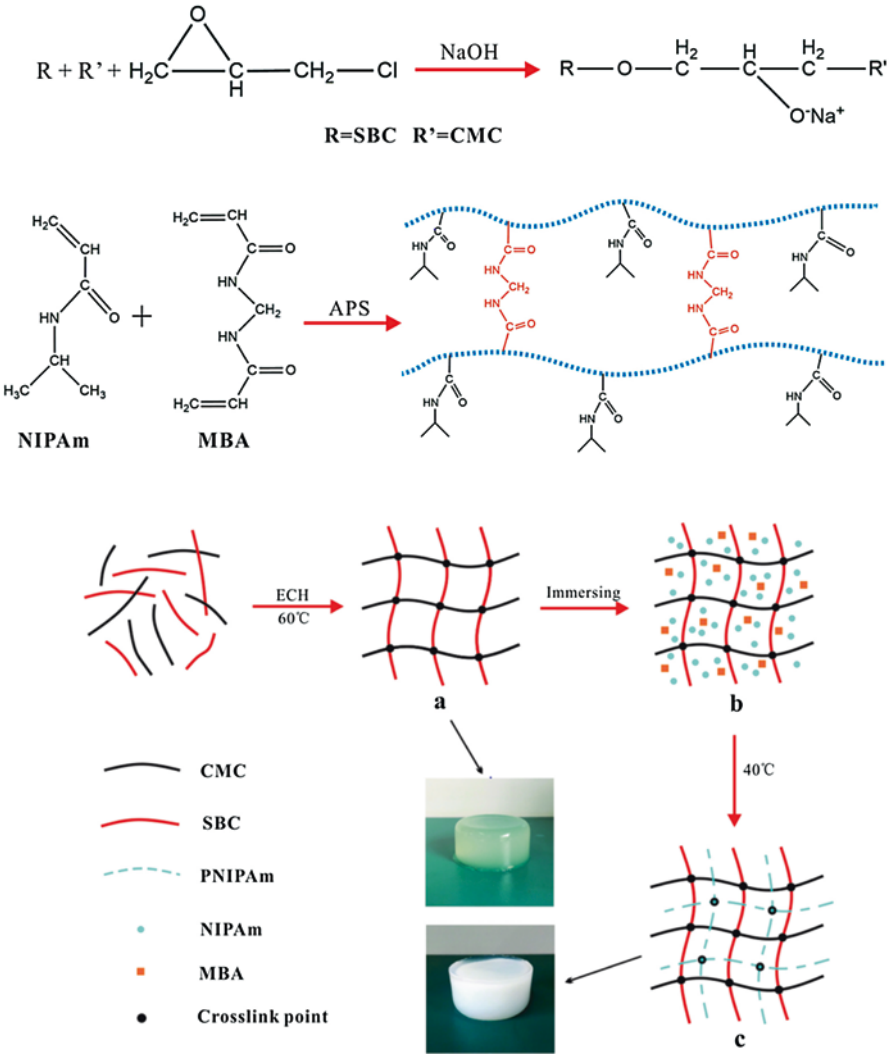

Fig. 11.1 Schematic representation of dual-responsive SBC-CMC-PNIPAM IPN-hydrogel synthesis. (Adapted from Pan et al. 2018)

Similarly, Mamaghani et al. (2018) have developed gelatin methacrylate, PEGDA, and graphene oxide nanoparticle containing IPN hydrogel to improve the mechanical strength and swelling behavior of the hydrogel. Various studies reported the poor mechanical strength of gelatin methacrylate hydrogels which limit sit application in hard tissues. Therefore, the author first time combines the nanoparticles with IPN to enhance the mechanical strength and swelling ability of gelatin methacrylate hydrogel and make it suitable for application in hard tissue like bone regeneration (Kadri et al. 2016). The IPN cross-linking was done by mixing gelatin

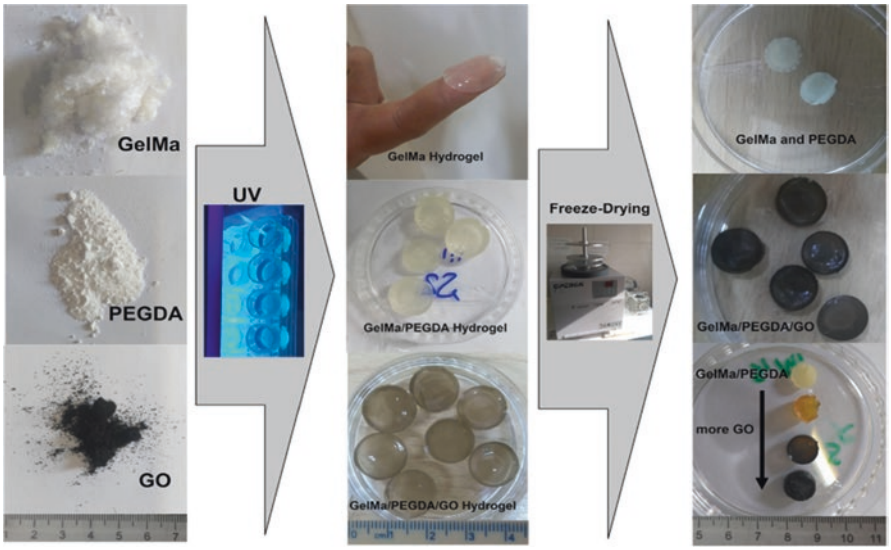

Fig. 11.2 Schematic representation of the formation of PEGDA- and gelatin methacrylate-based hydrogel. (Adapted from Mamaghani et al. 2018)

methacrylate with PEGDA which was further mixed with graphene nanoparticle with constant agitation to prepare nanoparticle containing IPN hydrogel. Ciba was added to this mixture as photo-initiator. Thereafter, the uniformly distributed mixture of polymers was kept at UV radiations (360 nm) which result in the formation of cylindrical hydrogel (Fig. 11.2). The result demonstrated that an increasing amount of PEGDA and graphene oxide enhances the mechanical properties of the IPN hydrogel while reduces the swelling behavior. The varying ratio of gelatin methacrylate, PEGDA, and graphene oxide was used to get a formulation with optimum swelling and mechanical property (Mamaghani et al. 2018).

Previously, Hu et al. (2017) also imply a semi-IPN technique for the fabrication of salecan and PAH hydrogel to improve its cell adhesion behavior. The amalgamation of salecan with PHA produces a more porous structure which increases the swelling ability of the hydrogel. In this study, the salecan and PHA-based semi-IPN hydrogel were prepared by using free radical polymerization. The N,N′-methylene diacrylamide was used as a cross-linking agent, and ammonium persulfate and TEMED were added as redox-initiating agents (Hu et al. 2017) (Fig. 11.3).

Another similar approach was carried out by Wang et al. (2011). They prepared PNIPAM-alginate-laponite-based semi-IPN nanocomposite hydrogel to facilitate the cellular sheet detachment in tissue engineering. The IPN network improves the mechanical property of the nanocomposite, and PNIPAM is attributed to the thermoresponsiveness of the system as well as serves as a monomer for the synthesis of IPN hydrogel. It offers rapid detachment of the cell sheet without affecting the cell viability and thus widely applied in tissue engineering.

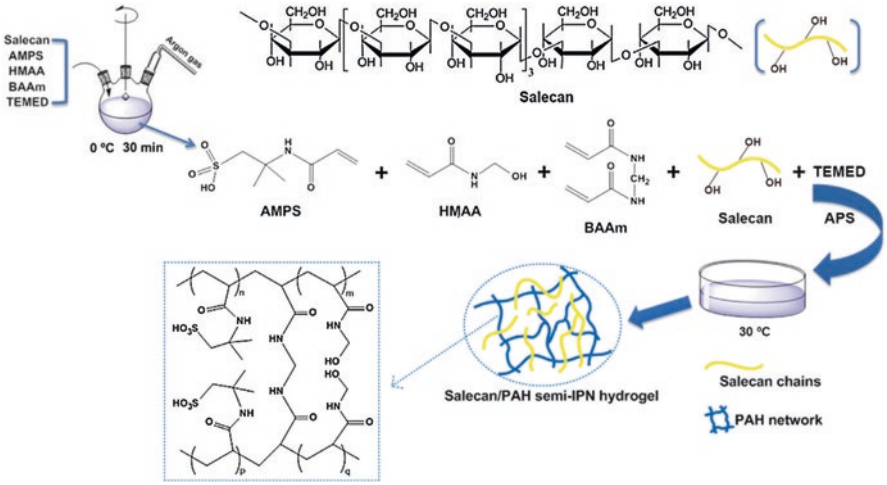

Fig. 11.3 Synthesis of salecan and PAH-based semi-IPN hydrogel

11.5.1.2 IPN Films

Interpenetrating network film is a novel strategy used in the various biomedical field. Mehta and Kaith (2018) developed gelatin- and agar-based semi-IPN film as a carrier for intestine- and colon-specific delivery of amoxicillin and carbamazepine. The gelatin and agar attributed to the biodegradability and biocompatibility of the carrier system. It also imparts good viscoelastic and thermoelastic property and produces nontoxic by-product upon metabolism (Ekici and Saraydin 2007). The interpenetrated cross-linked polymeric network of the IPN film effectively regulates the drug diffusion via ionic interaction between the ionic groups of the polymer and charged surface of the drug (Alvarez-Lorenzo et al. 2005).

Mehta and team utilize the γ-radiation as an initiator for the synthesis of the semi-IPN film via graft copolymerization. Initially, the agar and gelatin were mixed thoroughly, and then acrylic acid (monomer) was added dropwise to the previous mixture in the presence of MBA (cross-linking agent). The γ-rays initiate the reaction and result in the formation of semi-IPN hydrogel film. The detailed scheme of IPN film synthesis is demonstrated in Fig. 11.4. The prepared system is pH-responsive, i.e., releases the drug through pH-responsive swelling-controlled diffusion in the intestine and colon region (Fig. 11.5). The study shows that the IPN increases the mechanical strength of the carrier system which improves the drug loading and also provides delayed release of the drug specifically in intestine and colon (Mehta and Kaith 2018).

