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Review Article

PREPARATION METHODS AND PROPERTIES OF HYDROGEL: A REVIEW

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ABSTRACT

Hydrogels are three-dimensional cross-linked polymer network that can respond to the fluctuations of the environmental stimuli. These biomaterials can incorporate large quantum of biological fluids and swell. When swelled, they are soft & rubbery and resemble the living tissue, exhibiting excellent biocompatibility. Today, drug delivery experience several challenges where hydrogel could be one potential answer to those. Thanks to the unique properties of hydrogel for which they are widely exposed to different biomedical fields. Hence the preparation techniques of hydrogel biomaterial and the evaluation of the properties are of utmost significance. Literature reveals that this three dimensional architecture could be homo-polymeric, co-polymeric, semi- interpenetrating and interpenetrating polymer networks (IPN) based on preparation methods. Polymeric blends like semi-IPN have also been investigated to satisfy the specific needs of biomedical field. Such blends have shown superior performances over individual polymers. Unique biocompatibility, flexible methods of synthesis and tailor able physical properties have made the hydrogels to be used as a drug delivery device to tissue engineering scaffolds. As scaffolds they should provide structural integrity like tissue constructs and as a drug carrier it should have sufficient mechanical strength to control and protect the drug and proteins until they are delivered to the specific sites of the biological system. Hence, the evaluation of swelling, mechanical and biocompatible properties consider more attention before the hydrogels are applied. In this review article an attempt has been made to describe the available methods of hydrogel synthesis along with their inevitable properties.

Keywords: Hydrogel, homo-polymer, Co-polymer, Interpenetrating network, Swelling, Mechanical, Biocompatible.

INTRODUCTION

Recently hydrogels have gained considerable attention. Hydrogels are three-dimensional cross-linked polymer network that are smart enough to respond the fluctuations of environmental stimuli (pH, temp, ionic strength, electric field, presence of enzyme etc.) and swell or shrink accordingly. In the swollen state, they are soft and rubbery, resembling the living tissue exhibiting excellent biocompatibility [1]. Hence these biomaterials are widely used in different field of pharmaceutical and biomedical engineering [2].

With the advent of medical science large numbers of new therapeutic moieties are discovered and demands specialized carrier for their delivery into specific sites of the body. It is always with the formulation scientists to delve further and to design newer devices for the drug molecules which could serve even better. This quest has ended up with the construction of engineered biomaterials, hydrogel. Hydrogels are capable of delivering genetically engineered pharmaceuticals, *viz.* protein and peptides and improve the therapeutic efficacy and safety of drugs administered by conventional methods. Depending on the preparation methods this three dimensional architecture of hydrogels could be homo-polymeric, co-polymeric, semi-interpenetrating and interpenetrating polymer networks. Recently, thermoplastic co-polymeric biodegradable hydrogels with optimum mechanical strength have been designed for biomedical applications including drug delivery system [3].

Over the years, blends have been investigated to satisfy the specific need of biomedical field. Such polymeric blends showed superior performances than the individual polymers and the range of application has been extended. Carbohydrate based polymer blend is one of them and being investigated to develop controlled release formulations [4].

Semi-interpenetrating polymer networks (semi-IPN) is a way of blending two polymers where only one is cross-linked in the presence of another to produce an additional non-covalent interaction between the two polymers [5]. Semi-IPNs have been developed as a convenient technique for preparing multi-polymeric material and provided an alternative option to modify the properties of natural polymer-based hydrogels [6]. Semi-IPN materials are unique 'alloys' of cross-linked polymers and form dual polymer network and exhibited surprising properties superior to either of the two single polymers alone.

The chemical and physical cross-linking methods and properties of multi-polymers have been of great practical interest and translated into the development of interpenetrating network (IPN). IPNs are defined as a combination of two polymers in network form, at least one of which is synthesized and/or cross-linked in the immediate presence of the other. IPN hydrogels encompasses the advantages of both the conventional as well as novel drug delivery systems by offering a biocompatible, convenient and stable drug delivery system for molecules as small as non-steroidal anti-inflammatory drugs or as large as proteins and peptides.

Hydrogels due to their unique biocompatibility, flexible methods of synthesis, range of constituents and desirable physical characteristics, are widely used in different biomedical fields. They can serve as scaffolds which provide structural integrity to tissue constructs, control drug and protein delivery to tissues and serve as adhesives or barriers between tissue and material surfaces. Hence, the properties of hydrogels are important for tissue engineering and other areas of biomedical field. Among these properties one must evaluate the swelling, mechanical and biocompatible properties before the hydrogel biomaterials are applied. Author has attempted to describe briefly different methods of hydrogel preparation along with application. The essential properties that must be evaluated, was also under the purview of this review article.

PREPARATION METHODS OF HYDROGEL

Based on the methods of preparation, hydrogels may be classified as (1) homo-polymer (2) copolymer (3) Semi-interpenetrating network (4) interpenetrating network. Homo-polymer hydrogels are cross-linked networks of one type of hydrophilic monomer unit, whereas copolymer hydrogels are produced by cross-linking of two co-monomer units, at least one of which must be hydrophilic to render them swellable. Finally, interpenetrating polymeric hydrogels are produced by preparing a first network that is then swollen in a monomer. The latter reacts to form a second intermeshing network structure.

