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REVIEW

Methods of synthesis of hydrogels . . . A review

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Abstract Hydrogels are being investigated recently for the bioactive molecules (in particular pharmaceutical proteins) controlled release, such as matrices, and for the living cells encapsulation. Biodegradable nature of hydrogels has created much interest for drug delivery systems. The original three-dimensional structure disintegrates into nontoxic substances to ascertain an excellent biocompatibility of the gel. Chemical cross-linking is the highly resourceful method for the formation of hydrogels having an excellent mechanical strength. Cross-linkers used in hydrogel preparation should be extracted from the hydrogels before use due to their reported toxicity. Physically cross-linked methods for preparation of hydrogel are the alternate solution of cross-linker toxicity.

Contents

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1. Introduction

Hydrogels are polymer networks that take in and keep huge quantities of water. There are hydrophilic groups in the polymeric network, which become hydrated in aqueous media thus forming hydrogel structure. Because the term ''network'' is implied, cross-links must be present for the prevention of the dissolution of the polymer chains before use. Hydrogels may also be explored by the rheological manner. The solutions of water soluble polymers in low or intermediate concentrations where no considerable entanglement of chains occurs, normally exhibit 'newtonian' behavior. Furthermore, as cross-links between polymeric chains are introduced, networks obtained show viscoelastic and sometimes purely elastic behavior. Because of its ability to absorb water, hydrogels are under research to explore the fundamentals of swollen polymer networks and also have wide application in many technical areas, such as materials for protein separation and contact lenses, dies for encapsulating cells and devices for controlled release of proteins and drugs. For biodegradability of the hydrogels, labile bonds are introduced in the hydrogels that may be present either in the cross-links or in the network backbone. Unstable linkages may be cleaved in physiological conditions, either chemically or enzymatically, mostly by hydrolysis (Park et al., 1993). The enormous attention is the parameters control by which the degradation characteristics can be adapted. As the gels are used, this is of extreme importance that the hydrogels have excellent biocompatibility and degradation products produced have a low toxic potential. It means that either the substances produced can be excreted by glomerular filtration or can be metabolized into nontoxic products. Briefly, hydrogels hold excellent biocompatibility. Its water loving surface has less propensity for cells and proteins to stick to these surfaces. Furthermore, the elastic and soft nature of gels minimizes irritability to the neighboring tissues (Park and Park, 1996; Smetana, 1993; Anderson and Langone, 1999; Anderson, 1994). The properties of degradation products produced may be modified by the proper and rational selection of the starting materials of hydrogel.

Physical and chemical cross-linking methods of hydrogels preparation will be discussed in detail. In relation to their preparation methods, the properties and some possible applications of the hydrogels are also discussed. Covalent bonds are present between polymer chains in chemically cross-linked hydrogels whereas physical interactions exist between polymer chains, in physically cross-linked gels, to prevent from dissolution before use.

2. Physically cross-linked gels

Increased interest in physically cross-linked hydrogels in current era is due to the absence of cross-linkers used for synthesis. Table 1 gives some examples of physically cross-linked hydrogels (Polymer, method type and loaded drug are given in each case). Following are the different methods to synthesize physically cross-linked hydrogels.

2.1. By hydrogen bonds

Polyacrylic acid and polymethacrylic acid make complexes with polyethylene glycol. These complexes have hydrogen bonding between the oxygen of the polyethylene glycol and the carboxylic group of polyacrylic acid/polymethacrylic acid (Eagland et al., 1994). Hydrogen bonding is found not only between polymethacrylic acid and polyethylene glycol, but also in poly (methacrylic acid-g-ethylene glycol) (Bell and Peppas, 1996 and Mathur et al., 1998). Hydrogen bonds are formed only if the protonation of carboxylic acid groups occurs which shows pH dependent swelling of the gels.

Table 1 Some examples of physically cross-linked hydrogels: Polymer, method type and loaded drug are given in each case.

Sr. no.	Polymer	Method type	Loaded drug	Reference
1	PEG and PBT	Melt polycondensation of PEG and PBT	Lysozyme	Bezemer et al. (2000a)
2	Pullulan	Hydrogel nanoparticles	Adriamycin	Akiyoshi et al. (1996)
3	Polyacrylamide	Antigen–antibody interaction	IgG	Miyata et al. (1999)

2.2. From amphiphilic graft and block polymers

Amphiphilic graft and block polymers have ability to self-assemble in aqueous media to form hydrogels and polymeric micelles, in which the polymers hydrophobic parts are self-assembled. Hydrophilic diblock polymers produce lamellar phases, micelles etc. (Forster and Antonietti, 1998). Multiblock polymers may contain hydrophobic chains having hydrophilic grafts or a water-soluble polymer backbone to which hydrophobic segments are attached.

2.2.1. Polymers of PLGA and PEG

The biodegradability of polylactic acid or its polymer with glycolic acid and biocompatibility of polyethylene glycol motivated many researchers to create block polymers composed of these components, and for the purpose of drug delivery, to construct hydrogels from them. Release of drug may be motivated by degradation phenomena and passive diffusion.

