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Collagen and Keratin as a Components of Hydrogels

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Abstract
Smart hydrogels, or stimuli-responsive hydrogels, can and should play an important role as excellent drug carriers. These three-dimensional structure materials are composed of crosslinked hydrophilic polymer chains that are able to dramatically change their volume and other properties in response to environmental stimuli, such as pH, temperature and solvent changes. The main goal of individual research projects was the development of new hydrogels based on polypeptides. These hybrid materials are crosslinked hydrophilic polymers capable of absorbing large amounts of water, saline or physiological solutions. Superabsorbent hydrogels based on natural materials are non-toxic, biocompatible and biodegradable. Unfortunately, their mechanical properties in the swollen state are weak. In this article, we present a review of literature on the synthesis of smart hydrogels based on keratin or collagen with synthetic materials.

Keywords
biopolymers, collagen, keratin, smart hydrogel, applications.

1. Introduction

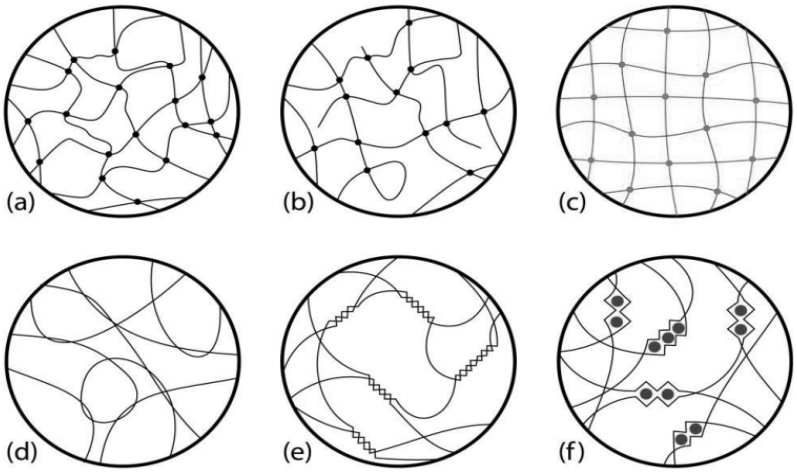
The first hydrogels appeared in the 1960's. Then two scientists, Wichterle and Lim, synthesised a material with high affinity for water, namely, a poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel, used in applications for permanent contact with human tissues. Since then, the amount of research on hydrogels for biomedical applications has increased year on year [1].

Hydrogels are polymeric materials with a distinct three-dimensional structure containing a solvent that fills the spaces between the macromolecules. The most significant characteristic of hydrogel is its swelling ability. The amount of liquid absorbed in hydrogels is related to the presence of specific groups such as –COOH, –OH, $–CONH_2$, –CONH–, and $–SO_3H$, as well as to the capillary effect and osmotic pressure [2]. The properties of hydrogel also depend on the type and structure of the monomer used in the synthesis of the hydrogel and on the density of its crosslinking. The liquid share in the hydrogel can be from 40 to 99% of the polymer dry matter. It performs a transport function for substances that diffuse inside [2, 3].

The basic properties of hydrogel e.g. mechanical properties, dimension stability, degree of swelling depend on the method of obtaining the material. Figure 1 (a–c) shows an example of chemically, and (d–f) physically crosslinked hydrogels. The presence of chemical or physical crosslinks prevents such materials from dissolving in water and allows them to swell. In the case of chemically crosslinked hydrogel, the covalent bonds formed during crosslinking can create an ideally chemically bonded network or non-ideally chemically bonded network with polymer chain self-loops and free ends. Double network gel, consisting of two distinct networks, each being only covalently linked and forming an interpenetrating network structure, can be obtained. Chemical bonding prevents the hydrogel from being reshaped and resized. The physical structure of this polymer depends on the strength of the covalent bonds, as well as on the rigidity and intermolecular force within the polymer chain [4, 5]. The physically crosslinked hydrogel possesses physical junction domains associated with chain entanglements, hydrophobic interaction, hydrogen bonding, crystallinity, and ionic complexation [6, 7]. This kind of physical connection between polymer chains allows solvent casting and/or thermal processing, while the swelling of these hydrogels mostly depends on the changes in thermodynamic parameters, such as temperature, pH, salt type and/or ionic strength. The major disadvantage of physically crosslinked hydrogels is their weak mechanical properties in the swollen state [8-10].