1) Homo-polymeric Hydrogel

Homopolymers are referred to polymer networks derived from single species of monomer. It is the basic structural unit and comprising of any polymer network [7]. Homopolymers may have cross-linked skeletal structure depending on the nature of the monomer and polymerization technique. Cross-linked

homopolymers are used in drug delivery system and in contact lenses. One possible way of preparing homo-polymeric hydrogel film is the use of poly (2-hydroxyethyl methacrylate) (polyHEMA) as a monomer, polyethylene glycol dimethacrylate as cross-linking agent and benzoin isobutyl ether as the UV-sensitive initiator. The film was prepared in de-ionised water and treated with UV radiation (λ = 253.7 nm, 11 mm distance from the source for 20 minutes). The film was then immersed for 24 h in water until it is fully saturated in order to remove toxic or unreacted substances that could damage a living tissue. Besides contact lenses, pHEMA can also be applied in artificial skin manufacturing and burn dressings, as it ensures good wound-healing conditions. It is also used for bone marrow and spinal cord cell regeneration, scaffolds for promoting cell adhesion and in artificial cartilage production [8-10]. Another low molecular weight cross-linking agent used in the synthesis of polyHEMA hydrogel is 1,1,1-trimethylol propane trimethacrylate. The hydrogel obtained with this agent is soft and contains 30-40 % of water & distinguished by its high oxygen permeability. These properties have translated its use in contact lenses, as matrices for drug delivery system and soft tissue implants. If the mechanical properties of the hydrogel are improved its application could further be extended. Cretu *et al*. has improved the hydrogel by synthesizing the amphiphilic material or introducing hydrophobic compounds (caprolactone) into its structure [11].

Polyetheleneglycol (PEG) based hydrogels are responsive towards external stimuli and hence these smart hydrogels are widely used in drug delivery system. Chemically cross-linked PEG hydrogels are used as scaffolds for protein recombination and functional tissue production. It is a suitable biomaterial for the efficient and controlled release of drugs, proteins, biomolecules and growth factor [12-14].

Lin and Anseth [13] have drawn attention to a new method of PEG hydrogel formation called 'Click' chemistry. This strategy based on a step-growth mechanism is distinguished by its rapid and specific reaction as well as versatility with respect to bio-conjugation. In this typical reaction, macromers bearing azide and alkyne functional groups are 'clicked' together in the presence of catalysts to form stable covalent linkages. This method produces PEG hydrogels with good mechanical properties and permits independent control of physical and chemical properties of the PEG hydrogels.

Polyvinyl alcohol (PVA) hydrogels can be obtained by alternating cycles of freezing and thawing. The PVA material prepared by this method has a greater mechanical strength than that obtained by UV radiation as the cross-linking agent. Functional groups of PVA are more accessible which broadens its range of application.

Polyvinyl pyrrolidone (PVP) hydrogels obtained by radiation technique could be applied for wound healing applications. Benamer *et al*. [15] prepared PVP solutions and carried out the irradiation using a 60Co source at a dose rate of 3.2 Gy/minute.

Polyacrylic acid (PAA) is another homopolymeric hydrogel [16]. Its commercial version contains 2.5 % of PAA and 97.5 % of water. It is stable and has optimal elasticity property. When used as an endoprosthesis, it was designed to be non-toxic, non-inflammatory and to imitate surrounding soft tissue.

2) Co-polymeric hydrogel

Co-polymeric hydrogels are composed of two types of monomer in which atleast one is hydrophilic in nature. Gong *et al*. [12] synthesized the biodegradable triblock poly(ethylene glycol)-poly(ε-caprolactone)- poly(ethylene glycol) (PECE) co-polymeric hydrogel for the development of drug delivery system. The mechanism involve here is the ring-opening copolymerization of ε-caprolactone. In the triblock synthesis mPEG was used as initiator, stannous octoate as catalyst and hexamethylene diisocyanate as coupling agent. This co-polymeric block is capable to form hydrogel when it is applied *in-situ*. The study reveals that the hydrogel was bio-degradable and bio-compatible. It was capable of releasing both the hydrophobic and hydrophilic drugs including proteins over a sustained period of time. This thermosensitive hydrogel has also been evaluated for cell encapsulation and tissue repair applications.

In another study, Kim and his co-workers attempted PEG based hydrogels to evaluate its feasibility to be used as a drug delivery system [14]. They prepared copolymers of methacrylic acid (MAA) with PEG-PEGMA by free-radical photopolymerization using tetra (ethylene glycol) dimethacrylate as cross-linker. The cross-linking occurred in the presence of an initiator, 1-hydroxycyclohexyl phenyl ketone in nitrogen atmosphere for 30-minutes under UV light. The hydrogel was successfully loaded with insulin. The authors claim that the swelling behavior and consequently the rate of release strongly depend on the molecular weight of PEG.

Cascone *et al*. [17] describe the synthesis of PVA based hydrogel as scaffolds for tissue engineering. A 10% aqueous solution of PVA was exposed to a high temperature in an autoclave (120°C/1h) and then mixed with a suitable modifier such as gelatin, dextrin, hyaluronic acid, collagen, dextrane or chitosan. The mixture was then subjected to eight cycles of freezing (253 K/1 h) and thawing (RT/30 min). This freeze-thawing cycle leads to the formation of PVA crystals which act as crosslinks between the polymer chains. The hydrogel was also evaluated as a matrix for drug delivery system.