On the other hand, Wang et al. (2013) used the semi-IPN hydrogel film for the surface modification of palladium-free metalized PVC plate. The metalized PVC plate was developed by electroless nickel plating technique and modified with chitosan-based semi-interpenetrating network film. This is a novel strategy to fabricate metalized plastic which is nowadays very popular as food packaging material, an electromagnetic device, electromagnetic shielding material, and other industries

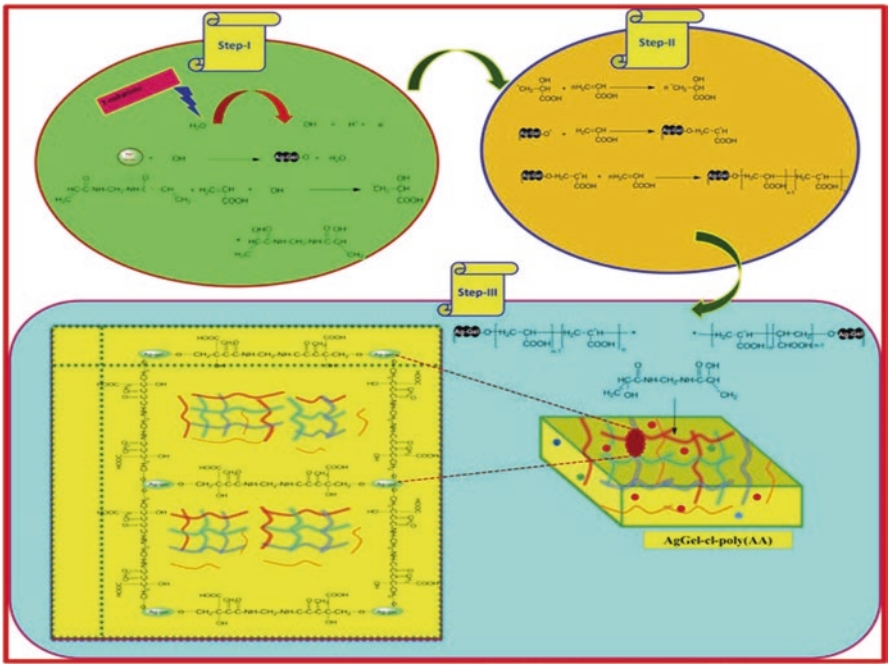

Fig. 11.4 Schematic representation of the synthesis of agar- and gelatin-based semi-IPN hydrogel film. (Adapted from Mehta and Kaith 2018)

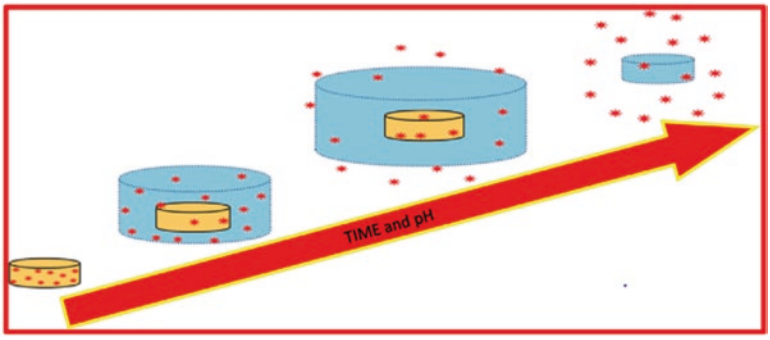

Fig. 11.5 Figure showing drug release from agar- and gelatin-based semi-IPN hydrogel film upon swelling into the biological fluid with increasing pH and temperature. (Mehta and Kaith 2018)

(Ramankiw1997). The semi-IPN film was chemically attached to the surface of PVC through -COO, -CO-NH, and C-O-C bond to immobilize the Ni atom via N-Ni bond. The Ni replaced the Pd and produced Pd-free PVC plate. This approach offers a low-cost technique for the commercial fabrication of the metalized plastics (Wang et al. 2013).

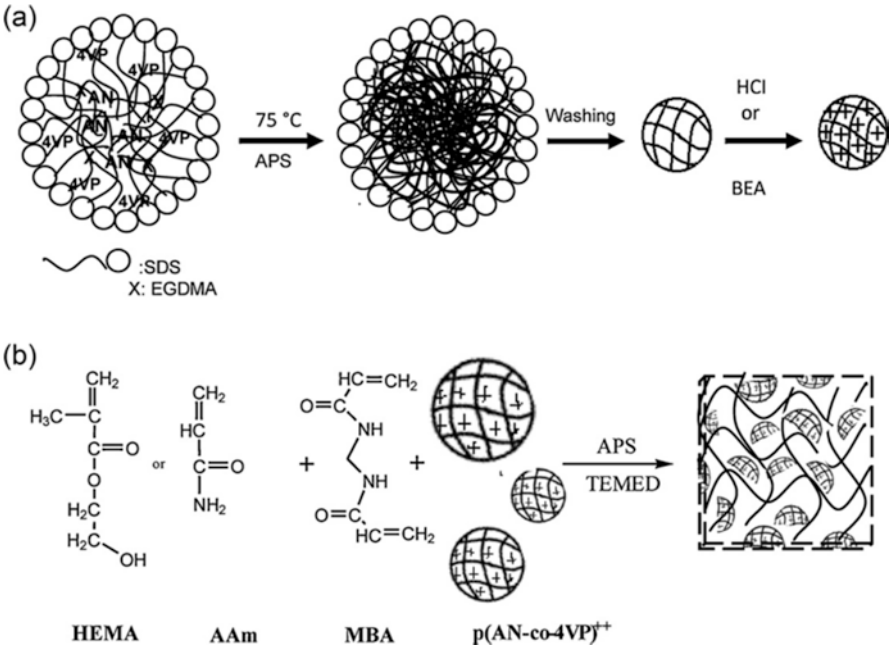

Fig. 11.6 Schematic illustration of (**a**) poly(AN-co-VP) nanoparticle synthesis and (**b**) poly(AN-co-VP) nanoparticle-loaded hydrogel semi-IPN composite synthesis

In recent years, the focus of the biomedical scientists shifted toward the development of inherent antimicrobial material like silver NP, copper NP, Ag-TiO_2 NP, silver nanocomposite, etc. for effective treatment of microbial infections (Sambhy et al. 2006; Cen et al. 2004; Weickmann et al. 2005). Using this strategy, Silan et al. (2012) synthesize various antimicrobial core-shell NP including poly(AN), poly(AN-co-APTMACI), poly(AN-co-NIPAM), and poly(AN-co-VP) and used these NP in development of microgel, nanogel, or hydrogel IPN composite. The nanoparticles were synthesized via microemulsion polymerization method. The nanoparticle-based IPN composites were prepared by employing HEMA and acrylamide as polymeric matrix and APS as process initiator (Fig. 11.6). The prepared nanoparticle-loaded hydrogel IPN composite offers an effective antimicrobial material which provides prolonged action against a variety of bacteria.

11.5.1.3 Microsphere

Microspheres are micron-sized polymeric drug carrier system used for controlled delivery of the drug. It offers various advantages over conventional drug delivery system like improves drug bioavailability, controls the rate of drug release, provides prolonged or sustained drug delivery, reduces the side effect and dosing frequency, and increases patient compliance (Malik et al. 2017). However, the poor drug loading efficiency and low stability limit its application as a drug carrier system. Thus, various strategies like the use of natural polymers and amalgamation with the IPN

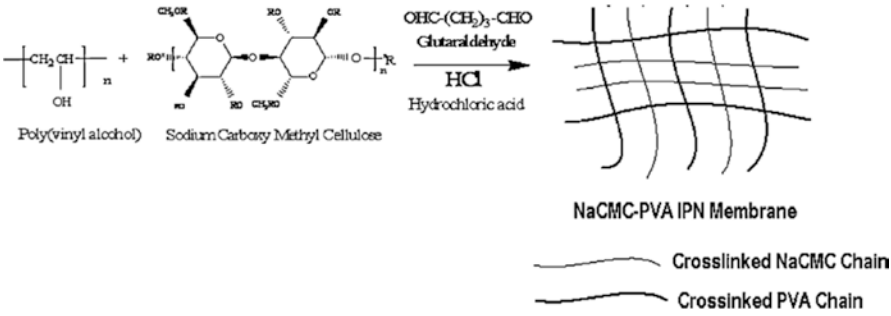

Fig. 11.7 Synthesis of sod. CMC- and PVA-based IPN hydrogel microsphere in presence of glutaraldehyde. (Adapted from Banerjee et al. 2012)

system can enhance its efficiency (Shanthi and Mahato 2010). Various studies performed using the combined IPN microsphere systems are discussed below.

Banerjee et al. (2012) worked on the development of sod. CMC- and PVA-based IPN hydrogel microsphere for controlled delivery of diclofenac sodium. The IPN hydrogel microsphere was prepared by emulsion cross-linking method. The NaCMC and PVA were responsible for the formation of the 3D interpenetrating cross-linked network which improves the mechanical strength of gel, improves the swelling behavior, and controlled the rate of drug release (Fig. 11.7).

Studies showed that the IPN system, based on natural polymers, offers great advantages as drug delivery carriers (Lohani et al. 2014; Kulkarni et al. 2001). Employing such a strategy, Kaity and Ghosh (2015) investigated the potential of LBG as a prime component of IPN-based delivery system. They prepared and optimized BH-loaded IPN microspheres for controlled delivery of the drug. LBG and PVA are the polymers responsible for IPN network formation. The microspheres prepared with LBG demonstrated poor drug loading is owing to poor aqueous solubility of LBG. Thus, carboxymethylated LBG was used to improve the solubility and hence the encapsulation efficiency of the system. The LBG-PVA IPN microspheres and CMLBG-PVA IPN microspheres were prepared by using emulsion cross-linking method in the presence of glutaraldehyde, as a cross-linking agent. The study suggested that the IPN microsphere, prepared from natural polymers, provides a promising drug delivery system which is biodegradable and exerts less or no toxicity to the in vivo models. However, the limitation of this system like poor encapsulation efficiency and phase separation could be resolved by using suitable derivatives of LBG.

Similarly, Jana and team (2015) also worked on LBG-based IPN microspheres for prolonged delivery of aceclofenac. They have prepared alginate-LBG IPN microspheres by calcium ion-induced ionic gelation method. The study illustrated that the prepared alginate-LBG IPN microsphere significantly enhances the drug encapsulation efficiency of the microspheres and prolonged the drug release. Also, the ionotropic gelation method was a simple, convenient, reproducible, and economical method of IPN microsphere preparation (Jana et al. 2015).

Soni and Ghosh (2017) prepared pullulan- and PVA-based IPN microemulsion for controlled delivery of pirfenidone by glutaraldehyde-assisted emulsion cross-linking method. The pullulan-and PVA-based IPN microemulsion represents a unique combination of natural and synthetic polymers which produce a promising drug carrier system with desirable properties. The pullulan is responsible for the biocompatibility, biodegradability, and nontoxic nature of the microsphere (Choi et al. 2017). On the other hand, the PVA imparts thermal and mechanical strength to the system, controls the drug release behavior, and offers ease of manufacturing (Sionkowska 2011; Kaity et al. 2013). The study illustrated that the prepared IPN microsphere reduces the drug dose and dosing frequency and releases the drug in a controlled manner (Soni and Ghosh 2017).

11.5.1.4 Nanoparticles

These are nanosized, polymeric structures which encapsulate or entrap the drug molecules in the polymeric matrix and serve as a potential carrier system. In the past few years, the biomedical application of nanoparticles or nanocomposites in drug delivery, tissue engineering, and biotechnology as diagnostic as well as an analytical tool has been significantly increasing (Craparo et al. 2011), although the application of nanoparticle is limited due to low drug loading, less control over the drug release, poor stability, and toxicity (Brand et al. 2017). IPN system offers a promising approach to improve the drug loading, mechanical strength, and stability of the nanoparticles and nanocomposite materials (Wang et al. 2016).

