ABSTRACT

A considerable amount of work has been published on chitosan and its potential use in drug delivery systems. Chitosan has a cationic character because of its primary amino groups. These primary amino groups are responsible for properties such as controlled drug release, mucoadhesion, in situ gellation, transfection, permeation enhancement and efflux pump inhibitory properties. Due to chemical modifications, most of these properties can even be further improved. Chitosan Hydrogel has been recently developed to serve as semi synthetic or synthetic extra cellular matrix to provide an amenable environment for cellular adherence and cellular remodeling in three dimensional structures mimicking that of natural cellular environment. Additionally, hydrogels have the capacity to carry small molecule drugs and/or proteins, growth factors and other necessary components for cell growth and differentiation. In the context of drug delivery, hydrogels can be utilized to localize drugs, increase drugs concentration at the site of action and consequently reduce off-targeted side effects. This review classifies hydrogels and their method of preparation.

KEYWORDS: Chitosan, Hydrogels, Drug Delivery, Deacetylation, Photopolymerization.

INTRODUCTION

Recently there has been an increasing interest in developing medically relevant hydrogels. Such an interest is due to the wide range of suitable characteristics and preparation methods for hydrogels in medical and pharmaceutical industries.
Various natural and synthetic polymers have been studied in hydrogel researches. Chitosan is a natural cationic copolymer and has hydrophilic nature with ability of degradation via human enzymes which result in biocompatibility and biodegradability, the two biological properties commonly needed for biological devices. Chitosan-based hydrogels are potentially used to obtain tissue repair achievements.

Furthermore, they have been applied as delivery systems for the controlled release of active pharmaceutical ingredients. Many polymers used in hydrogel lattices, like chitosan, employ their mucoadhesive characteristics via interactions between opposite charges. This specific feature can provide the ability of tissue binding for the aim of specific drug delivery. In order to improve feasibility of chitosan for medical and pharmaceutical applications, several derivatives of chitosan with different ligands such as 4-azidobenzoic acid, methyl acroloyl glycine and poly ethylene glycol have been synthesized and studied. In this review, hydrogel structure and characterization of the systems compromised from chitosan derivatives prepared using different ligands will be briefly explained. Then, specifically chitosan-based hydrogels with biomedical and biopharmaceutical applications will be discussed and their cons and pros in comparison with each other would be interpreted.

**Hydrogels**

Hydrogels are cross-linked networks of the same or different types of polymers with high capacity for water absorption.

Hydrogel forming polymers have hydrophilic functional groups in their polymeric structure such as amine (NH2), hydroxyl [-OH], amide (-CONH-, -CONH2) and sulphate (-SO3 H). The hydrophilic groups enable the hydrogel to absorb water and watery fluids that results in hydrogel expansion and occupation of larger volume, the process which is known as swelling. During swelling, the cross-linked structure of hydrogels prevents the dissolution and destruction of the hydrogel cross-links. A schematic representation of hydrogel swelling is shown in Fig.1.
There are three states of water molecules in polymeric hydrogel, free, intermediate and bound water molecules. Free water molecules undergo freezing process at freezing points since no bond exists between free water and polymer functional groups. The amount of free water molecules is dependent on the hydrogel structure that ultimately influences the swelling ratio. Thereby, a compact hydrogel structure contains lower quantity of free water. Second state of water or intermediate water can form weak interactions with functional groups in polymeric chains. Hydrogen bonding between polymeric chains and water molecules forms bound water. These water molecules are nonfreezing.[5]

The amount of water absorption in different types of hydrogel is varied Quantity and speed of water absorption is dependent on the following factors: i) cross-linking density, ii) chemical structure of the polymers and iii) environmental conditions.

Cross-linking density, the amount of cross-linked chains, governs the hydrogel swelling ratio and is inversely proportional to water quantity. In addition, presence of hydrophilic or hydrophobic functional groups on the polymer chain determines the swelling ratio.[6] Hydrogels have ability to swell in water or aqueous solutions. These structures can be similar to human body tissues because of high amount of water absorption.