Hydrogels can be also classified based on:
- the source (natural and synthetic hydrogels),
- the nature of the network (homopolymer networks, copolymer networks, interpenetrating networks, and double networks (Table 1),
- the electric charge present (neutral, anionic, cationic, ampholytic, hydrophobic modified, complex coacervates),
- the response (biochemically, chemically and physically responsive [8, 11].

Hydrogel that reacts to stimuli inspired by nature deserve special attention. Response hydrogel systems are also known as 'intelligent' or 'smart' polymers. These systems can respond to a change in the dynamic pH, temperature, electric field, amount of enzyme, amount of glucose, or to a change in antibodies in the environment. Responsive hydrogel systems can be divided into (Table 2):
- physically-induced release systems,
- chemically-induced release systems,
- other stimuli-induced release systems [23].



a ideal cross-linked network with all tetra-functional linkages; *b* non-ideal cross-linked network including molecular ends and loops; *c* ideal cross-linked double network gel; *d* physically entangled network; *e* physically entangled network with helix formation; *f* alginate-like network with divalent Calcium ions forming local bridges between adjacent chains

2 Schematic microstructures of gels to define types of cross-linking

Fig. 1. Examples of chemically (a–c) and physically (d–f) crosslinked hydrogels [7]

Type of hydrogel	Examples	References
Homopolymer	Poly(2-hydroxyethyl methacrylate)	[12]
	2-Hydroxyethyl methacrylate (HEMA)	[13]
	Poly(vinyl alcohol) (PVA)	[14]
	Polyacrylamide (PAAm)	[15]
	Polyetylene glycol (PEG)	[16]
Copolymer	Methacrylic acid (MAA)	[17]
	Carboxymethyl cellulose (CMC)	
	Polyvinylpyrrolidone (PVP)	
Interpenetrating network	Poly(N-isopropylacrylamide) (PNIPAAm)	[18]
	Polyacrylamide-grafted-gum ghatti (PAAm-g-GG) and sodium alginate	[19]
	Polyacrylamide-grafted-tamarind seed polysaccharide (PAAm-g-TSP)	
	Poli(vinyl alcohol)/chitosan	[20]
	Hyaluronic acid–methylcellulose (HA-MC)	[21]
Double-network hydrogels	Poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) as the first network and polyacrylamide as the second network	[22]
	Poly(2-acrylamido-2methylpropanesulfonic acid) as the first network and poly(N, N-dimethylacrylamide) (DMAA) or polyzwitterionic gels as the second network	

Table 1. Classification of hydrogel types based on the network nature

The intelligent hydrogels can occur in two states: swollen and shrunken. The transition between these two states caused by an environmental stimulus is defined as volumetric phase transition [24]. In the case of a swollen gel, during the volumetric phase transition effect the solvent is removed from the polymer structure. As a result, a drastic decrease in volume occurs in hydrogel. The phase transition can be regulated by the hydrophilic-hydrophobic balance. After the stimulus has subsided, there is a return to the initial state, which proves that this process is reversible. In the case of temperature-sensitive hydrogels, the phase transformation is closely related to the so-called lower critical solution temperature (LCST). Below this temperature, the formation of hydrogen bonds between the hydrophilic groups of the polymer and the water molecules stabilises the swollen gel structure. On the other hand, when the LCST is exceeded, the share of hydrophobic interactions increases and the hydrophobic-hydrophilic balance shifts, which results in breaking the hydrogen bonds and removing water from the polymer structure of the hydrogel (a contracted state is created). Amongst the stimuli responsive gels, thermoresponsive polymers like PNIPAAm, poly(dimethylaminoethyl methacrylate) (PDMAEMA) and poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) were studied [25, 26]. The sensitivity of the synthetic polymer to pH is a consequence of the presence of the amino group. Cationic polymers like as aminoalkyl methacrylate copolymer have higher water solubility at acidic pH than at neutral pH. Whereas anionic polymers such as hydroxypropylmethylcellulose phthalate (HPMC-P) with carboxyl groups have higher water solubility at basic pH than at acidic pH. The ratio of carboxyl groups to other groups can be manipulated to control the dissolution pH of the polymer. Common pH-responsive functional groups used in drug delivery are hydrazone, acetal, oher ester, and vinyl ether [27]. In pH responsive hydrogels the phase transformation may be related to the amino acid compositions of biopolymers and their pKa point [28]. A change in the charge on the polymer chains will lead to swelling and liquid release in a pH-responsive hydrogel based on synthetic material [27].