Utilizing the combined IPN nanocomposite technique, Wang and co-workers (2012) developed IPN-based hydrogel nanocomposite with antibacterial and antifouling behavior. The IPN network is prepared by the entanglement of agarose (a natural polysaccharide) and PEGMA. The agarose makes the system biocompatible and improves the stability of hydrogel (Khaing and Schmidt 2012; Meilander-Lin et al. 2005). Moreover, the IPN architecture reinforced the mechanical strength of the hydrogel (Wang et al. 2012; Silan et al. 2012). The ZnO nanoparticle contributed to the antibacterial property of the formulation (Shafiq et al. 2014), acts as filler to the polymeric network, and increases the mechanical behavior of the system (Wang and Li 2013). The IPN nanocomposite hydrogel was synthesized by a one-pot scheme under UV radiation. The study represents a simple and effective technique of IPN hydrogel nanocomposite preparation. The prepared biomaterial possesses good mechanical strength and antimicrobial property thus found suitable for wound dressing.

On a similar note, Gils et al. (2010) have developed superabsorbent semi-IPN hydrogel nanoreactors utilizing the green synthesis technique. The IPN architecture was synthesized with gum arabic (natural polymer) and poly(HEMA-co-AA) using MBA as a cross-linking agent and APS as an initiator. The glycine-copper sulfate chelate was used as the catalytic agent. Further, the prepared system was loaded with $AgNO_3$ nanoparticle (Fig. 11.8). Gum arabic is nontoxic and a biocompatible natural polymer which imparts antioxidant, nontoxic, and gelling properties of the IPN nanocomposite (Fent et al. 2009). The silver nanoparticle is responsible for the antibacterial property of the system. The results showed that the developed

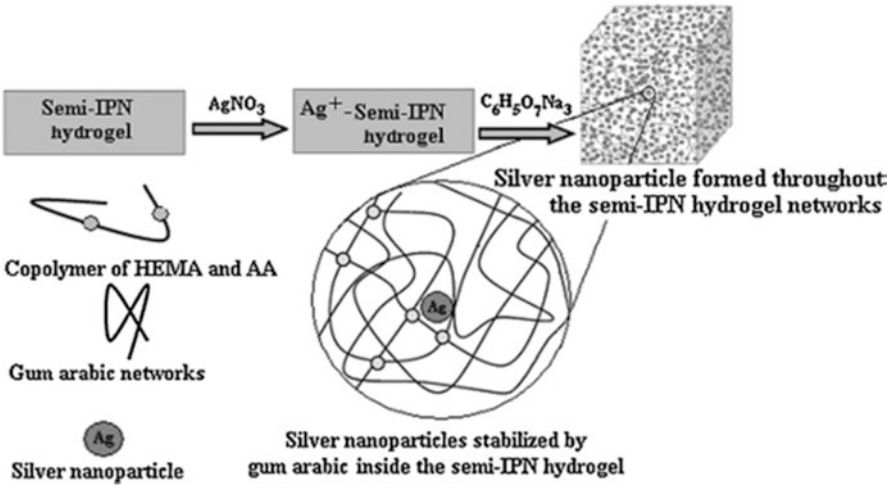

Fig. 11.8 Synthesis of gum arabic-based semi-IPN hydrogel-silver nanocomposite. (Adapted from Gils et al. 2010)

IPN-hydrogel nanocomposite exhibits excellent antibacterial property and great mechanical strength (Gils et al. 2010).

11.5.1.5 Sponge

The biomedical sponges are flexible, and soft scaffolds consist of interconnected porous polymeric materials used as a potential drug carrier system for local and systemic drug delivery (Chen et al. 2016; Parker et al. 2016). Commonly the sponges are prepared by using various biocompatible polymers such as chitosan, collagen, gelatin, and CMC (Kim et al. 2014; Foda et al. 2004). The amalgamation of sponges with IPN increases the stability, drug loading ability, release behavior, swelling, and mechanical properties of the carrier system. Using this approach, Racine et al. (2017) developed chitosan- and PEG-based semi-IPN sponges to control the drug release and enhance the properties of the sponge.

11.5.1.6 Tablet

In recent years, various modifications have been made in conventional oral tablets to control and sustain the rate of drug release for the prolonged duration (Agnihotri and Aminabhavi 2006). In this sequence, the polymeric matrix technology gained much attention to prepare a sustained-release oral tablet (Sarheed et al. 2015; Bajpai et al. 2002). Different synthetic and natural polymers are used to design the matrix tablet. Among these, the IPN system consists of the natural polymeric blend that is very popular in recent years for sustained oral drug delivery (Lohani et al. 2014). By immersing the oral tablets with IPN matrix, Jana et al. (2014) developed an IPN-based oral tablet for prolonged release of aceclofenac. The IPN network was synthesized by tamarind seed polysaccharide and chitosan in the presence of glutaraldehyde. TSP is a natural polysaccharide possessing excellent biocompatibility, stability at

acidic pH, and noncarcinogenic property. It also acts as a viscosity enhancer, mucoadhesive agent, emulsifier, or suspending agent (Kaur et al. 2012; Nayak and Pal 2011; Prajapati et al. 2013). Chitosan is also a natural, biocompatible polysaccharide, most commonly used as the polymer in drug carrier system (Li et al. 2008). The glutaraldehyde acts as a cross-linking agent. The aceclofenac-loaded IPN microparticles were firstly developed which were then compressed as an oral tablet. The study demonstrated that the IPN-based oral tablet significantly prolonged the drug release and reduces the dose as well as dosing frequency, thus minimizing the drug side effect. Similarly, Mandal et al. (2010) also worked on the development of IPN-based matrix tablet for sustained delivery of diltiazem hydrochloride. The IPN network was synthesized by cross-linking of polyacrylamide-grafted sodium alginate and sodium alginate in the presence of calcium ion. Further, the cross-linked polymeric network was compressed as a tablet via wet granulation method. The studies confirmed that the IPN matrix tablet offers a promising system for prolonged delivery of drug.

11.5.1.7 Capsule

Like oral tablets, capsules are also immersed in the novel technology to improve the drug performance, control the drug release behavior, and reduce the dose and thus the adverse effect of the drug. Various novel drug carrier systems like nanoparticles, microparticles, and hydrogels are frequently delivered in the form of oral unit dosage forms like tablet and capsules (Ramaraj and Radhakrishnan 1994). The efficiency of such carrier systems was enhanced by using IPN technique which stabilizes the system, improves the mechanical and physical strength, increased the drug loading, and prolonged the drug release (Petrusic et al. 2012). Ochi et al. (2014) demonstrated the development of thermosensitive IPN-based hydrogel capsules. The IPN system was developed by cross-linking of PNIPAM and calcium alginate. The PNIPAM was responsible for the thermoresponsive behavior of the system (Mohamad et al. 2015). The author developed the stimuli-responsive IPN hydrogel-loaded capsules in a core-shell structure via a concentric double-fluid nozzle, to make the capsule thermoresponsive. In this system, the capsule shell consists of PNIPAM-calcium alginate cross-linked IPN structure, while the core contains the buffer solution. MBA was used as a cross-linking agent and APS act as a reaction initiator. Both the polymeric solution and buffer solution were pumped out simultaneously form the double-layered nozzle. The capsule shell of thermoresponsive IPN hydrogel formed immediately in the presence of reaction initiator and accelerator TEMED with buffer solution encapsulated inside. This study presented a new technology for developing a promising IPN-based thermoresponsive hydrogel capsule.

Similarly, Tang et al. (2011) developed a potential method for preparation of PDMS−/poly(HEMA)-based silicon hydrogel IPN beads loaded capsules. The study illustrated that the sequential method successfully developed the silicone hydrogel IPN microspheres which possess capsule-like structure. The prepared system shows improved drug encapsulation and controlled drug release behavior for the prolonged duration; hence, it represents a promising strategy for the development of novel carrier system.

11.5.2 Nanocomposites

With advancements in research to overcome the limitations of hydrogels, nanocomposites have been developed. These are unique types of 3D structures wherein a polymeric network is coated or attached with metallic/nonmetallic nanoparticles. The overall result is improvement in desirable properties of hydrogels like strength and porosity. Such a combination also enhances the utility of both the components. Depending upon the nature of polymer and nanoparticle used, the length of hydrogel can be increased up to 1000% which is quite remarkable. They can also withstand comparatively greater compression force in relation to hydrogels (Haraguchi 2007).

The nanocomposite gels offer a good medium for growth of cells like fibroblasts and umbilical vein endothelial cells in vitro, and these cells can be easily recovered from the culture medium without enzymatic treatments. Wang et al. (2016) prepared a nanocomposite using PEG-based IPN structure with 4-azidobenzoic agarose. The prepared composites show enhanced mechanical properties with higher water vapor permeability compared to commercially available wound dressings. The hydrogel shows significantly higher antimicrobial activity due to the presence of zinc oxide. Similarly, silver and copper nanoparticle-coated polymeric hydrogel composites have also shown promising antibacterial activity (Thoniyot et al. 2015). Zhang et al. (2013) have also developed nanocomposites with controlled release of medications.

Nanocomposites of hydrogels with silicone nanoparticles show good prospects in controlling cellular functions. Chandra Babu et al. (2013) have developed silver nanocomposites of sodium carboxymethylcellulose- and polyacrylamide-based IPN hydrogels. These nanocomposites showed antibacterial activity against *Bacillus subtilis,* whereas the hydrogel alone was ineffective.

11.5.3 Scaffolds and Regenerative Medicine

Regenerative medicine is an emerging field of tissue engineering specially to avoid the risks and hurdles associated with transplants. The basic idea is to deliver cells and tissue constructs inside the body to develop healthy tissue(s) replacing the dead one. Extracellular matrix (ECM) is a key structure involved in maintaining tissue structure and homeostasis. Most of the protein portion of ECM is composed of collagen and proteoglycans. The role of ECM in growth and development is also supported by certain studies on animals. Murine species lacking ECM components like collagen and laminin have shown to die prematurely, and in certain cases, mice deficient in other ECM components like osteonectin show abnormal characteristics by birth. IPN-based hydrogels are known for their similarity in structure and composition with the extracellular matrix. Moreover, a wide framework of cross-linked polymeric hydrogels offers ample support for cell growth and maturation. The hydrogels are intended to provide structural support to cells with their tensile strength. Another approach toward tissue regeneration is by using IPN composites for cell delivery. Incorporation of bioactive molecules with IPN systems has shown potential in cellular interaction, thus manipulating cellular differentiation and so tissue regeneration.

For the purpose of tissue regeneration, hydrogels of varying features are designed by methods like micro-crystallization, hydrogen bonding, and physical interaction. The tensile strength of conventional IPN hydrogels can be modulated and improved by impregnating functional nanoparticles within the IPN matrix.

Polymeric scaffolds are a new field of tissue engineering and offer multiple pharmaceutical/medical applications. The polymeric matrices for scaffold formation can be prepared with high structural precision, using multiple polymers and assembly techniques, to regulate the desired features like stiffness, degradation, and porosity. Scaffolds are capable of producing 3D structures with desired architecture and properties. They are used in complex therapies and techniques like bone regeneration, graft generation, cornea and other organ replacements, etc. Many new techniques of modifying polymers into new complex structures have been tried and tested for producing scaffolds specifically for tissue engineering and regeneration of tissue structures (Dhandayuthapani et al. 2011).