Lugao *et al*. [18] obtained PVP hydrogels using different irradiation doses (5-15 KGy) and different additives: PEG (MW 600, 6000), Polyetheleneoxide (PEO) (400000) and glycerol. In the study, Glycerol and PEO were used to reduce the cross-linking density of the PVP network, whereas PEG increases the elasticity of the gels as a result of the plasticizing effect. Moreover, PVP/PEG hydrogels are sterile and non-cytotoxic, which makes PEG an ideal addition to biomedical hydrogels designed as a dressing material.

Wang *et al*. [19] suggested the use of cellulose or carboxymethyl cellulose (CMC) in the synthesis of PVP based hydrogel. CMC is water soluble and bio-compatible. Its low cost and high abundance were also the reasons for its blending with PVP. The PVP/CMC blend yields a hydrogel with good mechanical property (mechanical strength better than pure CMC and flexibility superior to that of pure PVP hydrogels). Its high water uptake capacity, enhanced bio-degradability and non-noxious quality translated it as perfect hydrogel dressing material.

Thomas *et al*. [20] synthesized a co-polymeric hydrogel by free radical copolymerization of two monomers namely acrylamide and acrylic acid using *N, N*-methylenebisacrylamide and potassium persulfate as the cross-linker and initiator respectively. The reactions were carried out in aqueous medium. The synthesized hydrogel was transparent in nature. Silver nanoparticles were embedded into this hydrogel. This hydrogel-silver nanocomposite exhibits antimicrobial activity.

A thermoplastic co-polymeric hydrogel based on γ-benzyl L-glutamate (BLG) and poloxamer was synthesized by polymerization of BLG *N*-carboxyanhydride, which was initiated by diamine groups located at the ends of poly(ethylene oxide) chains of the poloxamer. The resulting hydrogel was pH and temperature sensitive and characterized for drug delivery application [3]. The melting temperature (T_m) of the poloxamer in the copolymer was reduced with an increase of the PBLG block which is indicative of a thermoplastic property. The water contents of the hydrogel were dependent on the poloxamer content in the copolymers. Hydrogels water content was 31 and 41 wt % when the poloxamer quantum was 48.7 and 57.5 mol % respectively.

3) Semi- Inter Penetrating Network (Semi-IPN)

If one polymer is linear and penetrates another cross-linked network without any other chemical bonds between them, it is called a semi-inter penetrating network [5]. Semi-IPNs can more effectively preserve rapid kinetic response rates to pH or temperature due to the absence of restricting interpenetrating elastic network, while still providing the benefits like modified pore size & slow drug release etc. One example to justify the situation is the entrapment of linear cationic polyallylammonium chloride in acrylamide/ acrylic acid copolymer hydrogel which imparted both higher mechanical strength and fully reversible pH switching of theophylline release. This pH sensitive semi-IPN was synthesized by template copolymerization in the presence of *N, N'*-methylene

bisacrylamide as a cross-linking agent [21]. The network contained both covalent and ionic bonds. The covalent bonds retained the three-dimensional structure of hydrogel and the ionic bonds imparted the hydrogel with higher mechanical strength and pH responsive reversibility.

In another study [22], a semi-IPN hydrogel network was synthesized and evaluated as a nanoreactor for producing and stabilizing silver nanoparticles of 3-5 nm size. In this hydrogel, PVP chains were physically dispersed throughout PAA hydrogel network. The hydrogel-silver nanocomposites were characterized by using UV–vis, X-ray diffraction (X-RD), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The hydrogel-silver nano-architecture was found to have promising antibacterial effect.

Semi-IPN of gum arabic [23] and crosslinked copolymer of pHEMA was synthesized in the presence of ammonium persulfate and N, N-methylene bisacrylamide as an initiator and crosslinking agent respectively. The hydrogel was loaded with silver nanoparticles via *in situ* reduction of silver nitrate using trisodium citrate as reducing agent. The hydrogel-stabilized silver nanoparticles showed excellent antibacterial property.

Semi-IPN hydrogels, composed of alginate [24] and amine-terminated Poly (N-isopropylacrylamide) (PNIPAAm), were prepared by crosslinking with calcium chloride. From the swelling behaviors of semi-IPNs at various pH and FTIR spectra at high temperatures, the formation of a polyelectrolyte complex was confirmed from the reaction between carboxyl groups in alginate and amino groups in modified PNIPAAm. This alginate/PNIPAAm semi-IPN hydrogels are shown to be sensitive towards temperature, pH and ionic strength of swelling medium.

Polycationic semi-IPN hydrogels have also been used for drug delivery in the stomach. Semi-IPN of crosslinked chitosan and PEO showed more swelling under acidic conditions. This type of hydrogel has been investigated for the delivery of antibiotics such as amoxicillin and metronidazole in the stomach for the treatment of *Helicobacter pylori*.