Nowadays, hydrogel actuators and shape memory hydrogels have attracted increasing attention due to their promising potential applications in biomedicine [29], tissue or cell engineering [30], soft robotics [31], and as artificial muscles [32] and micro-swimmers [33].

Chemically responsive	Physically responsive	Biochemically responsive
pH Glucose Oxidant Solvent composition Ions and specific molecular recognition events	Temperature Electricity light Pressure Sound and magnetic field	Antigens responsive Enzymes responsive Ligands responsive

Table 2. Sub-classification of responsive hydrogels [23]

2. Biopolymers: collagen and keratin – their applications

In recent years, biodegradable materials derived from renewable resources have become more and more popular among scientists [34, 35]. The use of renewable resources ensures global sustainable development and a reduction in environmental pollution. Naturally occurring biopolymers are an interesting group of alternative materials to petroleum based polymers. The advantage of using biopolymers such as carbohydrates, proteins and lipids is their high availability, biodegradability, biocompatibility, and relatively low price. Proteins are of particular interest because of their diverse structure, which contains various amino acid chains providing many functional groups as compared to other biopolymers such as lipids or carbohydrates. Structural fibrous proteins and respective hydrolysates, can be recovered from fisheries, poultry and waste from the leather industry and used in fields such as agriculture, food, biomedicine, pharmaceutics, and cosmetics [36-39].

The most common animal protein is collagen (a component of skin, bones and tendons) and keratin (the building block of hair, feathers or hooves) [40].

Collagen, a primary component of the extracellular matrix (ECM), includes 19 or 21 amino acids in the structure. A characteristic feature of collagen is the presence of hydroxyproline (Hyp) and hydroxylysine (Hys). Hydroxyproline stabilises the helical structure in inter-chain connections with the participation of hydrogen bridges, while hydroxylysine is covalently bound to oligosaccharides. In addition, collagen is characterised by a high content of proline (Pro) and glycine (Gly) and a low proportion of aromatic amino acids (including tryptophan (Try), phenylalanine (Phe), tyrosine (Tyr)). Collagen macromolecules consist of three left-handed polypeptide chains that interwine around a common axis to the right and form a superhelix conformation. The most frequently repeated sequence in collagen are the following triads of amino acids in the polypeptide chain: Gly-X-Y, where X is typically proline and Y is typically hydroxyproline [41, 42]. This structure of collagen provides an ECM high strength as well as anisotropic mechanical properties [43].

Keratin is another biocompatible and biodegradable protein that can be used in the construction of new materials. At the molecular level, keratin possesses three different configurations (α-, β-, γ-keratin). The elastic nature of keratin fiber is due to the interaction between the α-helix and the β-sheet configuration. The remaining specific properties of keratin are determined by the presence of cysteine (Cys) in its structure. The presence of disulfide bonds is responsible for the low solubility of keratin in water, as well as for its high stability and resistance to enzymatic reactions [44-48]. Moreover, the presence of hydrogen and ionic bonds as well as the intermolecular interactions of polar and non-polar amino acids are also responsible for the high dimensional stability of keratin [48-51].

Due to the unique characteristics of collagen and keratin such as a very high level of biocompatibility and low cytotoxity, these biopolymers are widely used in biomedical applications that require structural integrity (artificial blood vessels and valves), in tissue engineering applications (3D scaffold) and as a drug delivery material (hydrogel) [52, 53].

Collagen hydrogel is a popular extracellular matrix material used in regenerative medicine. Collagen hydrogel, used as cell scaffolds, ensures high biocompatibility and bioactivity. Unfortunately, the disadvantage of collagen hydrogel is its isotopic and homogeneous structure, which is different from the anisotropic three-dimensional structure of native living tissues [54]. There are also known examples of chemical and physical hydrogels based on collagen in biomedical applications. Chemical hydrogel results from the covalent crosslinking of the side chains induced by enzyme present in the aqueous solution. Physical hydrogel is a thermal-induced self-assembly of fibril initiation and growth [55]. This type of hydrogels are formed as a result of the occurrence of hydrogen bonds, as well as hydrophobic or electrostatic interactions in amino and carboxyl telopeptides of collagen chains. Physical hydrogels based on collagen are characterized by poor dimensional and thermal stability as well as poor mechanical properties. The three-dimensional network of such a hydrogel can be destroyed by changing the temperature, ionic strength, pH or collagenase content [56]. In order to improve the features mentioned above, collagen combines with synthetic polymers, creating hydrogels based on crosslinked and/or interpenetrating polymer networks [53]. The weight ratio of synthetic polymer to collagen is important while designing hybrid hydrogels because it allows the mechanical properties, swelling and degradation of new hydrogels to be adapted to their intended use. The combination of collagen and synthetic polymers such as PEG [57], PAAm [58], poly(methyl methacrylate) (PMMA) [59], PVA [60], and polyvinylpyrrolidone (PVP) [61] are known.

