Gelatin-Based Hydrogels

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Taslim Ur Rashid, Sadia Sharmeen, Shanta Biswas, Tanvir Ahmed, Abul K. Mallik, Md. Shahruzzaman, Md. Nurus Sakib, Papia Haque, and Mohammed Mizanur Rahman

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Abstract

Hydrogels are crosslinked polymers that are able to absorb large amount of water, permit solutes within their swollen matrices, and provide sustained delivery of absorbed solutes. The use of various types of functional biopolymers as scaffold materials in hydrogels has become of great interest not only as an underutilized resource but also as a new functional material of high potential in various fields.

T. U. Rashid (✉) · S. Sharmeen · S. Biswas · T. Ahmed · A. K. Mallik · Md. Shahruzzaman · Md. Nurus Sakib · P. Haque · M. M. Rahman
Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering and Technology, University of Dhaka, Dhaka, Bangladesh
e-mail: taslim@du.ac.bd

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Among them, gelatin has been considered as highly potential candidate to be utilized as hydrogel component because of its hydration properties such as swelling and solubility; gelling behavior such as gel formation, texturizing, thickening, and water-binding capacity; and surface behavior like emulsion and foam formation, stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity. In addition, its properties of biocompatibility, low toxicity, antimicrobial activity, and biodegradability make it suitable for diversified biomedical applications. Many works have been reported in various scientifically reputable journals and publications worldwide that seem to have potential or satisfactory contribution of gelatin-based hydrogels. Numerous fields of application of gelatin hydrogels include, not limited to, usage as safer release system in agrochemicals, nutrient carriers for plants, drug and cell carrying devices, bioadhesives, wound healing, tissue engineering, etc. The purpose of this chapter is to compile the recent information on developments in gelatin-based hydrogel preparation, as well as new processing conditions and potential novel or improved applications.

Keywords
Gelatin · Hydrogel · Preparation · Application

1 Introduction

Recently a three-dimensional smart crosslinked polymeric network named hydrogel has gained considerable attention of the researchers. The characteristic properties of hydrogels such as desired functionality, enough smart response to the fluctuations of environmental stimuli (pH, temperature ionic strength, electric field, presence of enzyme, etc.), reversible swelling or shrinking and biocompatibility make them especially appealing to both materials and biological requirements [1–3]. Hydrogels based on natural or synthetic polymers have been of great interest regarding numerous fields of applications like biomedical engineering [4–7], tissue engineering [8–10], drug delivery [11], wound dressing [12], agricultural application [13, 14], pharmaceutical uses [15], food packaging [16], environmental applications [17], etc.

Hydrogels are not new but have been found in nature since life began on Earth. Many bacterial biofilms, which are extracellular matrix components, and plant structures are ubiquitous water-swollen motifs in nature [5]. Although less solubility, high crystallinity, unfavorable mechanical and thermal properties, unreacted monomers, and the use of toxic crosslinkers sometimes create barrier to the flourishment of hydrogel technology, the continuous development of new ideas to improve these properties with the combination of natural and synthetic polymers makes them a potential candidate in the field of biomedical applications. Different scientific approaches for the designing and processing of a specific hydrogel for a specific application are made to obtain maximum physicochemical properties, stimuli response, density, biodegradation, and biological and environmental response [5].

Both natural and synthetic polymers are now leading the field of source of hydrogels, but the use of the latter increases day by day. Among natural polymers, gelatin is one of the most common polymers to be used as structural networks of many hydrogels. It is a biodegradable natural polymer composed of different types of amino acids [18]. It is found in different parts of several mammals like cattle bones, pig skin, hides, and fish as well as in some plants and insects [19]. The structure of gelatin allows them to undergo interactions with other molecules and forms crystallites followed by further transformation into a three-dimensional network susceptible to immobilize the liquid [20]. Sometimes, chemical crosslinker enhances the gelation process through further networking [21, 22].

A wide variety of hydrogels made up of gelatin and other polymers have been reported yet in several scientific media [23–26]. This chapter attempts to summarize the recent development in this prominent field of polymer science. The first part of the chapter will briefly summarize the sources, structure, classes, and properties of hydrogels as well as their vast applications. Second part will emphasize on the structural properties of gelatin and gelatin-based hydrogels as well as their synthesis procedures. The third part will present most recent reports on the specific application of gelatin and modified gelatin-based hydrogels in different fields.