Cartilage regeneration using collage and hyaluronic acid is attracting considerable attention in clinical research. These can be easily dispensed in the form of patches, gels and foams, etc. Perez et al. (2016) have designed IPN-based scaffold to improve the utility of hyaluronic acid (HA). Cross-linked HA and cross-linked poly(ethyl acrylate) scaffolds were combined to form the IPN-based scaffolds. In in vitro study using L929 fibroblast cell culture, the IPN scaffold showed the most improved metabolic activity. The IPN scaffolds also improved the cellular proliferation of fibroblasts with increase in the number of stress fibers. This approach is a unique one as the polymers of opposite nature were clubbed as IPN scaffolds and yield desirable results while retaining properties of both the individual materials. In another approach, Naseri et al. (2016) prepared IPN-based scaffold using polymers of similar characteristics, viz., alginate and gelatin with cellulose nanocrystals in order to offer suitable substitute of articular cartilage. Cross-linking with cellulose nanocrystals resulted in formation of dense networks with lesser space within.

Most of the hydrogels prepared to function as scaffolds are highly compatible in aqueous systems and, thus, allow permeation of nutrients and cellular wastes maintaining healthy cell metabolism. A poly(ethylene glycol)- and poly(acrylic acid)-based IPN scaffold is successfully designed by Parke-Houben et al. (2015) with the purpose of integrating and supporting artificial cornea. The inverted colloidal crystal scaffolds were prepared by microsphere templating technique. The idea was to prepare interconnected scaffolds with high porosity to promote the proliferation of stromal cells. The corneal fibroblast cells were successfully grown in the hydrogel culture.

11.5.4 Others

The swelling ability of hydrogels makes it suitable in prostheses. Endoprosthesis is designed for treatment of individuals having blockage/obstruction in biliary tract. A hydrogel shaped as ring is placed around the endoprosthesis on the sides. By swelling the hydrogel keeps the device in position and protects damage. In one clinical

study, Brunner et al. implanted carboxymethylcellulose hydrogels into 122 subjects evaluated for around about 5 years. The results revealed substantial radio translucency and integrity of the implants. They were also easy to replace in case of defects. In case of breast cancer patients, hydrogels showed better patient satisfaction as compared to silicone implants, and they hardly show any complications (Kilinc et al. 2018).

Another prospective application of hydrogels is construction of contact lenses. Preparation of contact lens requires pure and clear materials with suitable mechanical strength and permeability for oxygen. They should be able to be sterilized and must be noncarcinogenic. Contact lenses are generally classified as "hard" or "soft" depending on their elasticity. Although hard contact lenses are more durable, users do not prefer them, since they require an adaptation period. Hard contact lenses are resistant to hydrophobic substances such as poly(methyl methacrylate) (PMMA) or poly(hexafluoroisopropyl methacrylate) (HFIM). Despite this, soft contact lenses are resistant to hydrogels.

Manufacturing bio-printed constructs is another potential application of IPN hydrogels. Bio-printing benefits from new biomaterials which can address the requirements for the fabrication of well-defined 3D constructs. These can be used for the preservation of cell viability and possess required mechanical strength. Pescosolido et al. (2011) evaluated the suitability of semi-IPN, based on hyaluronic acid and hydroxyethyl-methacrylate-derivatized dextran (dex-HEMA), to form 3D hydrogel bio-printed constructs. The rheological properties of the solutions allowed proper handling during bio-printing, whereas photopolymerization led to stable constructs of which their mechanical properties matched the wide range of mechanical strengths of natural tissues. Importantly, excellent viability was observed for encapsulated chondrocytes. The results demonstrate the suitability of hyaluronic acid/dex-HEMA semi-IPNs to manufacture bio-printed constructs for tissue engineering.

11.6 Conclusion

Biomaterials prepared from interpenetrating polymeric networks are currently one of the primary fields of research in polymers. The interpenetration of two or more polymers in the IPNs makes them versatile in nature. The hydrogels prepared from IPNs show diverse properties including increased strength, stability, biocompatibility, stimuli responsiveness, and others. They are now being readily utilized for important biomedical applications like drug delivery, organ transplant, and tissue regeneration. The scope is further widening, and now there are new emerging applications of IPN systems like forming of eye lenses, prostheses, and bio-printing technology. The future of IPNs is thus very exciting and full of expectations.

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Paper award, 2013; Outstanding faculty of the year award (2012) by G.D.R., Educational society etc. He has two research projects awarded from Fast Track, Young Scientist Start-Up Grant (2013) SERB, DST, New Delhi, and CGCOST, Raipur. He is a reviewer of various esteemed journals. Dr. Ajazuddin has organized and participated in various national and international seminars and workshops as a resource person and subject expert.

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Bio-nanocomposite IPN for Biomedical Application

12

Nur Arifah Ismail and Mohd Hasmizam Razali

Abstract

Bio-nanocomposite interpenetrating polymer networks (IPN) have gained great attention in the last decades, mainly due to their biomedical applications. This chapter is an overview of the recent design and modification of bio-nanocomposite IPN and their applications as wound dressing. Prior to that, the introduction of biopolymers, polysaccharide biopolymer, composite and nanocomposite, as well as bio-nanocomposite was presented. Bio-nanocomposite IPN based on polysaccharide biopolymer (chitosan, cellulose, and gellan gum) was discussed in detail regarding their preparation methods, physiochemical properties, and performance as wound dressing for skin tissue regeneration.

Keywords

Bio-nanocomposite · IPN · Biomedical · Wound dressing · Tissue regeneration

12.1 Biopolymers

Biopolymers are the polymers that are consisting of chain-like molecules with covalent bond of monomeric units. The prefix "bio-" represents that biopolymers are biodegradable. Hence, biopolymers are capable to be degraded by the action of naturally occurring organisms leaving behind organic by-product such as CO_2 and H_2O (Reddy et al. 2016). Biopolymers can be largely divided into different categories based on the manufacturing method as shown in Fig. 12.1. They include (i) natural biopolymers, for example, plant carbohydrate like starch, carrageenan,

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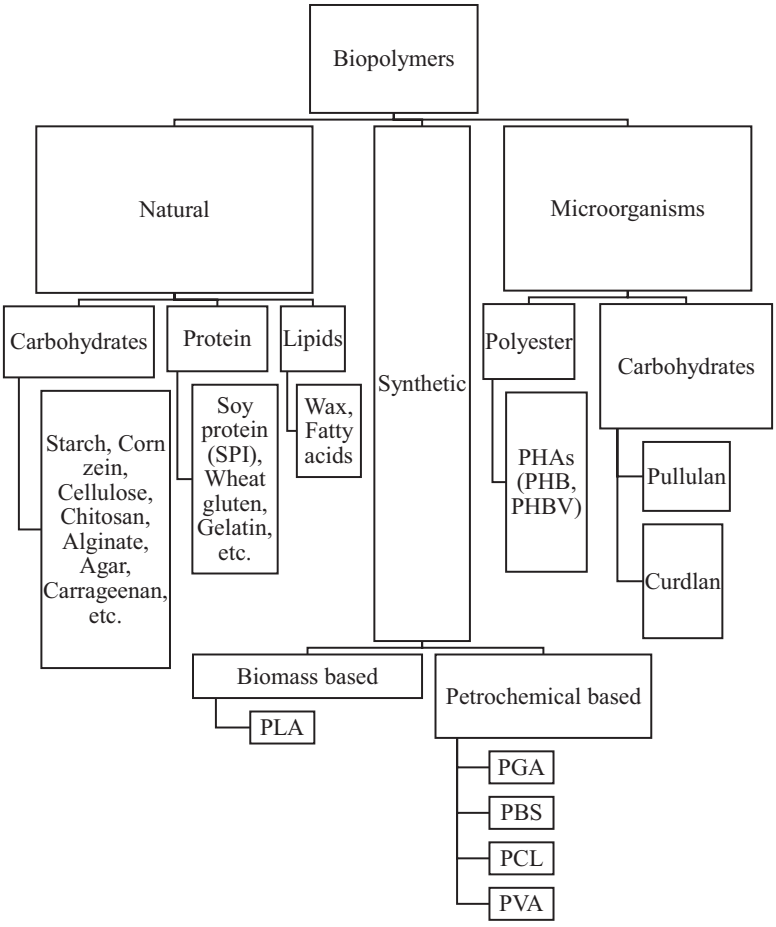

Fig. 12.1 Types of biopolymers. (Reproduced with permission from Ref. (Reddy et al. 2016))

chitosan, agar, alginate cellulose, etc., and animal or plant origin proteins like wheat gluten, soy protein, whey protein, gelatin, casein, corn zein, collagen, etc.; (ii) synthetic biodegradable polymers, for example, poly(glycolic acid) (PGA), poly(e-caprolactone) (PCL), poly(vinyl alcohol) (PVA), poly(L-lacticide) (PLA), poly(butylene succinate) (PBS), etc.; and (iii) biopolymers formed by microbial fermentation like microbial polyesters, for example, poly(hydroxyalkanoates) (PHAs) including poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PBHV), poly(β-hydrocxybutyrate) (PHB), etc., and microbial polysaccharides, such as pullulan and curdlan (Othman 2014).

12.2 Polysaccharide Biopolymer

Polysaccharides such as gellan gum (Coutinho et al. 2010), alginate (Jeon et al. 2009), gelatin (Nichol et al. 2010), and chitosan (Amsden et al. 2007) are among the biopolymers that have been explored widely due to its ability to form hydrogels, which can maintain the good viability of cells. Polysaccharides are polymers or macromolecules that contain two simple sugars in their chain. Polysaccharides can be divided into four types which are from bacterial, fungal, plant, and animal (Table 12.1). Since the polysaccharides are from natural sources, they are environmentally degradable and bioabsorbable (Prajapati et al. 2013). Apart from that, they are also nontoxic, are of low cost, and are renewable, which have potential to be used as key ingredients for mankind biomaterials production (Kalia 2016).

Polysaccharides have been used as viscosity modifiers or drug delivery agents in pharmaceutical compositions (Xia et al. 2009). The polysaccharide solution can be converted to the gel state by adding cations such as Ca^{2+} ions into alginate to form a gel (Smith et al. 2007). It also has been used in the encapsulation of probiotics for targeted gastrointestinal delivery (Cook et al. 2012). Apart from that, the polysaccharides are largely used as functional biopolymers in food technology, biomaterials, and biotechnology. The ability of polysaccharides to control the rheology of aqueous system makes them suitable to use in biopolymer industry (Lopes-da-Silva 2012). Previous studies have stated that the different polysaccharides can associate in aqueous dispersion in several degrees via intermolecular non-covalent interactions (hydrogen bonds and electrostatic interactions) (Higgins et al. 2011). Furthermore, the novel materials can be achieved by modifications of polysaccharides because of their appealing and tailorable properties and also due to the potential to be used in variety of applications (Lopes-da-Silva 2012).