However, the use of pure keratin for the synthesis of hydrogels is limited due to its weak mechanical properties and instability in an aquatic environment. The crosslinking of such a hydrogel occurs either physically or through the formation of disulfide bonds. It is a slow process and the properties of the hydrogels obtained are still not satisfactory, which limits

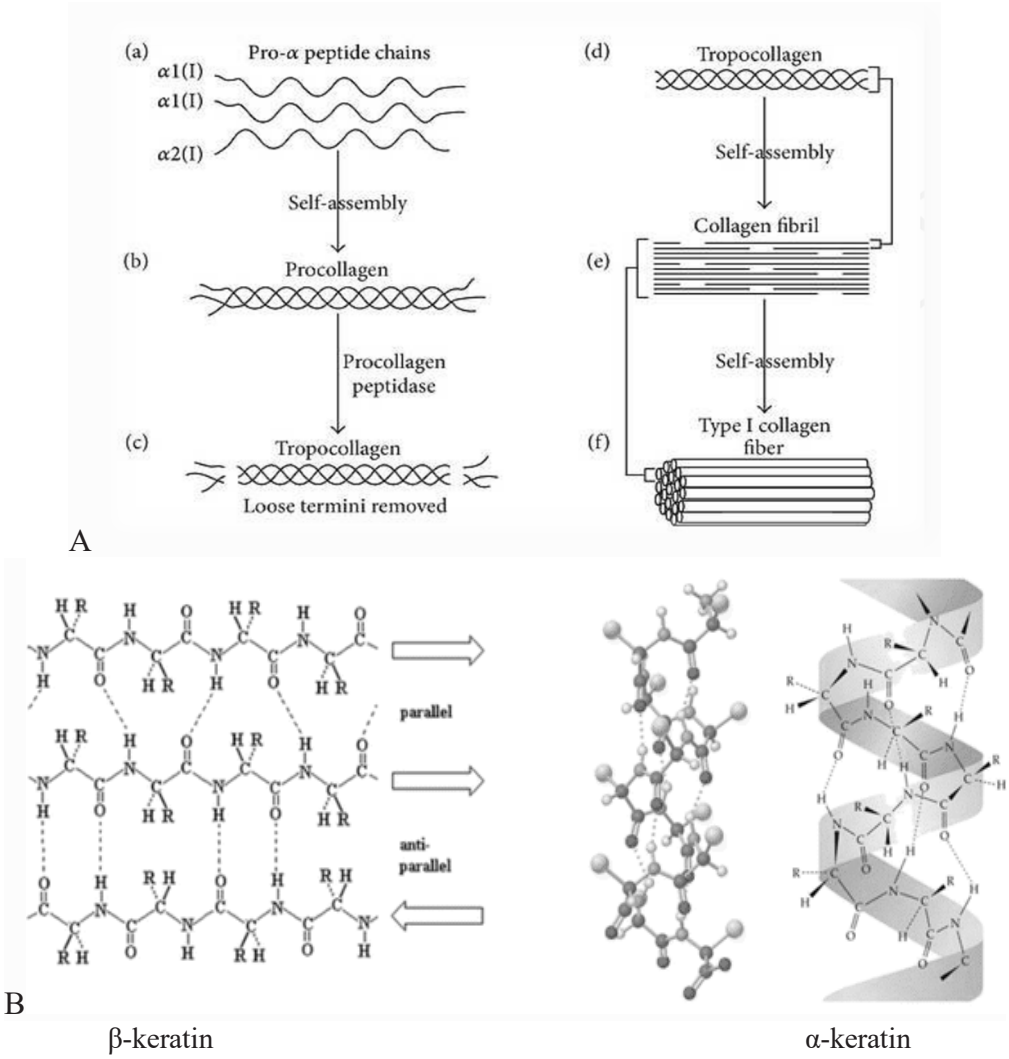


Fig. 2. Structure of collagen (A) and keratin (B) [39, 51]