Hydrophobic hydrogels with hydrophobic chains such as poly (Lactic acid) (PLA) or poly (Lactide-co-glycolide) (PLGA) or those prepared via polymeric modifications to enhance polymer hydrophobicity have lower water capacity than hydrophilic lattices. Swelling ratio of hydrophilic hydrogels varies with polymer hydrophilic density. Environmental conditions such as pH, temperature, certain chemicals,[7] light, pressure and electrical field[3] are influential on hydrogel swelling. The environmental factors control the swelling kinetics and
could be modified to modulate the swelling properties of the hydrogels. For example protein-based systems are susceptible to acidic gastric environment. Therefore, pH sensitive swelling hydrogels that are swollen in intestinal pH are useful devices for the purpose of oral delivery.

**Classification of hydrogels**

Hydrogels are classified into natural or synthetic polymeric based.

Natural hydrogel constructs are often made of polysaccharide or protein chains. Polysaccharides have hydrophilic structure which is a favorable property of hydrogel preparation. Some examples of polysaccharide-based hydrogels are hydrogels made of alginate, cellulose, chitin, chitosan, dextran, hyaluronic acid, pectin, starch and xanthan gum. Collagen, silk, keratin, elastin, resilin and gelatin are protein chains that form natural hydrogel lattices.

Synthetic polymers such as poly (vinyl alcohol), polyacrylamide, poly (ethylene oxide) and poly (ethylene glycol) have been used for hydrogel formation. Natural polymers usually present higher biocompatibility compared to synthetic polymers, as they undergo enzyme controlled biodegradation by human enzymes like lysozyme and produce biocompatible byproducts.[8] On the other hand, synthetic polymers are chemically stronger than natural ones, because of hydrolysable moieties with slower degradation rate. This feature provides more prolonged lifetime in human body.[9]

**Formation of Hydrogels**

Hydrogels are prepared via physical cross-linking or chemical (permanent bonds). Methods for chemical cross-linking of hydrogels include i) radical polymerization,[10] ii) photopolymerization,[11] iii) enzymatic reactions, iv) and covalent cross-linking via linkers such as aldehydes.[12] In contrast, physical cross-linking forms a nonpermanent network with physical interactions such as hydrogen or electrostatic bonds, crystal formation and physical entanglements.[13] So the physically cross-linked hydrogels can be formed via ion interactions, using graft copolymers,[14] crystallization and stereocomplex formation.

**Applications of hydrogels in pharmaceutical sciences**

Hydrogels are widely used in food industry, agriculture and pharmaceutical fields. In pharmaceutical area, they are applied for systemic and localized drug delivery and tissue engineering.
Drug delivery

Hydrogels are used as platforms for drugs delivery as well as gene delivery. Hydrogels can encapsulate macromolecule drugs like proteins into their polymeric chains. Polymeric network of hydrogels protects drugs from fast dissolution and eventually control release rate from matrices.

Hydrogels can be administered via oral, ocular, nasal, vaginal and subcutaneous routes. Hydrogels are also utilized extensively in tissue repairs. Hydrogels have also been developed as artificial cartilages, contact lenses, artificial corneas, biosensors and surgical aids. Synthetic materials are also applied as alternative to extracellular matrix.

Chitosan-based hydrogels

Chitosan

Chitosan is obtained by partial deacetylation of insoluble naturally available chitin, obtained from exoskeletons of crustaceans, fungi and insects. Chitin has rigid crystalline structure due to hydrogen interactions between acetamide groups and hydroxyl groups. Chitin is not readily applicable due to its high level of acetylated groups and rigid structure as well as poor solubility in aqueous solutions. When chitin is partially deacetylated and converted to chitosan (Fig. 2), the amount of amino groups and its aqueous solubility is enhanced. There is a proportional increase in chitosan deacetylation, and enhancement of bio-compatibility and biodegradability.

Figure 2: Chitin is extracted from crab shell from which chitosan is made by N-deacetylation
The polysaccharide structure of chitosan is made of glucosamine and N-acetylglucosamine. Glucosamine is generated from glucose in body and it can produce glucosaminoglycans (GAGs), which is a part of extracellular matrix and cartilage tissue.

The charge density of chitosan depends on degree of deacetylation which represents the amino group density. Indeed, pH of the chitosan solution represents the quantity of ionized amino groups.