A semi-IPN hydrogel of guar gum (GG) [25] and poly (methacrylic acid) was prepared at room temperature using water as solvent. 5-aminosalicylic acid (5-ASA) was loaded in the hydrogel and the entrapment efficiency was above 85%. It exhibited minimum swelling in acidic pH due to the formation of complex hydrogen-bonded structure and maximal swelling due to the electrostatic repulsion due to the ionization of the carboxylic groups in pH 7.4 medium. This results into a minimum release of 5-ASA at pH 2.2. *In-vitro* study reveals that the degree of degradation depends on the concentration of cross-linking agent and content of GG. The enzymatic degradation of hydrogels by cecal bacteria can accelerate the release of 5-ASA entrapped in the hydrogel at pH 7.4.

PVP based hydrogel [26] has been attempted as very promising thermosensitive material. The most vital shortcoming of PVP hydrogel as thermosensitive material is that it does not exhibit thermosensitivity under normal condition. The volume phase transition temperature (VPTT) of the semi-IPN hydrogel prepared from PVP and CMC was determined by swelling behavior and differential scanning calorimetry (DSC). The results showed that the VPTT was significantly dependent on CMC content and the pH of the swelling medium. VPTT occurred in buffer solution of pH 1.2 but did not appear in alkaline medium. Bovine serum albumin (BSA) as a model drug was loaded and the *in vitro* release were carried out in different buffer solutions. The results suggest that PVP/CMC semi-IPN hydrogels could serve as potential candidates for protein drug delivery in the intestine.

4) Inter Penetrating Network (IPN)

IPNs are conventionally defined as intimate combination of two polymers, at least one of which is synthesized or cross-linked in the immediate presence of the other [27]. This is typically done by immersing a pre-polymerized hydrogel into a solution of monomers and a polymerization initiator. IPN method can overcome thermodynamic incompatibility occurs due to the permanent interlocking of network segments and limited phase separation can be obtained. The interlocked structure of the cross-linked IPN components are believed to ensure stability of the bulk and surface morphology [28,29]. The main advantages of IPNs are relatively dense hydrogel matrices can be produced which feature stiffer and tougher mechanical properties, controllable physical properties and more efficient drug loading compared to conventional hydrogels. Drug loading is often performed in conjunction with the polymerization of the interpenetrating hydrogel phase [30]. IPN pore sizes and surface chemistries can also be controlled to tune the drug release kinetics, interaction between the hydrogel and the surrounding tissues along with its mechanical properties [31]. Interpenetrating phases with different degradation profiles and/or different swelling responses to physiological conditions can be used to provide multiple controls over the swelling responses of hydrogels and thus the drug release kinetics [32]. IPNs can moderate the effect of environmental changes on hydrogel responses and hence drugs burst release because of their ability to restrict the equilibrium swelling of either or both of the interpenetrating phases according to the elasticity (i.e. cross-linking density). Chivukula *et al*. has investigated highly cross-linked interpenetrating hydrogel network sensitive to pH fluctuations hydrogel and a hydrolysable hydrogel restricting the typical rapid swelling response of a pH responsive hydrogel. It facilitates linear swelling followed by an abrupt pH change from pH 7.4 to 2 [33]. Such responsiveness is particularly suitable for minimizing burst release of drugs in oral delivery applications. Lightly cross-linked chitosan/PNIPAM interpenetrating network has been studied which significantly increases the loading capacity of diclofenac compared to pure PNIPAM hydrogel while maintaining the sharp thermosensitivity of the PNIPAM phase to regulate the release kinetics [34].

In another example, polyethyleneglycol diacrylate (PEGDA) hydrogels modified with β-chitosan, which has improved biocompatibility. The hydrogel is formulated by adding 10 % aqueous solution of PEGDA into 2 % solution of chitosan in acetic acid. The mixture is cross-linked by UV radiation to form the hydrogel. This hydrogel displays IPN structure and contains 77-83 % of water.

Polyurethane (PU) is another classic biomaterial. An attempt was made by Kim *et al*. to extend the application of PU hydrogels. For this reason the IPN of PU and polyacrylamide (PAA) was obtained which can control water absorption [35]. The PU and PAA were mixed together and the respective cross-linking agent's viz. vinylpyrrolidone and methylenebisacrylamide were added followed by exposure of the mixture to UV radiation. This type of PU hydrogels are used for DDS, wound dressing material, artificial muscles, sensor systems and bio-separators.

Liu *et al*. have synthesized series of IPN hydrogels to impart sensitiveness towards temperature and pH fluctuations [36]. The investigators have incorporated one pH sensitive polymer, polyaspartic acid into the PNIPAAm hydrogel system for improving its response rate to environmental temperature. The morphologies and thermal behavior of the prepared IPN hydrogels were studied by both SEM and DSC. The IPN hydrogels showed a large and uneven porous network structure, without showing the structure of PNIPAAm hydrogel. The swelling experiments reveals that IPN hydrogels exhibited much faster shrinking and re-swelling with respect to the composition ratio of the two network components. These fast responsive hydrogels foster potential applications in biomedical fields.