their use in many areas [62]. Therefore, it is necessary to add a synthetic polymer or to introduce another natural polymer which will ensure adequate mechanical strength and increased stability of the final product. There are hydrogels based on keratin and synthetic polymers such as PVA [63], PEG [62] and PAAm [64]. In the case of keratin-based and synthetic polymer hydrogels, the presence of many amino acids in the hydrolysed keratin provides the possibility of generating non-covalent interactions (most often hydrogen bonds), thereby increasing the adhesive properties of the hydrogel produced. As the content of hydrolysed keratin in the polymer composition increases, the adhesion strength of the hydrogel to various substrates, such as aluminum, titanium, glass, silica rubber, wood, pigskin and plastic increases. Additionally, hydrolysed keratin is colorless and does not adversely affect the transparency of the hydrogel [62].

As a result of particular physical and chemical properties of hydrogel based biopolymers, such as: swellability, flexibility, softness, and biocompatibility, there is growing research interest in hydrogel synthesis, developing its properties, and thus increasing its implementations in different fields.

3. Controlled-release hydrogel systems based on collagen and synthetic polymers

The earliest works on collagen-based responsive hydrogels predominantly involved free radical polymerization of a combination of hydrolyzed collagen, acrylic acid (AA) and acrylamide (AAm). Pourjavadi and Kurdtabar developed a collagen-based highly porous hydrogel by neutralizing the grafted poly(acrylamide-co-acrylic acid) after gel formation. The formed hydrogel presented a pH-responsiveness character, as a result of which a swelling-collapsing pulsatile behaviour was recorded at pH 2 and pH 8 [65]. Currently, a new era for drug delivery systems has come, with a variety of new drug release systems appearing rapidly, and more and more attention being paid to intelligent (also called on-demand) drug delivery systems or smart drug delivery systems. Noppakundilograt *et al*. presented data involving hydrolyzed collagen-grafted-poly[(acrylic acid)-co-(methacrylic acid)] (HC-g-poly(AA-co-MAA)) hydrogel for drug delivery. They

investigated the effect of the content of the (N,N,N',N'-tetrametyloetylenodiamine) (TEMED) on the water absorbency in HC-g-poly(AA-co-MAA) hydrogel. It was found that the water absorbency of this hydrogel was both pH- and temperature-dependent [66].

Ding *et al*. synthesized new thermo- and pH-responsive hybrid gels consisting of PNIPAAm and collagen nanofibrils. Such hydrogel used as drug delivery was obtained by *in situ* polymerization (Figure 3). The results obtained suggested that the content of PNIPAAm had an important effect on the drug delivery abilities. In another work pH-sensitive hydrogel based on fish scale collagen and carrageenan was used as a novel allopurinol drug loading system to improve the bioavailability of allopurinol. This system may be used to treat gout and high levels of uric acid in the human body. results obtained proved that the hydrogen bonds of the functional groups in allopurinol with the functional groups in carrageenan and fish scale collagen led to the enhancement of drug release control as well as to the bioavailability of allopurinol loaded into the carrageenan/collagen hydrogel. The dissolution of these hydrogel beads in different simulated body fluids increased by 1.6 to 6.7 times compared to the crystalline allopurinol [67]. In article [68] sensitive polymer based on PNIPAAm and dominant fibrous protein of connective tissue, such as collagen, was synthetised. It was found that the reproducible method of extraction of collagen was essential to obtain stable products. In the case of the sample containing too much PNIPAAm (>20%), electrospinning could not be done because of the too high viscosity of this hydrogel [68].

Collagen is a typical matrix polymer that makes up one third of all proteins in tissues. It is an important component of the extracellular matrix of most connective tissues in mammalian bodies. This biopolymer is extracellular and mainly has a structural role. Hydrogels containing a natural extracellular matrix, such as collagen components, are very attractive as scaffolds for regenerative medicine applications due to their fundamental bio-interactive properties. Ravichandran *et al.* created a modular platform to engineer a multi-component ECM mimicking scaffold based on methacrylated collagen building blocks. In this study, novel covalently crosslinked thermo- and pH-responsive collagen hydrogels were synthetised under physiological conditions. Hydrogel was prepared by free radical co-polymerisation of methacrylated type-I collagen and N-isopropyl acrylamide (NIPAM) in the presence of N,N′-methylenebis(acrylamide) (MBA). The covalently linked double network was biocompatible and stimuli-responsive, showing volume-phase transition. Vitamin E succinate and bovine serum albumin were encapsulated easily in the gels as a model protein and small drug molecules, respectively [69]. In turn, Zhang *et al.* focused on the use of synthetic polymers in combination with isolated collagen, with properties corresponding to those of collagen, which is a component of the extracellular matrix. It is a characteristic spatial grid system that regulates many functions, such as: maintaining the elasticity and integrity of the tissues and tensile strength. In the case of collagen-PNIPAAm copolymer, the presence of the synthetic polymer contributed to the inhibition of collagen fibrillogenesis, but did not cause the loss of biocompatibility.