Biopolymers are widely investigated in skin tissue engineering as well as for preparation of artificial scaffolds (Garg et al. 2011). Biopolymers are isolated from a variety of sources including animal, plant (seaweed), fungal, or bacteria as shown in Table 12.1. The natural sources of polymer are soluble in aqueous or buffer solutions and are biodegradable in vivo. The collagen and hyaluronic acid (HA) that come from animal sources play a major role, and they are important components for skin regeneration. Thus, both of them have been fabricated as sponges, films, matrices, and gels for wound healing applications (Kawai et al. 2000; Yamamoto et al. 2001; Slavkovsky et al. 2010; Gao et al. 2010; Anilkumar et al. 2011). Their utilization was increased due to its biodegradability and biocompatibility properties. Currently, collagen scaffolds were widely used especially for burn injury wound

Table 12.1 Types of polysaccharides (Ige et al. 2012)

Types	Examples
Bacterial	Xanthan, dextran, gellan gum, and cellulose
Fungal	Pullulan, elsinan, yeast, and glucan
Plant	Starch, alginate, agarose, and tannin
Animal	Chitin, chitosan, collagen, hyaluronic acid, lignin, and tannin

care in promoting the strong attachment and proliferation of keratinocytes and dermal fibroblasts. Various skin templates based on the collagen were available for these skin injury types including Biobrane™, MatriDerm®, Apligraf®, Integra®, and others.

Subsequently, hyaluronic acid (HA) is a natural polysaccharide composed of N-acetylglucosamine and glucuronic acid sugar units. It is a structural component of skin ECM and has low immunogenicity. Thus, it has been considered as an ideal biomaterial for wound repair (Slavkovsky et al. 2010; Gao et al. 2010; Anilkumar et al. 2011). Generally, natural sources of polymers have poor physical properties and have some limitations for medical application. Thus, in order to overcome these limitations, the HA were introduced into some modifications on its chemical structure to enhance their resistance from degrading. As a result, two commercial products were produced which are Hyalomatrix and Hyalosafe which are used in burn treatment for dermal substitutes. Hence, due to the advantages of natural polymer that included similarity with natural ECM of the tissue, biocompatibility, easy processing, and commercial availability, it has been used broadly as biomaterials sources. It is included in the development of composite scaffolds based on the pullulan in enhancing the proliferation and differentiation of cells for tissue regeneration (Singh et al. 2016). Prior to the enhancement, some modifications on their surface (e.g., surface deposition of hydroxyapatite, incorporation of small functional groups, arginine-glycine-aspartate ligands, cell recognizable molecules like gelatin, and others) were introduced (Burdick and Anseth 2002; Dadsetan et al. 2012; Hutson et al. 2011; Phadke et al. 2012). Apart from that, currently, there have been some reports regarding the production of functional food packaging based on the pullulan that was enhanced with lysozyme nanofibers for antibacterial and antioxidant additives (Silva et al. 2017). Not limited to that, the natural polymer based on pullulan also has been explored for targeting and controlled release of doxorubicin (Doxo) to hepatocellular carcinoma cells (HCC) (Balasso et al. 2017). The bioconjugate for HCC targeting was obtained by pullulan reprogramming based on the backbone oxidation and conjugation of targeting peptide and Doxo through a releasable linker.

Further, owing to the unique structure and physical properties, microbial exopolysaccharides hold a vital role in food, pharmaceutical, and other industries applications. Their applications included as emulsifiers, stabilizers, binders, gelling agents, coagulants, lubricants, film formers, and thickening agents (Paul et al. 1986). Based on the previous reports, gellan gum from *Sphingomonas paucimobilis*, xanthan from *Xanthomonas campestris,* and *Azotobacter chroococcum* are among the biopolymers that are currently used for commercial product and have been subjected for extensive study (Sutherland 1994).

Gellan gum (GG) is a *Sphingan* group linear anionic heteropolysaccharide secreted by members of bacterial genus *Sphingomonas* (formerly *Pseudomonas*) *elodea* (Nishinari 2003; Pahwa et al. 2010; Hungerford et al. 2012). Previously, it was referred via code names as S-60 and PS-60 and was produced from microorganism that was isolated from the elodea plant tissue and then known as *Pseudomonas elodea* (Miles et al. 1984). It consists of a repeating unit of tetrasaccharide:

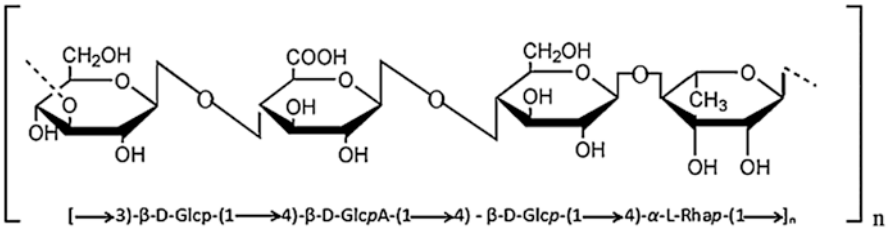

Fig. 12.2 Chemical structure of gellan gum. (Reproduced with permission from Ref. (Manjanna et al. 2010))

1,3-linked *β*-D-glucose, 1,4-linked *β*-D-glucuronic acid, 1,4-linked *β*-D-glucose, and 1,4-linked α-L-rhamnose (Fig. 12.2). The approximate composition is glucose 60%, rhamnose 20%, and glucuronic acid 20%. Apart from that, it contains considerable amounts of non-polysaccharide material such as cell protein and ash, which can be removed by filtration or centrifugation (Jansson et al. 1983; O'Neill et al. 1983).

GG becomes the subject of interest since its discovery in 1980 regarding their ability to form transparent gels even at low concentrations (Rodrıguez-Hernandez et al. 2003). In addition, their properties can form strengthened gels even at low pH compared to traditional gelling agents like agarose and carrageenan that reduce gelling capacity at low pH (Moritaka et al. 1995; Yamamoto and Cunha 2007). Consequently, the discovery was continuing, and in 1994, the production of GG was produced by *Sphingomonas paucimobilis* and was classified in α-4 subclass of the proteobacteria (Takeuchi et al. 1994). Yet, in 1992, this material was approved by the US Food and Drug Administration (USFDA) and the European Union (it is labeled as E 415 in EU regulation) for use in the food industry (Pszczola 1993).

12.3 Composite and Nanocomposite

The concept of composite materials is to combine different materials to produce a new material with performance and efficiency unattainable by the individual constituents. Composite term is practically used for newly developed biomaterials. A composite material consists of either two or more physically and chemically distinct, suitably arranged, or distributed materials with an interface separating them. Composite materials have a bulk phase, which is continuous, called the matrix, and one or more dispersed, noncontinuous phases, called the reinforcement, which usually has superior mechanical or thermal properties to the matrix. The region between the two can be simply a surface, called an interface, or a third phase, called an interphase (Iftekhar 2004).

Composite can be classified based on the characteristics of the reinforcement. It included shape, size, orientation, composition, distribution, and manner of incorporation of the reinforcement. In detail, for biomedical purpose, it can be classified into fiber-reinforced or particle-reinforced composites. Thus, it exists either as

polymer matrix composites (PMCs), ceramic matrix composites (CMCs), or metal matrix composites (MMCs) (Iftekhar 2004).

Currently, different types of composites were applied for various biomedical applications (Salernitano and Migliaresi 2003) including:

(i) Cardiovascular applications
(ii) Applications in dentistry
(iii) Oral and maxillofacial surgery
(iv) Applications in tissue engineering
(v) Applications in orthopedics

Nanocomposites, a high-performance material, exhibit unusual property combinations and unique design possibilities. Nanocomposites are composites in which at least one of the phases shows dimensions in the nanometer range (1 nm = 10^{-9} m) (Camargo et al. 2009; Roy et al. 1986). The definition of nanocomposites has broadened significantly to encompass a large variety of systems to two-dimensional, three-dimensional, and amorphous materials, made of distinctly dissimilar components and mixed at the nanometer scale. Typically, nanocomposites are from clay, carbon, or polymer or a combination of these materials with nanoparticle building blocks (Okpala 2013).

Nanocomposite materials can be classified, according to their matrix materials, in three different categories (Camargo et al. 2009):

(i) Ceramic matrix nanocomposites (CMNC)
(ii) Metal matrix nanocomposites (MMNC)
(iii) Polymer matrix nanocomposites (PMNC)

Nanocomposite systems have been extensively studied since the 1990s, and accordingly, there has been a steady and continuous increase in the number of publications on the subject, including reviews from time to time (Schmidt et al. 2002; Wypych et al. 1997; Fischer 2003; Pandey et al. 2005; Alexandre and Dubois 2000). In spite of this growth, the majority of the reviews describe the current status of only one type of nanocomposite. Thus, there are only two reviews on CMNC (Sternitzke 1997; Choi and Awaji 2005), three on CNT-reinforced nanocomposites (Peigney et al. 2000; Thostenson et al. 2001; Andrews and Weisenberger 2004), and a quite large number on PMNC (Alexandre and Dubois 2000; Sternitzke 1997; Wang et al. 2004; Jordan et al. 2005). In the case of PMNC, reviews deal with processing aspects, including those on layered silicates (Alexandre and Dubois 2000; Ray and Okamoto 2003), conducting and biodegradable polymer-based systems (Pandey et al. 2005; Ray and Okamoto 2003; Gangopadhyay and De 2000), fiber-reinforced (Peigney et al. 2000; Thostenson et al. 2001) and structure/morphology/property aspects (Pandey et al. 2005; Sternitzke 1997), as well as with applications and perspectives, including key opportunities and challenges in the development of structural and functional fiber nanocomposites (Pandey et al. 2005; Alexandre and Dubois 2000; Ray and Okamoto 2003).

12.4 Bio-nanocomposite

Bio-nanocomposites are made of natural polymers (biopolymers) and inorganic solids, having dimensions in the nanometer range (1–100 nm) (Visakh and Thomas 2010; Williams and Wool 2000; Darder et al. 2007). The bio-nanocomposite term was extremely used in 2004 and also known as nanobiocomposites, green composites, and biohybrids (Kalsoom Khan et al. 2017). Bio-nanocomposites are prepared by various methods which are solution intercalation, in situ intercalative polymerization, melt intercalation, and template synthesis. By solution intercalation method, the biopolymer or bio-prepolymer, such as starch and protein, is added into the solvent which is completely soluble in solvent. The inorganic nano fillers such as silicate platelets are swollen in a solvent such as water, chloroform, or toluene. When the biopolymer and solution of swollen nanoparticles are mixed, the polymer chains intercalate and displace the solvent within the interlayer of the silicate. Upon solvent removal, the intercalated structure remains, resulting in a formation of biopolymer/layered silicate bio-nanocomposite (Zhao et al. 2008; Shchipunov 2012).