In-situ polymerizable hydrogels are extensively investigated to implement new biomedical and pharmaceutical approaches. Polysaccharide hydrogel [37] based on calcium alginate (Ca-Alg) and dextran methacrylate derivative (Dex-MA), show potential application in the field of pharmaceutics. The semi-IPN obtained by dispersion of Dex-MA chains into Ca-Alg hydrogel leads to a hydrogel with rheological properties quite different from those of Ca-Alg. This allows injection of the semi-IPN easily through hypodermic needle. The UV curing of the semi-IPN results into cross-linking of methacrylate moieties, leads to an IPN hydrogel strong enough for modulated delivery of bioactive molecules like protein.

In another example [38] of polysaccharide based *in-situ* forming hydrogel, calcium alginate was interpenetrated within dextran-hydroxyethylmethacrylate-derivative (dex-HEMA). The IPN hydrogel was evaluated for protein release and the behavior of embedded cells. Degradation time of the IPNs after photocross-linking could be tailored from 15 to 180 days from the change of concentration and the degree of substitution of dex-HEMA. It was also observed that though there is an initial burst release of BSA the IPNs could extend it over 15 days. Encapsulation of expanded chondrocytes in the IPN revealed that cells remained viable and able to redifferentiate, as was demonstrated by the deposition of collagen type II. These results suggest that the IPNs are attractive materials for pharmaceutical and biomedical applications due to their tailorable mechanical and degradation characteristics, their release kinetics and biocompatibility.

Yunxiao Liu *et al*. [39] have investigated a new class of gelatin-dextran based hydrogel suitable for 3D encapsulation of Smooth Muscle Cells (SMC) and 2D culture of Endothelial Cells (EC). In this study a bifunctional dextran modified with methacrylate and aldehyde groups (Dex-MA-AD) mixed with gelatin and crosslinked under UV to produce IPN hydrogels which can encapsulate vascular SMCs. The Dex-MA-AD component imparted the hydrogels with elastic properties that are superior to PEG-based hydrogels. The incorporation of gelatin into the hydrogel provided cell adhesive and enzymatically degradable properties. It also significantly increases the compressive modulus and strength through the Schiff base contribution to the crosslink density. These hydrogels promote adhesion of vascular ECs in 2D culture and supported spreading and proliferation of vascular SMC in 3D culture up to 14 days. The attractive mechanical properties of this new class of IPN hydrogel coupled with 2D and 3D biocompatibility with vascular cells make this a promising material for 3D scaffolds for vascular tissue engineering and regeneration.

PROPERTIES OF HYDROGEL

Hydrophilic gels called hydrogels receive considerable attention for their use in the field of pharmaceutical and biomedical engineering. This material can be used as a carrier for drug and other therapeutic bio-molecule only if it is biodegradable, biocompatible and non-toxic *in situ*. Thus once the biomaterials are prepared one must evaluate the characteristic properties like swelling behavior, mechanical properties and toxicity studies etc so that the hydrogel could be used successfully in the concerned biomedical field.

1) Swelling properties

All polymer chains in hydrogels are cross linked to each other either physically or chemically and thus, considered as one molecule regardless of its size. For this reason, there is no concept of molecular weight of hydrogels and therefore, sometimes called infinitely large molecules or super macromolecules. A small change in environmental condition may trigger fast and reversible changes in hydrogel. The alteration in environmental parameters like pH, temperature, electric signal, presence of enzyme or other ionic species may lead to a change in physical texture of the hydrogel. These changes may occur at macroscopic level as precipitate formation, changes in size and water content of hydrogels. The difference in concentration of mobile ions in the hydrogel interior relative to external solution (osmotic pressure), changes in solvent pH, drives the volume change. Hydrogels with acidic or basic functional groups respond to the fluctuations in the external environmental pH. Degree of ionization of the functional groups dictates its swelling profile and hence the volume change. Polyacrylic acid is such type of pH sensitive hydrogel where swelling ratio changes due to the ionization of carboxyl groups on the polymer chain.

In other experiment, temperature-induced phase transitions and microenvironment of PNIPAM based hydrogels were studied in water using 9-(4-N,N-dimethylaminophenyl) phenanthrene (DP) as an intramolecular fluorescence probe. Fluorescence behavior of the DP-labeled PNIPAM gels depended on the concentrations of monomer and cross-linker. Thermo-responsive behavior of the PNIPAM hydrogel was affected by copolymerization of NIPAM with a hydrophilic monomer N,N-dimethylacrylamide (DMAM) and a hydrophobic monomer methyl methacrylate (MMA). Incorporation of DMAM raised the lower critical solution temperature (LCST) of the PNIPAM hydrogel and MMA lowered it. The results indicate that the NIPAM-DMAM copolymer hydrogel with higher LCST are more open with water-swollen nature above their LCST and the NIPAM-MMA co-polymer hydrogels with lower LCST are less open along with water shrunken nature below their LCST when compared with PNIPAM homo-polymer hydrogel [40].