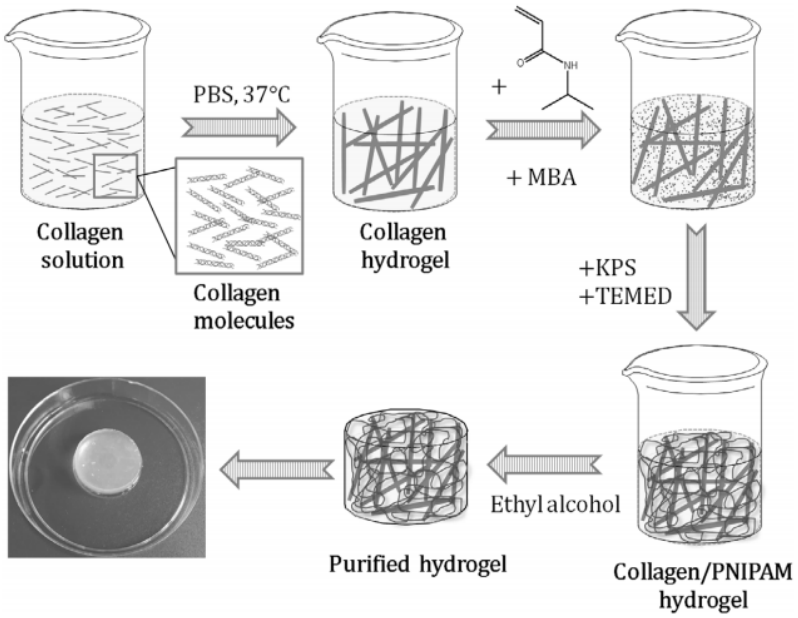


Fig. 3. Fabrication scheme of collagen/PNIPAAm hydrogel [67]

These studies demonstrated that the lower critical solution temperature value of the PNIPAAm-g-collagen copolymer was higher (~ 38.5°C) than the LCST value of pure PNIPAAm [70]. The increase in LCST could be due to an increase in the hydrophilic nature of the copolymer after the introduction of collagen [71]. The increase in LCST is most often attributed to the inclusion and strong interaction of hydrophilic groups. The introduction of carboxyl groups into the PNIPAAm macromolecule results in an increase in LCST of 2-3°C [72]. In contrast, the inclusion of hydrolyzed methacrylate gelatin moieties containing hydrophilic carboxylic acid groups increases the LCST of thermo-sensitive microstructures from 35.4 to 36.9°C [73]. Although collagen/synthetic intelligent systems show great potential in the aforementioned applications, their limitations cannot be ignored.

4. Controlled-release hydrogel systems based on keratin and synthetic polymers

There are known controlled-release hydrogel systems based on keratin and synthetic polymers, such as: PNIPAAm, PHPMA [49, 74].

In the case of PNIPAAm copolymer and keratin (functionalized with vinyl groups) obtained during free radical polymerisation, it was shown that the presence of keratin in the copolymer did not affect the LCST value; therefore it is justified to introduce keratin to improve the properties of the hydrogel, with complete control of the structures affecting LSCT. The degree of hydrogel swelling and its mechanical properties depended on the amount of keratin attached and its functionalisation. Additionally, the hydrogels obtained showed a porous structure, which enables their use as materials that absorb cations from aqueous solutions [49].