2 Hydrogels

Hydrogels are three-dimensional polymeric network which can take in and preserve 20 to 40 times, (even more in some cases) more water or biological fluids compared to their dry weight [7]. Hydrophilic groups in the polymeric network, either physically or covalently crosslinked with each other, become hydrated in an aqueous medium to form reversible hydrogel structure [27].

Based on the properties and application, hydrogels can be classified according to numerous ways and means. This classification depends on, sources of hydrogels, method of preparation, physical and chemical properties, ionic charges, swelling nature, biodegradation rates, and their nature of crosslinking [28]. In the following figure (Fig. 1), a detailed classification of hydrogels according to their different properties is stated.

Based on nature of the crosslink junctions of hydrogels, they can be mostly divided into two classes: physically crosslinked hydrogels and chemically crosslinked hydrogels. The polymeric composition of hydrogels directs to the development of some important categories of hydrogels. They can be classified as homopolymeric, copolymeric, and interpenetrating polymer network hydrogels. On the basis of their solubility, hydrogels can be classified into two groups in the water and enzymes. *Biodegradable hydrogels* have the capability of breaking down to simpler molecules, inside the body, with both water and enzymes. *Non-biodegradable hydrogels* are not broken down by the water and enzymes [29]. Hydrogels can be either “smart” or “conventional” based on their responses to unexpected changes in environment. Smart hydrogels are able to respond to external stimuli through sudden changes in the physical nature of the network. A small change in physical or

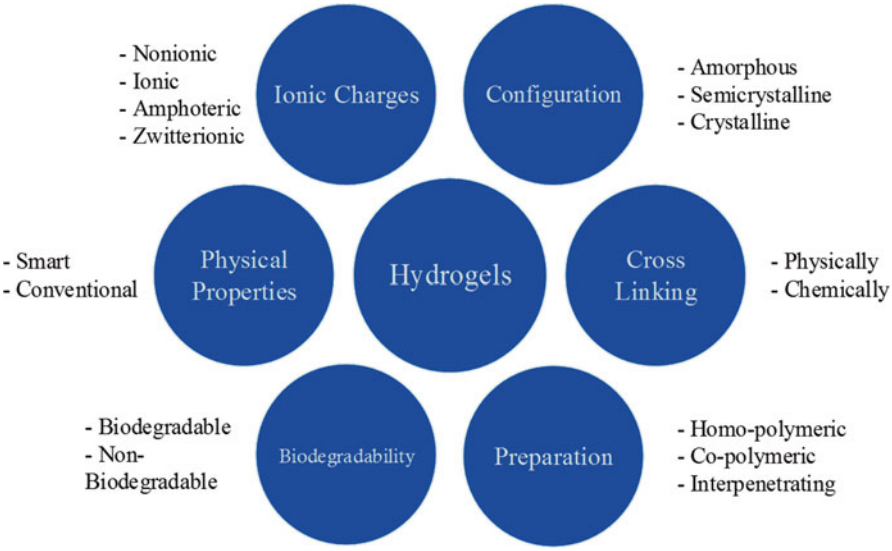

Fig. 1 Classification of hydrogels

chemical conditions can lead to a very sharp and large property change response. Physical and chemical stimuli include pH, light, pressure, ionic factors and chemical agents, temperature, electric, and magnetic field [30]. Conventional hydrogels do not show any kind of response to abrupt change in environment. Because of their distinct biocompatibility, physical properties, and flexible methods of preparation, hydrogels can be used in different areas. There are numerous amounts of original papers, research works, and reviews focused on the synthesis, properties, and applications of hydrogels. This section comprises the basic features and application areas of hydrogels.

2.1 Sources of Hydrogels

Hydrogels can be prepared from both natural and synthetic sources. The feasibility of use of hydrogels is still restricted due to their lower mechanical strength and fragile nature. As a result, to increase gel strength, service life, and water absorption capacity, natural hydrogels are gradually replaced by synthetic hydrogels [31].

2.1.1 Natural Hydrogels

Hydrogels formed from natural polymers can be classified into two main groups: polysaccharides and polypeptides. These hydrogels typically possess inherent biocompatibility and biodegradability. But, natural polymers are often expensive, and fine structural alterations of them are often limited because of their complex structures and delicate nature.

Polysaccharide–Based Hydrogels

As cellulose has abundant hydroxyl groups, it can be used to prepare hydrogels easily with fascinating structures and properties. Main advantages of these types of hydrogels are that they are environment friendly renewable polymeric materials with biodegradable property [32].

Chitosan is a cationic copolymer, which has hydrophilic character with capability of degradation results in biocompatibility and biodegradability, which make it a very useful source of natural hydrogels. These highly potential hydrogels have a great use in tissue engineering and drug delivery [33].