2) Mechanical properties

Mechanical properties of hydrogels are very important from the pharmaceutical and biomedical point of view. The evaluation of mechanical property is essential in various biomedical applications viz. ligament and tendon repair, wound dressing material, matrix for drug delivery, tissue engineering and as cartilage replacement material. The mechanical properties of hydrogels should be such that it can maintain its physical texture during the delivery of therapeutic moieties for the predetermined period of time. Changing the degree of crosslinking the desired mechanical property of the hydrogel could be achieved. Increasing the degree of crosslinking a stronger hydrogel could be achieved though the higher degree of crosslinking decreases the % elongation of the hydrogels creates a more brittle structure. Hence there is an optimum degree of crosslinking to achieve a relatively strong and yet elastic hydrogel. Copolymerization with co-monomer, may result into hydrogen bonding within the hydrogel which has also been utilized by many researchers to achieve desired mechanical properties. Recently, Grassi *et al*. determined the mechanical properties of calcium alginate hydrogel. The mechanical characterization consisted of the relaxation experiments (normal stress relaxation at constant deformation) to determine the hydrogel linear viscoelastic range and to define the relaxation spectra and Young modulus by using the generalized Maxwell model. On the basis of Young modulus and Flory's theory, it was possible to determine the hydrogels cross-linking density. This value was then used to estimate the average polymeric mesh size according to the equivalent network theory [41].

3) Biocompatible properties

It is important for the hydrogels to be biocompatible and nontoxic in order to make it applicable in biomedical field. Most polymers used for this purpose must pass cytotoxicity and *in-vivo* toxicity tests. Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. Biocompatibility consists basically of two elements: (a) bio-safety i.e. appropriate host response not only systemic but also local (the surrounding tissue), the absence of cytotoxicity, mutagenesis, and/or carcinogenesis and (b) bio-functionality i.e. the ability of material to perform the specific task for which it is intended.

This definition is particularly relevant in tissue engineering since the nature of tissue construct is to continuously interact with the body through the healing and cellular regeneration process as well as during scaffold degradation. If this requirement is not met, the hydrogel can be fouled or there may be damage and scarring to connected tissues, whether those tissues are immediately adjacent or linked by vasculature. Toxic chemicals that may be used in the polymerization of synthetic hydrogels present a challenge for *in vivo* biocompatibility if conversion is not 100%. Furthermore, initiators, organic solvents, stabilizers, emulsifiers, unreacted monomers and cross-linkers used in polymerization and hydrogel synthesis may be toxic to host cells if they seep out to tissues or encapsulated cells. For example, Irgacure 2959, a typical photo-initiator used in many free radical photo-polymerizations, has been shown to decrease cell viability when used in concentrations over 0.1% [42]. To remove hazardous chemicals from preformed gels, various purification processes should be followed such as solvent washing or dialysis. *In situ* gelation of scaffolds, usually with oligomers and pre-polymers, presents a special challenge since reactants used to synthesize the gel are injected into the body while still in a pre-polymer solution. Utilization of this technique is ideal for its minimal invasiveness but requires special attention to ensure all components used are safe and reasonably nontoxic. Though natural polymers are frequently

regarded to have superior biocompatibility over synthetic one, yet the presence of synthetic cross-linkers and initiators used in the polymerizations of naturally derived monomers and pre-polymers are subject to the same toxicity concerns as purely synthetic hydrogels.

3.1) Evaluation of biocompatibility

In vitro cell culture tests are often used to screen the tissue compatibility of implantable devices. The cell culture methods are also known as cytotoxicity tests. Three primary cell culture assays are used to evaluate biocompatibility of the hydrogels include:

a) Elution (extract dilution) b) direct contact c) agar diffusion. These assays are described in the US Pharmacopeia and in standards published by the International Standards Organization. These are morphological assays and the outcome is measured by observation of changes in cell morphology.

The *in vivo* assessment of tissue compatibility of a hydrogel is the knowledge of chemical composition of the biomaterial and the conditions of tissue exposure (including nature, degree, frequency and duration of exposure). Principles generally applied to the biological evaluation of hydrogels are described as follows: The material(s) of manufacture; Intended additives, process contaminants, and residues; Leachable substances; Degradation products; Other components and their interactions in the final product; The properties and characteristics of the final product.

Most of the problems associated with hydrogel regarding toxicity, are the unreacted monomers, oligomers and initiators that leach out during application. So it is important to evaluate the toxicity of the hydrogel components like monomers, initiators and other building blocks used for its synthesis. Modifying the kinetics of polymerization and extensive washing of the resulting hydrogel can reduce the toxicity. The formation of hydrogels without any initiators and using alternate path like radiation may eliminate the problem of the residual initiator. PVA hydrogels synthesized by freeze-thawing method to induce crystallization also reduces cytotoxicity. The crystals formed act as physical crosslinks and are capable to withstand the load applied to the hydrogels. In a study, a biodegradable poly(ethylene glycol)-poly(epsilon-caprolactone)-poly (ethylene glycol) (PECE) triblock copolymer was successfully synthesized, which was flowing at low temperature (sol state) and turned to non-flowing state at body temperature (gel state). The toxicity of *in situ* forming PECE hydrogel as a potential opthalmic sustained drug delivery system was evaluated. The biodegradation of the hydrogel was studied in the eye compartment, its effect on cultured human lens epithelia, intraocular pressure and ocular tissues are also been included in the study. The results were in good agreement from biocompatibility and toxicity point of view [43].