In the in situ intercalative polymerization, the nanoparticle is dispersed in a liquid monomer or a monomer solution, so the polymer formation can occur between the intercalated sheets. Polymerization can be done either by heat or radiation, by the diffusion of a suitable initiator, or by an organic initiator or catalyst (Zhao et al. 2008; Shchipunov 2012), while via the melt intercalation process, the polymer is heated at specific temperature to get a molten mass and mixed with nanoparticle. It can be done by the extruder (Zhao et al. 2008; Shchipunov 2012). Lastly, the bio-nanocomposites can be prepared via template synthesis. In this method, biomolecules, parts and whole cells, and microorganisms serve as the template for inorganics which are generated from a precursor. The templating bio organics is in nanosized particle which is entrapped in mesoporous matrix. This method mostly required water-soluble polymers, and the resulting product may be chances of contamination due to side product (Camargo et al. 2009; Shchipunov 2012; Siqueira et al. 2010).

Bio-nanocomposites are developed based on biomaterial and nanoparticles. Biomaterial is usually obtained naturally from plant, animal, and microorganism. It contained mostly cellulose, lignin, and hemicellulose. While nanoparticles exist in spherical, tube, and platelets, it can be developed by large particle to form small particle having dimension in micro or nano size. Typically, the bio-nanocomposites development is to improve the thermal stability, to improve the packaging applications, to improve mechanical property (strength, elastic modules, and dimensional stability), to improve properties of the polymer matrix, and to improve the permeability.

Biomaterial is the most important bio-nanocomposite component that is obtained naturally from plant, animal, and microorganism. It contained mostly cellulose, lignin, and hemicellulose. Cellulose is a polysaccharide that is mostly found in plants and animals. Cellulose is a building material of long fibrous cells and highly strong natural polymer. Cellulose nanofibers are inherently a low-cost and widely available material. Moreover, they are environmentally friendly and easy to recycle by combustion and require low-energy consumption in manufacturing. Basically two types

of nano-reinforcements can be obtained from cellulose microfibrils and whiskers (De Azeredo 2009; Oksman et al. 2016). On the other hand, lignin is the second most abundant natural raw material and nature's most abundant aromatic (phenolic) polymer (Lora and Glasser 2002), whose main function is to cement the cellulose fibers in plants (Carrott and Carrott 2007). Hemicellulose, also known as polyose, is a matrix of polysaccharides, such as arabinoxylans, that exist along with cellulose in almost all the plant cell walls. It is a polysaccharide that is present in the biomass of most plants, about 20–30% dry weight of plants. Hemicellulose, combined with cellulose, provides physical and structural strength to the cell wall. In addition to glucose, the other structural components in hemicelluloses are xylose, galactose, mannose, rhamnose, and arabinose. Hemicellulose has shorter chains of 500 and 3000 sugar units with a branched structure.

12.5 Interpenetrating Polymer Networks (IPN)

An interpenetrating polymer network may be defined as any material which contains two or more polymers in the network form (Singh et al. 2012). IPN is obtained when at least one of the polymers is synthesized or cross-linked in the immediate presence of the other polymer without any covalent bond between them (Qadri et al. 2015). In other words, IPN may also be defined as the combination of two or more polymers in the network form in which one polymer is cross-linked in the presence of other (Rokhade et al. 2007). IPNs can be prepared through different techniques as given in the literature, but in situ technique proves that it is the most convenient technique. In this technique, all reactants are combined together, and reaction can take place with the formation of two networks which can be started at the same time (Vancaeyzeele et al. 2005). The procedure for the synthesis of IPNs can be divided into two categories, simultaneous synthetic method and sequential synthetic method. In simultaneous synthetic method, both monomers are mixed together to form polymer network simultaneously through different reaction routes. In sequential synthetic method, different network reactions are controlled sequentially by adding different monomers.

IPN can be made in many different ways. Brief definitions of some of the more important IPN materials are as follows (Shivashankar and Mandal 2012):

- Sequential IPN. Polymer network I is made. Monomer II plus cross-linker and activator are swollen into network I and polymerized in situ. The sequential IPN include many possible materials where the synthesis of one network follows the other.
- Simultaneous interpenetrating network (SIN). The monomers or prepolymers plus cross-linkers and activators of both networks are mixed. The reactions are carried out simultaneously, but by noninterfering reactions.
- Latex IPN. The IPNs are made in the form of latexes, frequently with a core and shell structure. A variation is to mix two different latexes and then form a film,

which cross-links both polymers. This variation is sometimes called an interpenetrating elastomer network (IEN).

- Gradient IPN. Gradient IPNs are materials in which the overall composition or cross-link density of the material varies from location to location on the macroscopic level. For example, a film can be made with network I predominantly on one surface, network II on the other surface, and a gradient in composition throughout the interior.
- Thermoplastic IPN. Thermoplastic IPN materials are hybrids between polymer blends and IPNs that involve physical cross-links rather than chemical cross-links. Thus, these materials flow at elevated temperatures, similar to the thermoplastic elastomers, and at use temperature, they are cross-linked and behave like IPN. Types of cross-links include block copolymer morphologies, ionic groups, and semicrystallinity.
- Semi-IPN. Compositions in which one or more polymers are cross-linked and one or more polymers are linear or branched are semi-IPN (SIPN).

12.6 Wound and Wound Dressing

Skin is the largest organ of human body which plays a critical role as a protective barrier against the environment, preventing infection and loss of water and electrolytes (Xu et al. 2015), and it is a coat of the body to protect an internal organ. It functions to prevent the body from dehydration as well as to protect from microbes and stabilize body temperature. The damaging of skin will destroy its protective functions directly conducted to microorganism infections. Once the microorganism enters the body through the damaged skin, it will form colonies and infect the wounded site; thus, the healing process will be delayed and cause our life in risk. Actually, skin wound involved the disruption of skin structure and function like acute burns of the skin and chronic ulcers. While wound can be defined as any injury that damages the skin layer and disrupted its protective function, it is one of the major health-related problems around the globe (Sudha et al. 2012). It is an unpreventable event in our life that involved physical damage, chemical injury, and microbial pathogenic infections which lead to loss of cellular and functional continuity of living tissues. Recently, wounds are among major worldwide clinical problems because of morbidity associated with prolonged periods required for (1) repair and regeneration of the injured tissue, (2) bleeding, (3) risk for infections, and (4) septicemias, keloids, and scar formation (Sivaranjani and Philominathan 2016). Generally, the wound infections are commonly caused by plenty of bacteria or fungi. Gram-positive (*Staphylococcus*) and gram-negative (*Pseudomonas aeruginosa*) are the most common bacteria responsible for the majority of wound infections (Sivaranjani and Philominathan 2016). This is because both of the type of bacteria can easily contaminate the surface of wounds and access the underlying tissue and then delay the healing process (Sivaranjani and Philominathan 2016).

Although our technology has achieved a higher level in medical field, wound healing is still a challenging part since it remains an inefficiently managed area caused by various factors. Usually, the healing process contains four main stages including hemostasis, inflammation, proliferation, and remodeling (Figs. 12.3 and 12.4). The failure of any stages in the healing process may lead to the formation of hypertrophic scars, keloids or the chronic scarring. So, the most important part in

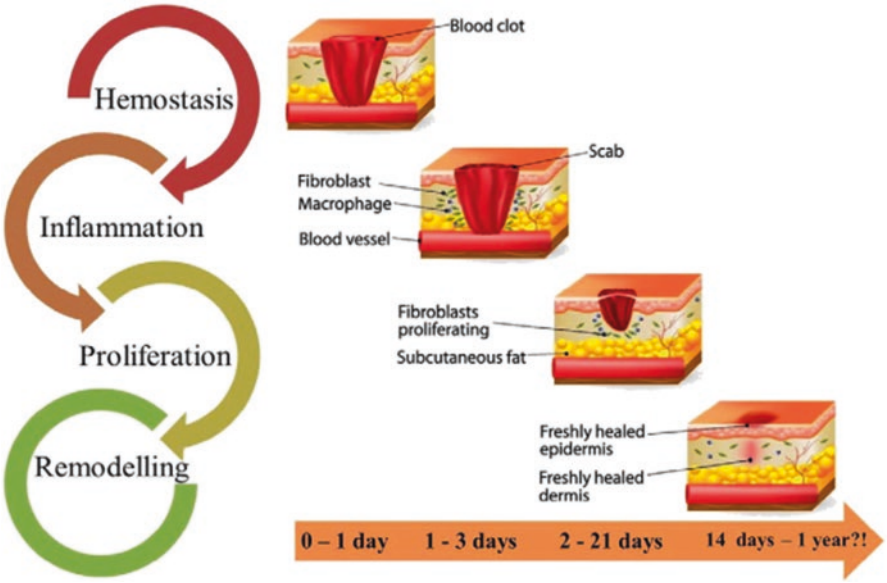

Fig. 12.3 The skin wound healing cascade. (Reproduced with permission from ref. (Korrapati et al. 2016))

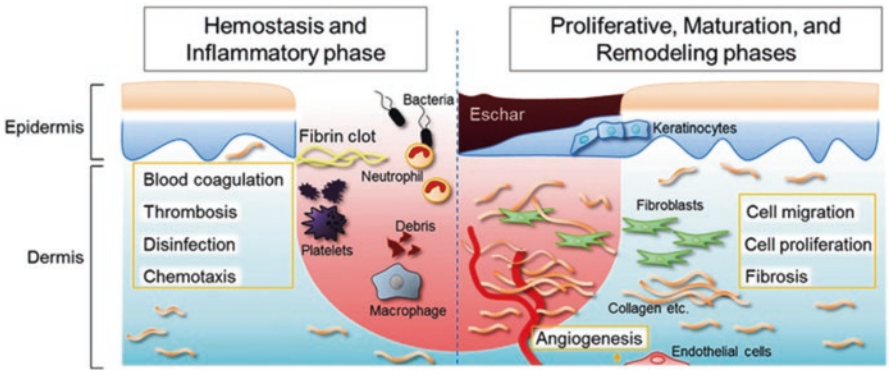

Fig. 12.4 Schematic illustration of various cellular events occurring during wound healing. (Reproduced with permission from Ref. (Kurahashi and Fujii 2015))

healing process that attracts attention around the globe is to speed the recovery process as well as to minimize the scar formation. This situation given leads to the introduction of wound dressing materials.

Wound dressings have been used to cover the wound, while the active ingredient inside the material prevents the wound from any infections and promotes the healing process. An ideal wound dressing is the dressing which can absorb the exudates and maintain the moisture to facilitate the wound healing from the inflammation, granulation, and reduce tissue needs during remodelling stages (Cencetti et al. 2012). Traditionally, most of the wound dressings have been used as a medium to dry the wound exudates by allowing the moisture to evaporate and to prevent bacterial infections. The sources of dressing materials are commonly found from either nature (alginate, chitosan, and others) or synthetic (bandages, cotton wool, lint, and gauze) that was designed with different absorbance degree functions in order to suit the variety of wound types. In order to fulfill the demand conditions for fastening the healing process, the design of dressing experienced drastic changes from traditional to advanced therapies to enhance the healing environment. This is because, once the optimum wound healing environment was disturbed, for instance, the wound gets dehydrated it may delaying the healing process. Therefore, the development of biomaterials with various structural modifications to prevent the wound from infections and dehydration has become the attention of the researchers around the world.

