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Review Article

A REVIEW ON NATURE AND PREPARATION OF HYDROGEL BASED ON STARTING MATERIAL

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ABSTRACT

Hydrogels are three dimensional crosslinked architectures with proven multifaceted applications. Nature has utilized these structured biomaterials instrumentally as mucus, vitreous humor, cartilage, tendons and blood clots. Thanks to the unique properties of hydrogel which could be tailored to apply them into different field of pharmacy, pharmaceutics and biomedical engineering. Hence the methods of hydrogel preparation and careful evaluation of properties are of utmost significance. Based on literature survey, preparation methods of hydrogels may also be classified on the basis of starting materials. Thus, hydrogels may be prepared from monomers, pre-polymers and polymers. Physical and chemical crosslinking methods are involved in the production of hydrogels for intended application. Physically developed hydrogels though less strong when compared with chemically crosslinked hydrogels, but free from unwarranted use of toxic chemicals. Physically crosslinked hydrogel formation is attributed to bonds formed by crystalline junctions, hydrogen bonding, phase-separation or other associations. Chemically crosslinked hydrogels on the other hand involves the reaction between functional groups present on polymer backbone with multifunctional crosslinking agents. Many of the hydrogels are product of schiff-base formation in which co-valent bond is involved. Co-polymerization of monomers and low molecular weight pre-polymers results into hydrogels formation. Ionic species and radiation takes active part in the initiation process of co-polymerization. Ceric ion and gamma radiation are the significant contributors in the catalysis process. This review article attempts to discuss naturally available biopolymer based hydrogels along with synthetically derived one and incorporates the findings from literature regarding methods of hydrogels preparation based on starting materials.

Keywords: Hydrogel, Natural, Synthetic, Monomer, Pre-polymer, Polymer.

INTRODUCTION

Hydrogels are three dimensional crosslinked architectures with proven multifaceted applications. The hydrogels are known to have good water interaction and swell when exposed to aqueous environment. The swelling continues till the elastic force and retractile force comes to an equilibrium point. When swelled, they are soft & rubbery; resemble the living tissue and exhibit excellent biocompatibility [1]. These force balanced systems have unique properties which could be tailored to apply them into different field of pharmacy, pharmaceutics and biomedical engineering [2]. In my earlier communication, a detailed discussion on hydrogel properties has been made [3].

Polymers having functional groups in its structure or functionalization of a neutral polymer exhibit promised response towards environmental stimuli (pH, temp, ionic strength, electric field, presence of enzyme etc.) and swell or shrink accordingly. This 'on-off' behavior could be manipulated depending on preparation methods in place for intended application. Chemical and physical cross-linking methods are very popular and being used in the development of hydrogels. Different types of hydrogel preparation methods are available in the literature and many of them are being discussed in details.

Hydrogels due to their unique biocompatibility, flexible methods of synthesis and range of constituents are widely used in different biomedical fields. This review article attempts to discuss hydrogel preparation methods on the basis of starting materials, namely monomer, pre-polymer and polymer. The article also incorporate findings from literature regarding the nature of biopolymer based hydrogel in comparison to hydrogels of synthetic origin.

NATURE OF HYDROGEL

i) Natural hydrogel

Nature has made extensive use of structured and homogeneous soft solids. Mucus, vitreous humor, cartilage, tendons and blood clots are all forms of a material known as hydrogel and playing vital roles. Hydrogels are three dimensional networks of polymer materials that confines and supports water without dissolving in it. Hydrogels typically may contain 50 to 90 percent of water depending on preparation and degree of crosslinking. Biological hydrogel formation based on natural macromolecules like polypeptides, proteins, nucleic acid or polysaccharides are highly significant. Vitreous humor of eye and cartilage present in the body, both are protein based hydrogels and derived from collagen. Though the hydrogels are chemically almost identical but marked differences could be achieved by tailoring the structure of crosslinked network resulting into low viscosity transparent fluid to a tough, load-bearing construct. The vitreous humor is a delicate, transparent gel composed of highly-hydrated double network of protein fibrils and charged polysaccharide chains. By weight, vitreous is 99% water and 0.9% salts [4]. The remaining 0.1% is divided into protein and polysaccharide components. Most of the protein is found as or associated with 10-20 nm heterotypic collagen fibrils composed of collagen (type V/XI) core wrapped by thick layer of collagen type II (75% of fibril by mass).