Chitosan is a weak base with \( pK_a \) 6.5 which can be dissolved in dilute acidic medium. Because of the presence of amine and hydroxyl groups, chitosan molecules can form hydrogen bonds leading to the crystalline structure of the polymer.\(^{[15]}\)

Chitosan exists in different molecular weights and degree of acetylation. The average molecular weight of chitosan lies between 50-2000 KD. Hydrophilic polymers such as chitosan may undergo systemic absorption in human body, so the polymers should have proper molecular weight to eliminate by renal filtration. In vitro studies showed that chitosan can be degraded via several enzymes such as \( \beta \)-N-acetylhexosaminidase, chitosanase, chitinase and chitin deacetylase. In human body, chitosan can be biodegraded by lysozyme, acid, gastrointestinal enzymes and colon bacteria.\(^{[15]}\)

**Hydrogel preparation via chitosan cross-linking**

The intermolecular forces between polysaccharide chains of chitosan are hydrogen, hydrophobic and ionic interactions. These interactions are influenced by molecular weight and ionic strength.\(^{[16]}\)

Cross-linking of chitosan polymers is necessary to improve chitosan properties such as stability and durability for the aim of drug delivery. Chitosan based hydrogel networks are categorized based on the method of chitosan cross-linking and preparation.

**Preparation of chitosan hydrogels via chemical cross-linking**

Chemically cross-linked hydrogels are formed by covalent linking of the chitosan macromers, where the bond formation is irreversible. Chemical cross-linked hydrogels are found in four states of formation, a) chitosan cross-linked system, b) hybrid polymer networks (HPN), c) interpenetrating polymer networks (IPN) and d) semi interpenetrating polymer networks (SIPN). Fig. 3 shows schematic representation of these four states.
The simplest type of chemical hydrogel formation occurs when chitosan undergoes cross-linking reaction with another polymeric chain of its own. Second chain can be similar to or different from first structural unit in derivation.

Amines and hydroxyl groups situated on chitosan chains are responsible for chemical cross-linking. Chemical cross-linking can occur via cross-linkers or photopolymerization reaction.

**Cross-linking via cross-linkers**

Cross-linking can be formed between polymers themselves or between polymers and a cross-linker. Cross-linkers initiate cross-linking reaction between chitosan chains.\(^4\)

A few of customary cross-linkers include dialdehyde compounds such as glutaraldehyde and other reagents like genipin, palladium cation, diisocyanate and acrylic acid.

Glutaraldehyde has been extensively used for chemical cross-linking of chitosan. Glutaraldehyde is mainly used for cross-linking when a second polymer is added to chitosan for modification of its properties. In an attempt, Pluronic F127 has been used for modification of chitosan and this hybrid was cross-linked by glutaraldehyde for controlled delivery of 5-FU. Genipine is a natural, water soluble and bifunctional cross-linking agent made of a glucoside named geniposide by β-glucosidase enzyme.

Genipine is widely used as cross-linker in tissue fixation, food industries and drug delivery which is due to its, lower toxicity and higher biocompatibility versus other cross-linkers especially glutaraldehyde. There are a large number of studies on cross-linking of chitosan by genipine. Chitosan/gelatin networks cross-linked by genipine have been developed for articular cartilage tissue repair. Genipine-cross-linked chitosan hydrogels present slower degradation rate in comparison to glutaraldehyde-cross-linked hydrogels and higher biocompatibility, but there is the risk of incompatibility with the therapeutic agent loaded into the hydrogel.\(^{17}\)
**Cross-linking via photopolymerization**

The other method of forming covalently cross-linked chitosan hydrogels is photopolymerization. Photopolymerization is the process of changing liquid precursor solution to gel with the help of photoinitiators and visible or UV irradiation. This technique is used *in vivo* and *in vitro*. The polymeric reaction is controlled by adjusting the distance and duration of exposure. UV or visible light in reaction with molecules called photoinitiators, produce free radicals which initiates radical polymerization and forms cross-linked hydrogel. Photopolymerization has a distinct advantage over general methods of polymerization and that is in situ formation of hydrogels which can be used *in vivo* for several applications like in laparoscopic devices, following subcutaneous injection or in different surgeries.\[^{18}\]

By introducing azide and lactose moieties to chitosan, a photocrosslinkable derivative of this polymer has been synthesized. This modified chitosan can be used as a tissue adhesive in punctures.

Azide modified chitosan and vinyl benzoic acid derivatives of chitosan can also provide photo cross-linked networks for different applications.

**Physical cross-linking**

Physical cross-linking to form chitosan-based hydrogel networks is another class of crosslinking. Physical interactions can be ionic interactions, as in ionically cross-linked chitosan hydrogels and polyelectrolyte complexes, or can be secondary interactions such as networks named grafted chitosan hydrogels and entangled chitosan hydrogels.