Since the last two decades, various types of dressings were available in the market. The new types of dressing were available for each year, and the new becoming dressing types increased rapidly from 4 on 1998 to 262 on 2007. According to the MedMarket Diligence report, the global wound dressing market is estimated to be worth over $22 billion from 2014 to 2024. The concern of modern wound dressing types provided to improve cell growth as well as to prepare the optimum environment for wound healing. In order to achieve the satisfied conditions of the wound site, the dressings should have an appropriate water penetration and absorption to control the exudates. Table 12.2 listed some water vapor transmission rate values for a synthetic dressing.

Wound dressing has been designed to cover the wound to support for reepithelialization and prevent wound infection, skin desiccation, and further skin damage. Commercially, they can be found in four types of dressing which are biological dressings, conventional dressings, biosynthetic dressings, and antimicrobial dressings. Biological dressing consists of cadaver allograft skin (transplantation between individuals of the same species), xenograft (transplantation between individuals of different species), and human amnion, which have been adopted to temporarily cover wounds for reepithelialization. Biological dressings are efficient enough for further skin grafting; however, it cannot be used as permanent skin replacement due to the immunological disparities (Garfein et al. 2003). Apart from that, varied issues arise regarding the biological dressing including inconsistent quality, limited supply, and the risk of pathogen transfer (Atiyeh et al. 2005).

Consequently, the conventional dressing is a type of dressing that does not have any antibiotic or medication contents. It included Vaseline gauze or silicone sheets

Table 12.2 The water vapor transmission rates (WVTR) value for a few types of commercial dressing (Wu et al. 1995)

Dressing type	Trade name	WVTR (g m^{-2} d^{-1}), 24 h
Hydrocolloid	Biofilm®	6512 ± 445
	Comfeel®	285 ± 8
	Dermiflex®	76 ± 5
	Duoderm®	889 ± 49
	Duoderm CGF®	120 ± 19
	Granuflex®	216 ± 6
	IntraSite®	357 ± 29
	Metoderm®	809 ± 19
	Restore Cx®	476 ± 18
	Tegasorb®	136 ± 15
	Ultec®	534 ± 63
Hydrogel	Geliperm®	9009 ± 319
	Vigilon® (no film)	9360 ± 34
	Vigilon® (with film)	50 ± 19
Film	Bioclusive®	394 ± 12

that are widely used temporarily to cover the wounds during reepithelialization. However, these types of dressing tend to adhere on the wound surface and demand frequent changes leading to traumatization of the newly epithelialized surface and delay of the healing process (Liu et al. 2017a). Subsequently, the biosynthetic dressings are developed based on the materials that mimic the function of the skin as a replacement to the epidermis or dermis or both of them. It is Biobrane® and TransCyte®. Last but not least is the antimicrobial dressing that is widely used in burn management. It is applied in order to prevent the wound from infection through minimizing the bacterial colonization. Some of the antimicrobial dressings have been introduced to burn care. Basically their active ingredient comes from silver (e.g., Aquacel AG), nanocrystalline silver (e.g., ACTICOAT), cadexomer iodine (e.g., Iodosorb), or honey to prevent bacterial infection. Usually, the silver compounds are widely used in topical burn therapy, and it has contributed in reducing the incident of burn wound that induced sepsis and death.

Recently, the wound products are developed to replicate the extracellular matrix (ECM). The ECM is a major component in the dermis, and it provides a structural support for cells, growth factors, and receptors which play a major role in wound healing. Since the skin provides a route of delivery for local and systemic drugs, it is being efficiently used to deliver therapeutic products such as nanoparticles for treating skin diseases, for antimicrobial activity in wounds and burns, as well as for cancer treatment. Nowadays, due to the three crucial factors, one being an advancement in antibiotics, pathogenic bacteria and virus have become more resistant, and due to the outbreak of infectious diseases, it caused the pharmaceutical companies and researchers to continue searching for new drugs.

Consequently, an ideal wound dressing exhibits good biological compatibility, biodegradability, water absorption and retention properties, no cytotoxicity,

nonstick ability, and antibacterial effects. A good dressing can prevent the wound from being infected, allows gas exchange, could be removed easily, absorbs excrescent wound exudate, and remains a part of the exudate to maintain local moisture of the wound, which accelerates wound healing. Wound dressing based on the natural sources of polymer is getting wide interest among the researchers. This is because the polymer from natural origin is one type of biomaterials that have significant potential in contribution to the engineering of tissues especially to their resemblance to the ECM, their high chemical versatility, and their inherent interaction with biological systems (Goonoo et al. 2017).

The unique properties, biocompatibility, widespread availability, inherent biodegradability, and critical biological functions of natural polymer led to the application in various areas of our life. Simultaneously, natural polymers have been broadly investigated as excipients for pharmaceutical or biomedical purposes. Singh et al. (2017) have used an acacia gum together with polyvinylpyrrolidone and Carbopol in development of wound dressing material (Singh et al. 2017). An accelerated wound healing process has been shown in their study via histology analysis expected from inherent antioxidant properties of the materials used.

At the same time, current research is focused on the interaction between L929 fibroblast cells and polyelectrolyte-complex multilayer membrane (PCMM) interfaces. PCMM with gradient porous structure were fabricated by incorporation of alginate on both sides of porous chitosan membrane in order to create a moist healing environment. Their study shows an interesting results when the gradient porous structures of PCMM were helpful in accelerating wound healing. Good antibacterial activities of PCMM toward *Escherichia coli* also were observed (Sun et al. 2018).

During the past year, many techniques have been applied for making hydrogel, porous scaffolds, and films in developing new antibacterial wound dressings. Among them are synthesis of poly(aminoethyl)-modified chitosan (PAEMCS) by grafting of poly(aminoethyl) groups onto hydroxyl groups on chitin and then follow-up with removal of acetyl groups from the grafted polymer. A few characterizations were introduced onto the synthesized PAEMCS including rheological tests, swelling, antibacteria, and cytotoxicity. PAEMCS-based hydrogels not only maintained inherent numerous properties of chitosan but also possessed excellent hygroscopicity; high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, or *Staphylococcus epidermidis*; and good cytocompatibility toward L929 cells (Zhang et al. 2017).

According to the skin damage increased annually, the demand of wound healing material keeps attracting attention among the researchers. So the wound dressing based on electrospun poly(e-caprolactone) (PCL) nonwoven mat was combined with surface coating of nitric oxide (NO)-conjugated chitosan (CS). The realizing of NO enhanced the healing properties by regulating inflammation, improving angiogenesis, and enhancing collagen deposition that led to the promoted tissue regeneration and remodeling (Zhou et al. 2017). On the other hand, a multifunctional hybrid hydrogel of methacrylate arginine (M-Arg) and N-isopropyl acrylamide (NIPAAm) with temperature response, anti-protein adsorption, and antibacterial properties was prepared (Wu et al. 2018). In this study, they were preloaded with chlorhexidine

diacetate (CHX) into the hydrogel and then grafted with polyhexamethylene guanidine phosphate (PHMG) on the hydrogel surface for the antimicrobial properties (Wu et al. 2018).

Within this past year, wound dressing material based on biopolymers has been developed by using a different technique. Cellulose is one of the important polysaccharides that plays a key role in wound healing application. Recently, hydrogel membranes based on carboxymethylcellulose (CMC) were prepared by blending with polyethylene glycol (PEG) in the presence of citric acid (CA) to improve their properties. The CA that was cross-linked chemically and addition of PEG into the hydrogel affected the swelling degree and the mechanical properties of the materials. This is due to chemical reaction of CMC hydroxyl groups with CA and the formation of CMC-PEG hybrid nanostructures that influence the formation of a hybrid polymeric network. Biocompatibility study demonstrated the effectiveness of CMC-based hydrogels as a wound dressing material due to the no cytotoxicity observed based on over 95% of cell viability responses (HEK297T) via in vitro MTT assay (Capanema et al. 2018).

12.7 Bio-nanocomposite IPN and Their Application in Biomedical as Wound Dressing for Skin Tissue Regeneration

Bio-nanocomposite IPN relies on the presence of two or more naturally occurring polymers in network that are usually in continuous phase in combination with one or more reinforcing phases displaying at least one dimension in the nanometer scale. In general, throughout this chapter, we shall consider the bio-nanocomposites IPN materials devoted to biomedical as the combination of a structural part and functional part. The bio-nanocomposites IPN or structural part is previously defined as network composed of one or more reinforcing phases in nanometer scale dispersed within a macromolecular continuous phase. The biomedical or functional part consists of a biological entity such as living cells, enzymes, or other biomolecule hosts by bio-nanocomposites IPN.

Bio-nanocomposite IPNs are widely used in various applications in different fields, including the biomedical due to its well-established antimicrobial activity as well as good correlation for wound healing activity. In the recent works, several reports have emphasized the role of nanostructured materials together with biopolymer in wound healing and antimicrobial activities. Generally, utilization of nanostructured materials is to enhance the biological as well as the physical and chemical properties of the materials. Previously, chitosan (CS) biopolymer was abundantly incorporated with nanostructured materials due to their versatile properties which are high biocompatibility, excellent rate of biodegradability, effective antibacterial activity, hemostasis, tissue adhesive properties, as well as their essential role in the wound healing process (Kumar et al. 2018). Prior study was successfully loading CS with Fe_3O_4 nanoparticles (NPs), silver inlaid with gold nanoparticles (Au-Ag NPs), nano Ag/ZnO (zinc oxide), gelatin/C4S (chondroitin 4-sulfate)/ZnO,

modified ZnO/CO (castor oil), and PVA (heparinized polyvinyl alcohol)/ZnO NPs for wound dressing applications (Cai et al. 2016; Li et al. 2017; Lu et al. 2017; Cahú et al. 2017; Díez-Pascual and Díez-Vicente 2015; Khorasani et al. 2018). In the previous report, CS also was successfully cross-linked with genipin, and then a bilayer composite was designed by adding Ag NPs for the antimicrobial properties (Ding et al. 2017). The addition of Fe_3O_4 NPs into the CS/gelatin (GE) increased the mechanical properties of the composite nanofiber's membranes (Cai et al. 2016). The effective dispersion of fillers (Fe_3O_4) and the interaction between the fillers and polymer matrix (CS/GE) enhanced the mechanical strength of the membranes (Cai et al. 2016). Subsequently, previous study revealed incorporation of Ag NPs and ZnO NPs increased the collagen production resulting in reepithelialization occurring in open wounds that led to the rapid wound healing (Verma et al. 2017).

In previous study by Cremar et al. (2018), chitosan (CS)-based composite fine fibers were produced by a centrifugal spinning technology (Cremar et al. 2018). Then, the antimicrobial CS-based nanofiber dressings were developed by addition of silver nanoparticles (Ag NPs) and cinnamaldehyde (CA) in order to improve the antibacterial properties. Basically, in wound care management, CS acts as an analgesic drug and anti-inflammatory agent (Cremar et al. 2018). The CS can exhibit a pleasant and soothing effect when applied to an open wound (Ahmed et al. 2015). Hence, in order to increase the stability of composite system, the tannic acid (TA) was added as a cross-linking agent. Based on the observation, the composite shows good antibacterial activity against *Staphylococcus aureus* and no sign of cytotoxicity since 3T3 mouse embryonic fibroblast cells were able to grow normally during the evaluation (Cremar et al. 2018).