Considerable efforts have been made to materialize the behavioral features of these natural polymer based hydrogels. Viscoelasticity and permeability are the two very vital features of hydrogels present in human body. Viscoelasticity is the behavior that results from being halfway between rubber and water. Crosslinked polymer architectures i.e. hydrogels exhibit elastic response to rapid deformations, where the ability to transport fluid results in a compliant response to slow deformations, while still maintaining their shape over long periods. Viscoelasticity is one pivotal factor in cartilage's success as a load bearing surface. Cartilage is conformable under static loads, but maintains shape and elasticity when subjected to impact loads. Cartilage being hydrogel in nature, results in the generation of lubricating layer of fluid on its surface. This ultimately reduces the coefficient of friction on the bearing surface. In addition to that permeable feature of hydrogels allows them to be populated by cells and transport nutrients and solutes across spaces in the body.

Biopolymers have drawn considerable attention in designing hydrogel with many tailorable properties. One very widely applied biopolymer is alginic acid. This polysaccharide consists of alternating manuronic and guluronic acid backbone and exhibits gelation when comes in contact with divalent cations. An experiment on Sprague-Dawley rats has shown that sodium salt of alginic acid is a good carrier of Schwann cells. Schwann cells are known for its vital role in promoting neuronal growth, development and survival along with axon growth. Schwann cells-sodium alginate hydrogel transplant demonstrate the feasibility of treating spinal cord injury [5]. Another experiment carried out by Grogan *et al.* studied the effects of perfusion

and dynamic loading on human neocartilage formation in alginate hydrogels which ends with appreciable results [6]

Gelatin is another important class of natural polymer. It is protein in nature and derived from collagen extracted from animal tissue. When crosslinked with selective agent, it is capable of hydrogel formation. CCN family protein 2 (CCN2) is a unique molecule known for its utility in bone regeneration. It enhances the adhesion and migration of bone marrow stromal cells along with the growth and differentiation of osteoblasts. Kikuchi *et al.* reports the application of CCN2-gelatin hydrogel, together with collagen scaffold to the bone defect prepared in a rat femur model. The *in-vivo* experiment results in remarkable induction of osteoblastic mineralization and bone regeneration in 3 weeks [7].

An engineered hydrogel based on hyaluronic acid (a naturally-occurring body substance) when placed into spinal cord injured site, decreases scarring and promotes the realignment of spinal cord fibers. Hyaluronic acid forms scaffold like configuration which helps in the structural stabilization of spinal cord injured site [8, 9].

ii) Synthetic hydrogel

Synthetically derived hydrogels may be grouped into two broad classes; chemically and physically crosslinked hydrogel.

Chemically-crosslinked hydrogels are three dimensional polymer networks that have bonds between the chains. Many hydrogels are yield of covalent bonds. These hydrogels could also be formed directly from hydrophobic monomers. Monomers like vinyl pyrrolidone, methacrylic acid and poly-2-hydroxyethyl methacrylate [10] forms the basis of hydrogel formation and mostly used in the fabrication of contact lenses. Additionally, hydrogel could also be engineered by the crosslinking of hydrophilic polymer chains. Among the methods of crosslinking, utilization of radiation or hydrolysis of hydrophobic network is very popular. Although extensive research is going on to provide a safe biomaterial, chemically-crosslinked systems have certain drawbacks like reaction that generates the crosslinks results in increase of heat (exothermic) as the bond forms; unused materials remains which are reactive and potentially toxic; and bond forming reaction results in by-products that may have undesirable safety profiles.

Hydrogels formed from physical interaction present a broad class of materials. These physical bonds could be formed by crystalline junctions, hydrogen bonding, phase-separation or other associations. Strength of the hydrogel depends upon the strength of these physical bonds and their density. For example, tri-block copolymers can be constructed with hydrophobic end blocks and a hydrophilic center block. When placed in water, the end blocks associate into tight bundles and the hydrophilic blocks absorb water and expand. This results into a three dimensional architecture with a reasonable strength. Polyvinyl alcohol (PVA) is an interesting polymer that could be transformed into hydrogels by a variety of mechanisms. PVA can covalently be crosslinked to form hydrogel. On the contrary, PVA gel formation by hydrogen bonds is also very popular. Hydrogen bond formation results from the interaction between hydrogen atom and an electronegative atom, oxygen. Though the bond is relatively weak when compared to covalent bond, it can still be structurally stable. It is this bond which is used to stabilize folded proteins and provide water with its unique properties. These hydrogen bonds create crystalline junction points in the polymer chain and translate into hydrogel. PVA based hydrogels can be made by repeated freezing and thawing of PVA solution [11]. This allows the polymer chains to move into sufficiently close proximity to form the hydrogen bonds and subsequent crystalline junction points. With increase in number of freeze-thaw cycles, tougher hydrogel could be produced. PVA is used in variety of biomedical applications [12] including drug delivery, cell encapsulation, artificial tears, artificial vitreous humor, contact lenses and more recently as nerve cuffs.