Thermo-sensitive keratin hydrogels were synthesised by Zou et.al by grafting with PNIPAAm and loading with deferoxamine mesylate (DFO) to act against iron-induced brain injury after intracerebral hemorrhage (ICH). They showed that keratin hydrogels had the ability for nerve repair and drug delivery due to their biocompatibility, biodegradation, and cellular attachment. Thermo-sensitive keratin was synthesised via a thiol-ene reaction between the thiol group of the keratin and the ethylene bond of the NIPAM. In addition, DFO, a hexadentate chelator binding iron, was entrapped in the thermo-sensitive keratin hydrogel to relieve the iron-induced brain damage after ICH. The LCST of thermo-sensitive keratin hydrogel can be tailored from 28.5 to 31.8 ºC by varying the graft ratios of keratin to NIPAM [74].

A method for producing thermal responsive keratin-based hydrogel was described by Chen *et al*. Porous keratin extracted from chicken feather was modified for the fabrication of keratin-g-PNIPAAm. The modification process was based on the functionalisation of keratin with groups of the initiator. Reversible-deactivation radical polymerisation can be used for grafting PNIPAAm from keratin macroinitiator. The results showed that fast polymerisation and a high graft rate were achieved. Additionally, the graft rate could be adjusted by controlling the reaction time and monomer addition. A rough surface of keratin-g-PNIPAAm was obtained, which proves that the reaction was well performed. The thermal stability of the keratin-g-PNIPAAm was characterised by higher thermal stability compared to pure keratin, whereas grafted PNIPAAm chains exhibited a higher glass transition temperature. The results obtained show good hydrophilicity of keratin-g-PNIPAAm. In addition lower LCST was observed comparing to pure PNIPAAm [45].

A keratin-based biopolymer hydrogel with an interpenetrating network structure, pH-sensitivity and temperature sensitivity can be obtained by the two-step polymerisation of PNIPAAm and itaconic acid (IAc) in the presence of a crosslinker [75].

Keratin-g-PHPMA copolymers can self-assemble into micelles in water, and the introduction of keratin to PHPMA increases the stability of micelles due to the presence of disulfide bonds formed by the oxidation of thiol groups on keratin. The keratin-g-PHPMA micelles designed were found to be effective in encapsulating doxorubicin (DOX), the content of which can be controlled by the degree of keratin grafting in the copolymer. The DOX content of the micelles increases with the keratin content of the graft copolymer. The release behaviour of the DOX-loaded micelles is sensitive to trypsin. The presence of trypsin helps to achieve the complete release of the loaded DOX. Additionally, keratin-g-PHPMA copolymers show no cytotoxicity to cells. The results obtained proved that keratin-based graft copolymers can be used as excellent drug carriers in the treatment of cancer [46].

Eslahi *et al*. presented a novel thermal responsive hydrogel based on mineral nanoclay, wool-derived fibrous protein (keratin), triblock copolymer, and chitosan biopolymer, with potential applications for articular cartilage tissue engineering. The ability of the hydrogels to undergo *in situ* crosslinking and rapid gelation under physiological conditions was demonstrated. The new materials were biocompatible (>90%) with good cell adhesion depending on their formulation and microstructure, and they have a high capacity to be used as cartilage scaffolds [76].

Villanueva *et al*. merged hybrid material with keratin and zinc oxide nanoplates (nZnO). Keratin hydrogels and antimicrobial nZnO were designed for their potential use as a wound dressing. Smart gel biocidal agent nZnO will be released, to a greater extent, in the presence of microorganisms. Additionally, in the presence of microorganisms, the pH will also change. The authors proved that nZnO will be released to a greater extent if the wound becomes contaminated with bacteria. The product obtained had a good mechanical strength and was malleable. A biocide effect increased along with the antimicrobial agent concentration. Unfortunately, cytotoxicity also rose together with nanoparticle concentration. In designing such materials, attention should be paid to the following three effects: the biocide effect, cytotoxicity and mechanical strength [77].

5. Conclusions

This review outlines recent progress in the field of stimuli-responsive hydrogels based on keratin or collagen. Collagen and keratin, as biodegradable and nontoxic materials, are intensively researched as materials for the production of hydrogel matrices. Their unquestionable advantage is the diversified structural network that guarantees the presence of many functional groups. Unfortunately, they are characterised by a high rate of proteolytic degradation and poor mechanical properties. In order to improve the features mentioned above, polymer systems based on collagen or keratin are designed with synthetic polymers. Such a combination of collagen or keratin with synthetic materials allows to obtain new composite materials with attractive properties such as biocompatibility, degradability and nontoxicity. The stimuli-responsive hydrogels based on biopolymers and synthetic polymers can be used in biomedical applications that require structural integrity, in tissue engineering applications and as a drug delivery material.

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