**Ionically cross-linked chitosan hydrogel**

Since chitosan is a cationic polyelectrolyte polymer with ionizable amine groups, anions are often employed as ionic cross-linkers to engineer ionically cross-linked chitosan hydrogels. One of the examples is the multivalent counter ions such as phosphate bearing molecules like tripolyphosphate (TPP).\[^{19}\] This ionic cross-linking process which is also called ionic gelation of chitosan is mostly used for loading of low molecular weight drugs, but recently has been used for macromolecules as well.

**Chemical versus physical cross-linking**

Type of cross-linking determines the stability of hydrogels. Covalently cross-linked hydrogels with covalent cross-linkers have permanent feature that show resistance to
environmental variables. However these systems need extra process of purification to remove toxic unreacted cross-linkers.

Physically cross-linked hydrogels are more biocompatible due to the lack of chemical cross-linkers and well tolerated compared to covalently systems.

However they may have not high mechanical stability and they may react to environmental changes such as pH, temperature or ionic strength. This especial feature of physically cross-linked hydrogels is very useful for preparation of stimuli responsive systems that are sensitive to environmental conditions and can be used for drug delivery in specific conditions.[7]

Molecules used for modification of chitosan properties.

In order to improve chitosan based hydrogel properties, chitosan derivatives have been synthesized and evaluated. The functional amino groups on chitosan chains help the polymer to enter chemical reactions which generate derivatives with improved properties such as muco-adhesion, high drug loading and ability for gene transfer.[20] Some other chemical modifications have gained interest to prepare photopolymerizable chitosan derivatives or to improve water solubility of chitosan.

Chitosan and 4-azido-benzoic acid (Az-CS)

Azido-benzoic acid is one of the cross-linking agents and photoinitiators with two functional groups, azide and carboxylic acid.

The carboxylic acid group of benzoic acid enters in the reaction with amino group of chitosan which results in preparation of photosensitive chitosan derivative. Upon UV irradiation, the azide functional group is changed into nitrene and further process causes cross-linking for gel formation. The reaction of chitosan with 4-azido-benzoic acid and resulting photo-cross-linking. Study on Az-CS derivatives showed that the solution made gel in less than a minute under UV light and the resulting gel was very adhesive similar to fibrin glue. The resulting gel was also non-toxic in acute and chronic exposures.[21, 22]

Carboxylation of chitosan

As biodegradability is one of the essential features of biocompatible polymers such as chitosan, the extent of degradability is of importance. Dependent on chitosan chain length and its application, sometimes it is needed to enhance the degradation rate of chitosan.
One of the approaches is to synthesize carboxyl methyl chitosan with higher dissolution rate in aqueous environments and faster degradation in the presence of enzymes such as lysozyme. Fig. 4 depicts the chemical structure of carboxy methyl chitosan. Carboxy methyl chitosan is water soluble in a wide range of pH along with high viscosity, gel forming ability, low toxicity and acceptable biodegradability which makes it a good option for use in food and cosmetic products.

![Chemical structure of carboxymethyl chitosan](image)

Figure 4: Structure of carboxymethyl chitosan

The carboxymethyl derivative of chitosan can also be modified by photoinitiators and provide photopolymerizable derivatives.\textsuperscript{[23]} Low molecular weight carboxy methyl chitosan modified by photoreactive 4-azido benzoyloxy succinimide has been synthesized as antiadhesive agent in surgeries and tissue regeneration.\textsuperscript{[18]}

**CONCLUSION**

Numerous hydrogel structures have been prepared and characterized for biopharmaceutical and biomedical applications. The significant features of these networks for \textit{in vivo} applications include swelling ability, similarity to host tissues and mechanical strength as well as biodegradability. In addition to owning biocompatibility and biodegradability, biopolymers like chitosan, has potential abilities for structural modifications, which results in formation of new applicable derivatives. In addition to inherent properties of chitosan like antifungal activities and antibacterial, biocompatibility and biodegradability, different strategies to prepare chitosan derivatives make it a good carrier for pharmaceuticals, cosmetics and food products. Hydrogel preparation, modified performance and cross-linking mechanism should be related to aim, for example sustained release profile for drug delivery systems or porous structural appearance for tissue engineering applications.
Conflict of Interest
The authors have no conflict of interest.

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