PREPARATION OF HYDROGEL BASED ON STARTING MATERIAL

Hydrogels are a class of biomaterials that have multifaceted functionalities. The elastic and retractile force balanced system; hydrogel could be prepared from monomers, pre-polymers or existing hydrophilic polymers.

i) Hydrogel preparation based on monomers

Co-polymerization of hydrophilic monomers and polyfunctional co-monomer cross-linkers react to form hydrophilic crosslinked structures. Most commonly used monomers like (meth)acrylates and (meth)acrylamides are hydrophilic in nature. The first example reported [13] was (2-hydroxyethyl) methacrylate (HEMA) and ethyleneglycol bismethacrylate copolymer and hydrogel that has been used in the production of soft contact lenses and reservoir for drug delivery. In another study [14], HEMA was copolymerized with various hydrophilic cyclic monomers like cis-1,2-bis(2,3-epoxybutanoyloxy), cis-1,2-bis(10,11-epoxyundecanoyloxy), cis-1, 2-bis(trimethylsiloxy) and cis-1,2-bis(tert-butyldimethylsiloxy)-3,5-cyclohexadienes to prepare hydrogels. Crosslinked copolymers of acrylamide and methylene bisacrylamide are used frequently in the preparation of hydrogels for electrophoresis. Radiation, ultraviolet rays or chemical catalysts generally initiates polymerization reaction. The choice of suitable initiator depends on type of monomers and solvents being used. Chemical initiators include ferrous salts, sodium metabisulfite, ammonium per sulfate and hydrogen peroxide. Cobalt-60, Cesium-137 and electron beam accelerators are the sources of radiation used in the polymerization process.

Vinyl monomer polymerization is most frequently initiated by radical initiators like peroxides and azo-compounds. Radicals may be generated by heating, using redox initiators such as ammonium persulfate and N, N, N', N'-tetramethyl ethylene diamine (TEMED) or a photoinitiator. Aboulfazl Barati and his coworkers [15], synthesized a cationic hydrogel from methacrylamide (MAAm) monomer using radical copolymerization technique where ammonium persulphate (APS) and TEMED were used as initiator and activator, respectively. In the polymerization process the reaction between APS and TEMED forms an active molecule. TEMED activated molecule has an unpaired electron valance which reacts with MAAm, a cationic agent (Methacrylamido propyl trimethyl ammonium chloride) and crosslinker (N, N'-methylenebis-acrylamide). Additionally, radical polymerization process could also be initiated by high energy irradiation. Recently research is focused in the preparation of hydrogel based on the polymerization of water soluble monomers. Radicals generated by ultrasound initiates the polymerization process [16]. Polymerization of water soluble acrylic monomers in presence of viscosity enhancers falls in this category. Styrene, acrylonitrile, acrylamide and acrylic acid are the monomers whose polymerization could be achieved by this technique.

Photo-initiators are the light sensitive materials that exhibit high absorption at specific wavelength and produces radical initiating species. Photo-initiator selection takes into account, factors like biocompatibility, water solubility, stability and cytotoxicity. Utilization of photo-initiator in the process of polymerization and hydrogel preparation has been illustrated by Rivarola and his coworkers [17]. A visible light photo-initiator namely (tris (2,2′-bipyridine)ruthenium(II)/*N*,*N*-dimethylaniline) is shown capable of polymerizing *N*-isopropylacrylamide (NIPAM) and 2-acrylamido-2-methylpropanesulfonic acid (AMPS) in aqueous solution to render high molecular weight polymers and crosslinked hydrogels. The photo-initiator is specially designed to synthesis thermosensitive polymers and hydrogels. The initiator is efficiently used at temperatures below phase transition and allows the polymer chain to grow in its uncoiled state. It is reported that the polymerization and conversion rates are affected by the structure of monomers and decreases in the order of NIPAM > AAm > AMPS. This method produces smart hydrogels.