Subsequently, previous studies have fabricated cellulose (CE)-based filter paper (FP) composite scaffolds comprising of adsorbed CS and Ag NPs (Haider et al. 2018). The Ag NPs are incorporated in the CS layer of the composite scaffold. The biocompatibility of the scaffolds was assessed by subjecting pristine FP, CS-adsorbed FP (CS-FP), and Ag-loaded CS-FP (Ag-CS-FP) composite scaffolds to in vitro studies. Based on their study, it has been observed that NIH3T3 fibroblastic cells adhered and proliferated onto all the scaffolds. Additionally, the Ag-CS-FP composite scaffolds exhibited good antibacterial activity against both gram-positive and gram-negative bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) contributed with the presence of Ag NPs. Thus, it has been believed that the composite scaffolds have potential to be applied in biomedical field especially as a wound dressing agent due to their antibiotic-resistant strains and cell proliferation (Haider et al. 2018).

Recent advances in wound dressing discovery were continued by Chen et al. (2018) who develop composite sponge based on Konjac glucomannan (KGM) and Ag NPs (Chen et al. 2018). In this study, the Ag NPs were prepared with green deoxidizer egg white, and the KGM/Ag NPs composite sponge was obtained via freeze-drying method. Excellent water absorption and water retention were displayed by the composite sponge. Not limited to that, good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and good cytocompatibility between L929 cells were demonstrated by KGM/Ag NPs composite sponges (Chen

et al. 2018). The Ag NPs displayed excellent antimicrobial activity due to the large surface area of nanoparticles (Rajendran et al. 2018). The silver ions were released by the nanoparticles and then enter inside the bacterial cells targeting in damaging the respiratory chain, directly disturbing cellular divisions that led to cell death (Marambio-Jones and Hoek 2010). An accelerated wound healing progress also was observed on New Zealand white rabbits model by using KGM/Ag NPs composite sponge that promoted fibroblast growth and accelerated epithelialization (Chen et al. 2018).

In addition, the antimicrobial gelatin-based elastomer nanocomposite membrane was fabricated by addition of ciprofloxacin (CPX) and polymyxin B sulfate-loaded halloysite clay nanotubes (HNTs-B) into a gelatin elastomer (Shi et al. 2018). Both of these antibiotics were loaded together into the gelatin matrix in order to produce a synergistic antimicrobial effect. While addition of HNTs into the matrix was enhanced, the thermal stability and mechanical strength also supported to control drug release rate. The release rate would decrease at high concentration of HNTs since the tubular structure of HNTs can prevent the drug release inside the system (Shi et al. 2018). Halloysite ($Al_2O_3{\cdot}2SiO_2{\cdot}2H_2O$) is one of the economical viable clay materials that has been used for loading, storage, and controlled release of anticorrosion agents, drugs, and biocides (Joshi et al. 2012). Therefore, the HNTs could slow down the release rate of CPX and high-dissoluble polymyxin B sulfate, thus beneficial against long-term bacterial infection (Shi et al. 2018).

Further, the combination of biopolymer and nanostructured dressing materials continues to attract interest among the researchers. Lately, carrageenan was prepared by blending with metallic nanoparticles like zinc oxide and copper oxide to produce functional hydrogels and dry films (Oun and Rhim 2017). Another study with hydroxyapatite (HA) and multi-walled carbon nanotubes (MWCNTs) filled by iron (Fe) was synthesized by the "wet chemistry" method (Sukhodub et al. 2018). These composite materials provide the opportunity to be used in bioengineering of bone tissue to fill bone defects of various geometries due to its ability to prolong the drug release. The interaction mechanism between the surface of MWCNT+Fe and chlorhexidine (CH) enhanced the structural integrity of the matrix and prolonged the drug release to more than 24 h compared to the HA-Alg (alginate) only (Sukhodub et al. 2018). Basically, CH acts as an antibacterial agent against gram-positive and gram-negative bacteria and fungi (Kovtun et al. 2012).

In recent years, the incorporation of bioactive agents to common wound dressing agent has become potential drug for wound treatment and tissue engineering application. Nanocomposite hydrogel (NC) is one of the new approaches for the therapeutic delivery. Recently, gellan gum methacrylate (GG-MA) was combined with Laponite® XLG to develop novel NC that is potentially used for wound dressing application. This type of dressing was targeted for the delivery of therapeutic agents at the site of the injury especially for burn wounds that are commonly found in chronic infections conditions (Pacelli et al. 2016).

Polysaccharides like gellan gum (GG) is becoming an interesting topic among the researcher in the field of biomedical applications due to their non-toxic monomers releasing during degradation (Pasqui et al. 2012). Recent study by Bonifacio

et al. (2017) proposed the tri-component based on GG, glycerol (Gly), and halloysite nanotubes (HNTs) for soft tissue engineering application. Those three components were altered at suitable composition to control the hydrogel viscosity, mechanical properties, water uptake, as well as the biocompatibility of the materials. An in vitro study with human fibroblast cells displays a good correlation between the materials since higher metabolic activities and cell survival up to 7 days of incubation at 25% of HNTs are incorporated into the matrix (Bonifacio et al. 2017).

Besides that, GG was combined with Manuka honey and HNTs as potentials used for cartilage regeneration. Rather than evaluation on biocompatibility, this study was conducted in understanding the water uptake kinetics and degradation rate of the biomaterials. This is regarding the cartilage replacement properties that need an adequate fluid permeability and lubricant capability. Thereafter, since the degradation rate is a crucial aspect in tissue engineering, the hydrogels were performed in simulated synovial fluid, in phosphate buffer solution (PBS), and in lysozyme in order to evaluate the degradation rate of the materials (Bonifacio et al. 2018).

The new dressing generation was introduced based on the nanostructured formulation. For example, lately, the dressing based on the chitosan, synthetic polymer poly(N-vinylpyrrolidone) (PVP), and nanoparticle titanium dioxide was fabricated. Based on the evaluation, it presents an excellent antibacterial properties against four pathogenic bacteria and is biocompatible to the NIH3T3 and L929 fibroblast cells. Not limited to that, the scaffold was further evaluated by animal testing on albino rats. The results show an effective wound closure rate via the nanocomposite treatment compared to the pure chitosan and positive control as well as negative control groups. Regarding this in vivo evaluation, it is proven that the nanocomposite scaffold has a large potential to be applied as an effective wound dressing candidate (Archana et al. 2013a).

In addition, TiO_2-NPs also were introduced into chitosan and pectin due to the biocompatibility, antimicrobial activity, water-swellable nature, good healing efficiency, and tensile strength. Based on the morphological observation, the TiO_2-NPs materials were distributed well in the polymer matrix. Subsequently, chitosan that was incorporated with pectin and TiO_2-NPs shows an optimal level in control of the evaporation water loss from wound bed. Apart from that, this dressing material also absorbs more exudates and keeps the wound beds moist without risking dehydration or exudate accumulation. Consequently, based on the in vivo evaluation, the chitosan incorporated with pectin and TiO_2-NPs dressing materials show a significant rate for wound closure step. The evaluation shows an excellent healing process of open wounds compared to the rat model that was applied with conventional gauze and chitosan only (Archana et al. 2013b).

Basically, introduction of metal oxide into the biopolymer is one of the new strategies to enhance the properties of wound dressing materials. In the present study, TiO_2-NPs and curcumin were incorporated into the sodium alginate (SA)/polyvinyl alcohol (PVA) using gel-casting technique. TiO_2 were added into the composite scaffold to enhance the wound healing activity, while the curcumin is good for inflammation as well as contains antimicrobial and antioxidant property

(Prasanna et al. 2018). Moreover, TiO_2 nanocomposite dressing was prepared by freeze-drying technique (Mehrabani et al. 2018). This nanocomposite scaffolds were prepared through combination with chitin and silk fibroin. The scaffold shows well-defined interconnected structure with more than 90% of porosity. The high porosity of the matrix could contributed to the high swelling and water uptake which served better environment for woung healing. Besides that, the cytocompatibility, antibacterial, and antifungal properties of the materials were in good condition when a human Caucasian fetal foreskin fibroblast cells displayed great proliferation and attachment, while high antibacterial and antifungal activities are demonstrated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Mehrabani et al. 2018).

In the recent years, CS was often used to combine with TiO_2 nanostructured materials due to its biocompatibility, biodegradability, and polycationic, nontoxic, antimicrobial, and bioresorbable properties. Currently, Behera et al. (2017) and Fan et al. (2016) incorporated TiO_2 into CS in production of membrane and porous scaffold (Behera et al. 2017; Fan et al. 2016). TiO_2 concentration was added at different concentrations in order to observe their effect on mechanical and porosity of the composite dressing (Behera et al. 2017). Porosity usually related to the surface roughness of the materials, which is higher surface roughness, referred to the high level of porosity that led to the enhancement of cell attachment on the dressing materials (Misra et al. 2010). Subsequently, collagen (COL) also was added together with nano-TiO_2 into the biopolymer matrix to enhance fibroblast infiltration and dermal regeneration (Fan et al. 2016). It was found the red blood cells easily form cluster aggregation which is supported to stop bleeding once the TiO_2/COL-CS composite scaffolds were applied on the wound (Fan et al. 2016).

Additionally, TiO_2 is among of the metal oxides that have been considered for combination with biopolymer in many studies. In recent works, TiO_2 was prepared with GO and bacterial cellulose (BC) for development of the antibacterial hybrid materials. This materials show 91.3% of antibacterial rate against *Staphylococcus aureus* under UV irradiation due to the ability of GO-TiO_2/BC that could generate highly reactive ROS near UV and cause the apoptotic death (Liu et al. 2017). Apart from that, 3-D nanocomposite scaffolds were generated with the mixture of type I collagen (extracted from porcine skin) and polyvinylpyrrolidone (PVP) coated with titanium dioxide (TiO_2) nanoparticles using freeze-drying technique (Li et al. 2016). Interestingly, this scaffold was developed without any cross-linker or toxic reagents which contribute in controlling the porosity of the matrix. However, the stability of the materials was enhanced by formation of four main hydrogen bondings from collagen, PVP, and TiO_2 resulting to an adequate strength as dressing materials (Li et al. 2016). Not limited to that, TiO_2-NPs also were mixed with regenerated bacterial cellulose (RBC) to increase the capabilities of dressing materials for bactericidal activity and tissue regeneration process (Khan et al. 2015).

12.8 Conclusion

For more than 50 years, 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. Nowadays, bio-nanocomposite interpenetrating polymer network (IPN) had received a great attention last decade owing to their improved properties, which is suitable for biomedical application. It was demonstrated that bio-nanocomposite IPN based on polysaccharide biopolymer such as chitosan, cellulose, and gellan gum exhibited good biomaterial properties for wound healing. The incorporation on Ag, ZnO, and TiO_2 nanomaterials into the bio-nanocomposite IPN enhanced their biocompatibility, antibacterial, and antifungal properties which support the good performance of bio-nanocomposite IPN for biomedical application.

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