Future prospects

The specific requirements of advanced drug delivery could easily be met by hydrogels. Wide array of methods for the synthesis of these novel biomaterials has extended its application from drug delivery system to tissue engineering scaffolds, wound dressing material, bioseparators, gene delivery device and biosensors etc. Further delve into the fundamentals of multi-polymer based hydrogel and their properties, may give raise a novel approach for implementing the biomaterials in the biomedical field in a better way.

REFERENCES

1. Prashant PK, Vivek BR, Deepashree ND, Pranav PP. Hydrogels as a drug delivery system and applications: a review. Int J Pharm Pharm Sci. 2012; 4: 1-7.
2. Das N, Bera T, Mukherjee A. Biomaterial hydrogels for different biomedical applications. Int J Pharm Bio Sci. 2012; 3: 586-595.
3. Oh SB, Choi YK, Cho CS. Thermoplastic hydrogel based on pentablock copolymer consisting of poly(γ-benzyl L-glutamate) and poloxamer. J Appl Polym Sci. 2003; 88: 2649-2656.
4. Isiklan N. Controlled release of insecticide carbaryl from sodium alginate, sodium alginate/gelatin, and sodium alginate/sodium carboxymethyl cellulose blend beads crosslinked with glutaraldehyde. J Appl Polym Sci. 2006; 99: 1310-1319.
5. Zhang JT, Bhat R, Jandt KD. Temperature-sensitive PVA/PNIPAAm semi-IPN hydrogels with enhanced responsive properties. Acta Biomater. 2009; 5: 488-497.
6. Krishna Rao KSV, Vijaya Kumar NB, Subha MCS, Sairam M, Aminabhavi TM. Novel chitosan-based pH-sensitive interpenetrating network microgels for the controlled release of cefadroxil. Carbohydr Polym. 2006; 66: 333-344.
7. Iizawa T, Taketa H, Maruta M, Ishido T, Gotoh T, Sakohara S. Synthesis of porous poly (*N*-isopropylacrylamide) gel beads by sedimentation polymerization and their morphology. J Appl Polym Sci. 2007; 104: 842-850.
8. Sykova E, Jendelova P, Urdzikova L, Lesny P, Hejcl A. Bone marrow stem cells and polymer hydrogels-two strategies for spinal cord injury repair. Cell Mol Neurobiol. 2006; 26: 1111-1127.
9. Kubinova S, Horak D, Kozubenko N, Vanecek V, Proks V, Price J et al. The use of superporous Ac-CGGASIKVAVS-OH-modified PHEMA scaffolds to promote cell adhesion and the differentiation of human fetal neural precursors. Biomaterials 2010; 31: 5966-5975.
10. Bavaresco VP, Zavaglia CAC, Reis MC, Gomes JR. Study on the tribological properties of pHEMA hydrogels for use in artificial articular cartilage. Wear 2008; 265: 269-277.
11. Cretu A, Gattin R, Brachais L, Barbier-Baudry D. Synthesis and degradation of poly (2-hydroxyethyl methacrylate)-*graft*-poly (ε-caprolactone) copolymers. Polym Degrad Stab. 2004; 83: 399-403.
12. Gong CY, Shi S, Dong PW, Kan B, Gou ML, Wang XH et al. Synthesis and characterization of PEG-PCL-PEG thermosensitive hydrogel. Int J Pharm. 2009; 365: 89-99.
13. Lin CC, Anseth KS. PEG hydrogels for the controlled release of biomolecules in regenerative medicine. Pharm Res. 2009; 26: 631-643.
14. Kim B, Peppas NA. Poly(ethylene glycol)-containing hydrogels for oral protein delivery applications. Biomed Microdevices 2003; 5: 333-341.
15. Benamer S, Mahlous M, Boukrif A, Mansouri B, Youcef SL. Synthesis and characterisation of hydrogels based on poly(vinyl pyrrolidone). Nucl Instrum Methods Phys Res. 2006; 248: 284-290.
16. Christensen L, Breiting V, Vuust J, Hogdall E. Adverse reactions following injection with a permanent facial filler polyacrylamide hydrogel (aquamid): causes and treatment. Eur J Plast Surg. 2006; 28: 464-471.
17. Cascone MG, Lazzeri L, Sparvoli E, Scatena M, Serino LP, Danti S. Morphological evaluation of bioartificial hydrogels as potential tissue engineering scaffolds. J Mater Sci Mater Med. 2004; 15: 1309-1313.
18. Lugao AB, Rogero SO, Malmonge SM. Rheological behaviour of irradiated wound dressing poly(vinyl pyrrolidone) hydrogels. Radiat Phys Chem. 2002; 63: 543-546.
19. Wang M, Xu L, Hu H, Zhai M, Peng J, Nho Y et al. Radiation synthesis of PVP/CMC hydrogels as wound dressing. Nucl Instrum Methods Phys Res. 2007; 265: 385-389.
20. Thomas V, Yallapu MM, Sreedhar B, Bajpai SK. A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity. J Colloid Interface Sci. 2007; 315: 389-395.
21. Zhang YX, Wu FP, Li MZ, Wang EJ. pH switching on-off semi-IPN hydrogel based on cross-linked poly(acrylamide-*co*-acrylic acid) and linear polyallyamine. Polymer 2005; 46: 7695-7700.
22. Murthy PSK, Murali Mohan Y, Varaprasad K, Sreedhar B, Mohana Raju K. First successful design of semi-IPN hydrogel-silver nanocomposites: a facile approach for antibacterial application. J Colloid Interface Sci. 2008; 318: 217-224.
23. Gils PS, Ray D, Sahoo PK. Designing of silver nanoparticles in gum arabic based semi-IPN hydrogel. Int J Biol Macromol. 2010; 46: 237-244.
24. Ju HK, Kim SY, Kim SJ, Lee YM. pH/temperature-responsive semi-IPN hydrogels composed of alginate and poly(*N*-isopropylacrylamide). J Appl Polym Sci. 2002; 83: 1128-1139.