Hydrogels based on polyesters and polyamides are prepared by condensation or step-addition polymerization techniques. In this process a small molecule may split and results in the formation of an ester or amide bond. Condensation polymerization technique is reported in the preparation of PVA and egg-albumin based cryogels [18]. Literature reports a fast responsive ionic hydrogel which is produced using sodium salt of AMPS as the monomer and N, N'-methylene(bis)acrylamide (BAAm) as a crosslinking agent [19]. The gels when prepared well below (-22^{0}C) and in other case, above the bulk freezing temperature of water (25^{0}C), translates into cryogels and hydrogels respectively. The cryogels exhibit super fast swelling-

deswelling properties when exposed to water and acetone. An increase in the initial monomer concentration from 2.5 to 10% further increases the response rate of the cryogels due to simultaneous increase of porosity of the networks.

ii) Hydrogel preparation based on pre-polymers

Hydrogels have also been prepared based on low molecular weight hydrophilic polymers or oligomers. Polyurethane hydrogel formation from the reaction of α,ω-hydroxyl poly(ethylene glycol) with diisocyanate using a triol as a crosslinking agent, may be included as an example of this class.

Linear PVA was modified with glycidyl acrylate (Acr) to form a macromer that was radically crosslinked by photo-initiated mechanism [20]. The crosslinking reaction behavior of Acr-PVA macromer was found to be dependent on initial macromer concentration. Generally, 100% conversion of macromer is observed in just few minutes when photo-polymerization reaction was carried out in place. Swelling and mechanical properties were evaluated which confirmed that difference in crosslinking densities could be achieved by variations in the initial macromer concentration. A comparative study was conducted and verified the imperfections and structural differences in the photo-crosslinked networks, where PVA was crosslinked with glutaraldehyde and Acr-PVA was crosslinked in a semi-crystalline state. Short reaction time and versatile properties of the PVA gels paved them to be used in many potential applications.

PEG-grafted chitosan [21] aqueous solution exhibit thermally reversible phase transition behavior. The graft transforms from an injectable free-flowing sol at low temperature to a gel at body temperature. Aqueous solution of PEG-grafted chitosan could be prepared at physiological pH, thereby allows safe incorporation of bioactive molecules. This injectable thermo-reversible hydrogel is potentially suitable for wide range of biomedical applications including sustained *in vivo* drug delivery and tissue engineering.

Another attempt has been made to study, poly (N, N-dimethylacrylamide) grafted chitosan (PDMAAm-g-CT) hydrogel for DNA adsorption [22]. Poly (N, N-dimethylacrylamide) with terminal carboxylic acid group (PDMAAm-COOH) was synthesized first by free-radical polymerization reaction using mercaptoacetic acid as the chain-transfer agent. It was then grafted onto chitosan backbone. The PDMAAm-g-CT hydrogel thus produced were utilized in DNA adsorption experiments conducted at 40^0C in tris EDTA solution of pH 7.4. The data reveals that hydrogels made of higher PDMAAm-COOH content exhibit higher DNA adsorption capacity. PDMAAm-g-CT hydrogels comprising 80 wt % PDMAAm-COOH feed concentration show DNA adsorption capacity upto 4620 µg DNA/gm dry gel. Seven times higher adsorption capacity value unveils the suitability of the graft hydrogel when compared with CT alone.

Polysaccharide biopolymers have gained considerable attention due to its exceptional properties like biocompatibility, biodegradability, renewability and non-toxicity. Graft copolymerization of vinyl monomers onto polysaccharides backbone is one efficient route to prepare hydrogels. Vinyl monomers grafted onto polysaccharides such as starch, chitosan, sodium alginate, carrageenan and cellulose have shown hydrogel formation ability. Cerium in its tetravalent state is a versatile oxidizing agent and used most frequently in the graft copolymerization of vinyl monomers onto cellulose & starch. Under slightly acidic conditions, it forms a redox pair with anhydrous glucose units of polysaccharide to yield macro-radicals. Graft copolymerization of acrylic and methacrylic acids onto chitosan and methyl methacrylate on various natural substrates such as carboxymethyl cellulose, hydroxypropyl cellulose, carboxymethyl starch and hydroxypropyl starch in aqueous medium are very popular instances. Both the graft co-polymerization was initiated by ceric ion. The same radical system was involved in the grafting process. The graft copolymerization of sodium alginate with polyacrylamide using ceric ammonium nitrate initiator has also been reported. Catalyst potassium persulphate initiated graft copolymerization of acrylonitrile and methylmethacrylate onto chitosan has been reported in the literature [23]. Later, fatty acid grafting on starch has also been reported by Simi and Abraham [24] using the same catalyst, potassium persulphate.