2.2.2. Polymers of PBT and PEG

Feijen and coworkers studied multiblock polymers of PEG and a hydrophobic compound, poly (butylene terephthalate) (PBT) (Bezemer et al., 1999; Bezemer et al., 2000a,b). Melt polycondensation of butanediol, PEG and dimethyl terephthalate was used to synthesize such biocompatible polymers. For drug loading, the solutions of polymers were made in a hexafluoroisopropanol and chloroform (1:6) mixture and then W/O emulsion was prepared having the protein 'lysozyme' in water phase. The abovementioned emulsions were cast, to produce a film, or microspheres were synthesized using W/O/W emulsification method.

2.2.3. Hydrophobized polysaccharides

By hydrophobic modification, physically cross-linked hydrogels can be made from polysaccharides such as dextran, chitosan, carboxymethyl curdlan and pullulan. Hydrogels based on pullulan bearing cholesterol were focused by Sunamoto and coworkers (Akiyoshi et al., 1996; Taniguchi et al., 1999 and Akiyoshi et al., 2000). Monodisperse hydrogel nanoparticles with high water constituent (typically 80% w/w) were produced from pullulan bearing cholesterol upon dialyzing a solution from DMSO against PBS buffer. Insulin, BSA and α-chymotrypsin have been loaded and a hydrophobic anticancer drug adriamycin was loaded by simply mixing adriamycin and pullulan suspension (Akiyoshi et al., 1996).

2.2.4. Other graft and block polymers

Examples are: multi-block polymers of PEG-poly(γ-benzyl L-glutamate) (Cho et al., 2000), PEG-polyisobutylene (Kurian et al., 2000), poly(2-ethyl-2-oxazoline)-PCL which behaved like PEG–PCL hydrogels (Lee et al., 1998) and thermosensitive hydrogels from PEG–PNIPAAm (Lin and Cheng, 2001).

2.3. Cross-linking by crystallization

2.3.1. Crystallization in homopolymer systems

When aqueous solutions of polyvinyl alcohol (a natural hydrophilic polymer) are stored at room temperature, a gel is created, but, with a little mechanical strength. A tough and greatly elastic gel is produced when polyvinyl alcohol aqueous solution subjected to a freeze–thaw process (Yokoyama et al., 1986).

2.3.2. By stereocomplex formation

The homopolymers of L-lactic acid and D-lactic acid, respectively, are PLLA and PDLA (semi-crystalline substances). High molecular weight PLLA or PDLA, of either stereoisomer, has 170 °C Tm (melting temperature). In mixtures of high molecular weight PLLA and PDLA, a 230 °C Tm is observed, which is attributed to the stereocomplex formation.

2.4. Cross-linking by ionic interactions

Alginate may be cross-linked via calcium ions (Gacesa, 1988). Cross-linking is done at physiological pH and at room temperature. Alginate gels may be used as a matrix for protein release (Gombotz and Wee, 1998) and for the living cells encapsulation (Goosen et al., 1985).

2.5. Cross-linking by protein interaction

2.5.1. Genetically engineered proteins use

Tirrell and Cappello pioneered a new field in materials chemistry i.e. protein engineering (McGrath et al., 1992 and Cappello et al., 1990). The advantage of protein engineering is that the peptide sequence and therefore its physical and chemical properties may be controlled by rational design of the genetic code in synthetic DNA sequences. In addition to natural amino acids, synthetic amino acids may also be used (Yoshikawa et al., 1994).

Cappello and colleagues synthesized polymers of sequential block containing silk-like and elastin-like blocks repetition, in which silk-like segments (insoluble) are associated with the shape of aligned hydrogen bonded beta sheets through genetic engineering (Cappello et al., 1990 and Cappello et al., 1998). These biocompatible ProLastins are solutions in water which may be mixed with drugs and due to crystallization of the silk like domains undergo an irreversible sol to gel transition (with time) in physiological conditions.

2.5.2. By antigen–antibody interactions

In the presence of an additional cross-linking agent i.e. antibody, rabbit IgG was grafted to chemically cross-linked polyacrylamide (Miyata et al., 1999). In the presence of free antigen, the hydrogel showed slight swelling due to the polymer bound antigen replacement, resulting in the antibodies release along with a decrease in the cross-linking density.

3. Chemically cross-linked gels

Increased interest in chemically cross-linked hydrogels in current era is due to the good mechanical strength of chemically cross-linked hydrogels. Table 2 gives some examples of chemically cross-linked hydrogels (Polymer, method type and loaded drug are given in each case). Following are the different methods to synthesize chemically cross-linked hydrogels.

Table 2 Some examples of chemically cross-linked hydrogels: Polymer, method type and loaded drug are given in each case.