25. Li S, Liu X. Synthesis, characterization and evaluation of semi-IPN hydrogels consisted of poly(methacrylic acid) and guar gum for colon-specific drug delivery. Polym Adv Technol. 2008; 19: 371-376.
26. Lu S, Liu M, Ni B, Gao C. A novel pH- and thermo-sensitive PVP/CMC semi-IPN hydrogel: Swelling, phase behavior, and drug release study. J Polym Sci Part B: Polym Phys. 2010; 48: 1749-1756.
27. Lipatov YS. Polymer blends and interpenetrating polymer networks at the interface with solids. Prog Polym Sci. 2002; 27: 1721-1801.
28. Muniz EC, Geuskens G. Polyacrylamide hydrogels and semi-interpenetrating networks (IPNs) with poly(N-isopropylacrylamide): mechanical properties by measure of compressive elastic modulus. J Mater Sci - Mater Med. 2001; 12: 879-881.
29. Zhang XZ, Zhou RX. Synthesis of temperature-sensitive poly (Nisopropylacrylamide) hydrogel with improved surface property. J Colloid Interface Sci. 2000; 223: 311-313.
30. Mohamadnia Z, Zohuriaan-Mehr MJ, Kabiri K, Jamshidi A, Mobedi H. pH-sensitive IPN hydrogel beads of carrageenan-alginate for controlled drug delivery. J Bioact Compat Polym. 2007; 22: 342-356.
31. Yin L, Fei L, Cui F, Tang C, Yin C. Superporous hydrogels containing poly(acrylic acid-*co*-acrylamide)/*O*-carboxymethyl chitosan interpenetrating polymer networks. Biomaterials 2007; 28: 1258-1266.
32. Li S, Yang Y, Yang X, Xu H. *In vitro* degradation and protein release of semi-IPN hydrogels consisted of poly (acrylic acid-acrylamide-methacrylate) and amylase. J Appl Polym Sci. 2007; 105: 3432-3438.
33. Chivukula P, Dusek K, Wang D, Duskova-Smrckova M, Kopeckova P, Kopecek J. Synthesis and characterization of novel aromatic azo bond-containing pH-sensitive and hydrolytically cleavable IPN hydrogels. Biomaterials 2006; 27: 1140-1151.
34. Alvarez-Lorenzo C, Concheiro A, Dubovik AS, Grinberg NV, Burova TV, Grinberg VY. Temperature-sensitive chitosan-poly (*N*-isopropylacrylamide) interpenetrated networks with enhanced loading capacity and controlled release properties. J Controlled Release 2005; 102: 629-641.
35. Abraham GA, de Queiroz AAA, San Roman JS. Hydrophilic hybrid IPNs of segmented polyurethanes and copolymers of vinylpyrrolidone for applications in medicine. Biomaterials 2001; 22: 1971-1985.
36. Liu M, Su H, Tan T, Synthesis and properties of thermo and pH-sensitive poly(N-isopropylacrylamide)/polyaspartic acid IPN hydrogels. Carbohydr Polym. 2012; 87: 2425-2431.
37. Matricardi P, Pontoriero M, Coviello T, Casadei MA, Alhaique F. *In situ* cross-linkable novel alginate-dextran methacrylate IPN hydrogels for biomedical applications: mechanical characterization and drug delivery properties. Biomacromolecules 2008; 9: 2014-2020.
38. Pescosolido L, Vermonden T, Malda J, Censi R, Dhert WJ, Alhaique F et al. *In situ* forming IPN hydrogels of calcium alginate and dextran-HEMA for biomedical applications. Acta Biomater. 2011; 7: 1627-1633.
39. Liu Y, Chan-Park MB. Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering. Biomaterials 2009; 30: 196-207.
40. Iwai K, Hanasaki K, Yamamoto M. Fluorescence label studies of thermo-responsive poly(N-isopropylacrylamide) hydrogels. J Luminescence 2000; 1289: 87-89.
41. Grassi M, Sandolo C, Perin D, Coviello T, Lapasin R, Grassi G. Structural characterization of calcium alginate matrices by means of mechanical and release tests. Molecules 2009; 14: 3003-3017.
42. Bryant SJ, Nuttelman CR, Anseth KS. Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts *in vitro*. J Biomater Sci Polym Ed. 2000; 11: 439-457.
43. Yin H, Gong C, Shi S, Liu X, Wei Y, Qian Z. Toxicity evaluation of biodegradable and thermosensitive PEG-PCL-PEG hydrogel as a potential in situ sustained ophthalmic drug delivery system. J Biomed Mater Res B. 2010; 92: 129-137.