In addition to the above mentioned chemical systems, various redox initiating systems have also been tried for the synthesis of polysaccharide-based graft copolymers. A study by Behari *et al*. [25] revealed that the graft copolymerization of acrylamide onto xanthan gum could be initiated by the Fe^{2+}/BrO^3 redox system in aqueous medium under nitrogen atmosphere. They observed that grafting takes place efficiently when acrylamide concentration and temperature were 4.0×10^{-3} mol dm^{-3} and 35°C, respectively. Graft copolymerization on guar gum with N-vinyl formamide [26] and acrylic acid [27] has been established using potassium bromate/ascorbic acid and peroxydiphosphate-silver (I) redox pairs, respectively. More recently, graft copolymers of sodium alginate with itaconic acid has been prepared in aqueous solution using benzoyl peroxide as initiator [28].

Although radiation-based grafting is cleaner and more efficient than chemical initiation methods, they are harder to handle under technical conditions. Hence, few reports are available. Graft co-polymerization of 2- hydroxyethylmethacrylate onto chitosan films was carried out using 60Co γ-radiation to improve its blood compatibility. It was found that level of grafting could be controlled by the grafting conditions namely solvent composition, monomer concentration, dose rate and total dose. Literature reports another study conducted to graft N-isopropylacrylamide onto alginate, with varying dosages of 60Co γ-radiation onto alginate films in deionized water and methanol media. At 50 kGy of irradiation dose, N-isopropylacrylamide monomers were grafted on alginate with graft ratio of 18.7% [29]. Microwave irradiation was used in an instance for grafting polyacrylonitrile onto guar gum polymer. The grafting was carried out in water without using any radical initiator or catalyst and very short reaction time was required. The grafting was controlled by optimizing the reaction conditions and maximum percentage of grafting; about 188% was obtained in just 1.66 minutes [30].

iii) Hydrogel preparation based on polymers

Selective chemical crosslinking of hydrophilic polymers results in the formation of hydrogel. Sephadex is a three dimensional crosslinked network of dextran where epichlorohydrin has been used as a crosslinking agent. Sephadex is used as stationary phase in gel filtration chromatography. One very well known example of selective chemical crosslinking is the formation of gelatin hydrogel using polyaldehyde as a crosslinking agent. Polyelectrolyte like sodium alginate, chitosan etc could also be transformed successfully into hydrogel when crosslinked with specific di- or tri-valent counter ions. Gelation of sodium alginate by Ca^{2+} ion and chitosan by sodium tripolyphosphate ion are the classic examples of this category. Though chemical crosslinking is associated with few drawbacks, but comparatively stronger and permanent crosslinked architecture with tailorable properties could be achieved by this method. Glutaraldehyde is an example of crosslinking agent that is widely used in the preparation of hydrogels from -OH groups containing polymers like poly (vinyl alcohol). Amine functional polymers can also be crosslinked with glutaraldehyde under mild conditions whereby so-called Schiff bases are formed. Investigation on proteins like albumin, gelatin and polysaccharides also revealed the application of chemical cross linkers in the production of hydrogels. Scleroglucan is a branched homopolysaccharide and resistant to hydrolysis. This polysaccharide is crosslinked with borax to produce hydrogel [31]. Polyethylene oxide cross-linked with 4, 4'-diphenylmethane diisocyanate produces polyurethane hydrogel network and the said agent is also used in the preparation of interstitial hydrogel of poly (methylacrylate).

Physical entanglement of polymer chains is also capable of producing three dimensional networks. The hydrogels thus achieved are strong enough to execute intended action which however free from many toxic chemical reagents. Gelatin and agarose are the classic examples of two biopolymers that could be transformed successfully into hydrogels upon cooling from its aqueous solution. The gel formation is attributed to the helix-formation and association of the helices, forming junction zones. Currently, physical gels have drawn considerable attention due to temperature dependent sol-gel transition behavior.

FUTURE PROSPECTS

To meet the specific requirements of advanced drug delivery system, sophisticated delivery devices need to be fabricated. Crosslinked architectures of monomers, pre-polymers and polymers, known as hydrogel are currently most preferred delivery device. Biocompatible and biodegradable hydrogels have already established its suitability in biomedical fields of interest like drug delivery, tissue engineering scaffolds, wound dressing materials, gene delivery device and as biosensors. Novel conceptual assimilation of hydrogel preparation may lead to tailored properties, translating its innovative applications in the field of pharmacy, pharmaceutics and related field of biomedical engineering.

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