Sr. no.	Polymer	Method type	Loaded drug	Reference
1	Chitosan-PVA	Crosslinking with aldehyde	Nano-insulin	Zu et al. (2012)
2	Gelatin	Crosslinking with aldehyde	TGF-β1	Yamamoto et al. (2000)
3	Albumin	Crosslinking with aldehyde	Adriamycin	Willmott et al. (1984)
4	Chitosan	Crosslinking with aldehyde	Mitoxantrone	Jameela and Jayakrishnan (1995)
5	Dextran	Addition reaction	Hydrocortisone & prednisolone sodium phosphate	Brondsted et al. (1995)
6	PVA	Condensation reaction	Diltiazem hydrochloride	Ray et al. (2010)
7	Gelatin	Condensation reaction	Lysozyme	Kuijpers et al. (2000a)

3.1. Cross-linking by complementary groups chemical reaction

Hydrophilic polymers have certain hydrophilic groups namely NH_2, COOH, OH which may be used for the hydrogels development. The reactions such as an amine-carboxylic acid or an isocyanate-OH/NH_2 reaction or Schiff base formation, may be used to recognize covalent linkages between polymer chains.

3.1.1. Cross-linking with aldehydes

Hydrophilic polymers having –OH groups e.g. polyvinyl alcohol may be cross-linked through glutaraldehyde (Zu et al., 2012). To establish cross-linking, tight conditions are applied (low pH, methanol added as a quencher, high temperature). Alternatively, polymers having amine-groups may be cross-linked by the use of same cross-linker under mild conditions in which Schiff bases are formed. It was specially designed for the cross-linked protein synthesis, for example, gelatin (Yamamoto et al., 2000) and albumin (Willmott et al., 1984) and the amine containing polysaccharides (Jameela and Jayakrishnan, 1995).

3.1.2. By addition reactions

Bis or higher functional cross-linkers may be used to react with functional groups of hydrophilic polymers through addition reactions. Polysaccharides may be cross-linked by means of 1,6-hexamethylenediisocyanate (Brondsted et al., 1995), divinylsulfone (Gehrke et al., 1998), or 1,6-hexanedibromide (Coviello et al., 1999).

3.1.3. By condensation reactions

Polyesters and polyamides can be synthesized through condensation reactions among the –OH groups or –NH_2 with –COOH or derivatives, respectively. These reactions may be used for the hydrogel synthesis (Ray et al., 2010). A highly efficient reagent for cross-linking hydrophilic polymers having amide groups is N,N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC). Gelatin hydrogels were synthesized by Feijen and coworkers using EDC (Kuijpers et al., 2000a). To restrain any side reaction and to have a superior command on the hydrogel cross-linking density, N-hydroxysuccinimide was added during the reaction. Hydrogel was planned as a tool for antibacterial proteins release and was loaded into a prosthetic valve of Dacron. After synthesis, hydrogels were loaded with lysozyme and in vivo and in vitro lysozyme release was studied over a 2 day period. For loading capacity improvement, anionic polysaccharide (chondroitin sulfate) was also loaded into hydrogel (Kuijpers et al., 2000b).

3.2. Cross-linking by high energy radiation

High energy radiation e.g. gamma rays and electron beam may be used to polymerize unsaturated substances (Amin et al., 2012 and Alla et al., 2012).

3.3. Cross-linking by free radical polymerization

Chemically cross-linked hydrogels may be produced by free radical polymerization of polymerizable group derivatized hydrophilic polymers, besides free radical polymerization of vinyl monomers mixtures. To synthesize gels via this route, natural, synthetic and semi-synthetic hydrophilic polymers were applied. Using enzymes as catalyst, methacrylic groups have been introduced into the mono and disaccharides, which may be used for the hydrogel synthesis (Patil et al., 1996; Martin et al., 1998 and Patil et al., 1997). Moreover, by UV-polymerization, the hydrogel synthesis may be done (Hubbell, 1996), the planned structures may be synthesized and photo-reversible systems are also possible, which means that after exposing to UV light, preformed hydrogels degrade and so a drug is released (Andreopoulos et al., 1998).

3.4. Cross-linking using enzymes

An attractive method was devised to create PEG-based gels via using an enzyme by Sperinde et al. They functionalized glutaminyl groups with tetrahydroxy PEG (PEG-Qa). To aqueous solutions of poly (lysine-co-phenylalanine) and PEG-Qa, addition of transglutaminase resulted in the formation of PEG networks. Transglutaminase catalyzed reaction between the γ-carboxamide group of the PEG-Qa and the ε-amine group of lysine resulted in the formation of an amide bond (Sperinde and Griffith, 1997).

4. Conclusion

Novel hydrogel systems have been devised in current years. In terms of application, hydrogels are under research as matrices for the living cells encapsulation and for the pharmaceutically active proteins controlled release. Too many cross-linking methods have been devised and are currently available for

hydrogel synthesis. Physically cross-linked hydrogels are of huge interest for the labile bioactive substances and living cells encapsulation and entrapment, especially when hydrogel development does in the absence of organic solvents and under mild conditions. A number of physical cross-linking methods have been devised; there is undoubtedly a need for other methods. Supra-molecular chemistry principles will be used to devise new kind of gels with modifiable characteristics which may be synthesized preferably in aqueous environment. To give a way to the formation of hydrogel systems having a precise command on their microstructure and therefore characteristics thereof, protein engineering may also be useful.

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