Alginate is a well-known biomaterial, and it will continue its application as one of the most significant one among the numerous biomaterials used for hydrogel formation. Properties like biocompatibility, biodegradability, and nontoxic nature and wide applications in drug delivery and cell delivery systems make it a great source of hydrogels [34].

Polypeptide-Based Hydrogels

Collagen is the main extracellular matrix material of human and animals. Hydrogels based on collagen have a great use in tissue engineering and biomedical applications. Transparent collagen hydrogels can be used as a corneal substitute for cornea regeneration. Other polymers can be incorporated with collagen hydrogels to increase its mechanical strength and lifetime [35]. Gelatin-based hydrogels have been evolving as great multifunctional biomaterials. It is an easily digestible protein that contains all the essential amino acids. The use of gelatin over collagen is increasing day by day because of its inexpensiveness and high dissolving capability in water. The gels formed from gelatin are naturally biodegradable and show non-cytotoxicity toward human cells [36].

2.1.2 Synthetic Hydrogels

Synthetic polymers are generally derived from monomers like acrylamide, vinyl acetate, ethylene glycol, and lactic acid. Because of their broadly variable and easily altered properties, synthetic polymer-based hydrogels have been extensively studied nowadays. The structures of these types of hydrogels can be regulated by changing the preparation techniques as well as physical and chemical compositions. Properties including swelling ability, mechanical strength, biocompatibility, biodegradability, stability, and porosity can all be adjusted for specific application purpose [31].

2.2 Mechanism of Hydrogels

Hydrogels are crosslinked polymer networks swollen in a liquid medium. The polymer network acts like a matrix to hold the liquid together, and the imbibed liquid serves as a selective filter to allow free diffusion of some solute molecules. Hydrogels may absorb water from 10 to 40% up to thousand times of their dry weights [37–39].

In structure formation of hydrogels, crosslinks act as an integral part. Crosslink is a bond which links one polymer chain to other and results in a stable network structure. Hydrogels can be characterized into two classes depending on the types of crosslink junctions: the physically crosslinked and chemically crosslinked [40]. Addition of crosslinks can change the physical properties of the polymer depending on the degree of crosslinks usage. The crosslinked polymer becomes elastic at limited crosslinks but becomes rigid at high crosslinks. Crosslinks also decrease the viscosity and melting point and increase the glass transition temperature, strength, and toughness [41].

Crosslinking is not accurately a property of hydrogels, as it is more of a cause of all the other properties of the material itself. Basically, every characteristic of a hydrogel can be correlated to the crosslinking degree [45]. The crosslinking can occur by ultraviolet irradiation, heating, or chemical crosslinking via crosslinker with a huge ensemble of reactions [42]. It is possible to tune the property of the material by controlling the degree of crosslinking and optimize it for any different applications [43, 44].

In addition, mechanical properties of hydrogels are very important especially from the pharmaceutical and biomedical point of view. 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. The desired mechanical property of the hydrogel can be achieved by changing the degree of crosslinking [37, 45, 46].

Above all hydrogels must be biocompatible and nontoxic in order to make it applicable in biomedical field. Biocompatibility consists basically of two elements: (a) biosafety, i.e., appropriate host response both systemic and local (the surrounding tissue) and 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 [37, 47].

2.2.1 Physically Crosslinked Hydrogels

This type of hydrogels is physically crosslinked and also known as temporary gels or thermoplastic hydrogels. The reason for physically crosslinked hydrogels to maintain their stability is the presence of reversible physical transient junction domains, associated with hydrophobic interaction, chain entanglements, crystallinity, hydrogen bonding, and/or ionic complexation [48, 49]. As the use of crosslinking agent is avoided in this type of hydrogels, that's why it is receiving a greater interest in recent years. Different methods have been investigated to create physically crosslinked hydrogels, like ionic interaction, hydrophobic interactions, hydrogen bonding interactions, etc. [30]. *Alginate* is a well-known example of a polymer that can be crosslinked by ionic interactions. It is a polysaccharide consists of mannuronic and glucuronic acid residues (β-D-mannuronate (M) and α-L-guluronate (G) monomers) and can be crosslinked by calcium ions. The three-dimensional network with hydrophilic structure of these gels can be illustrated by the "egg-box" model where each calcium atom is coordinated to the carboxylates and hydroxyl groups of four G monomers from two adjacent chains of the polymers (Fig. 2) [50].

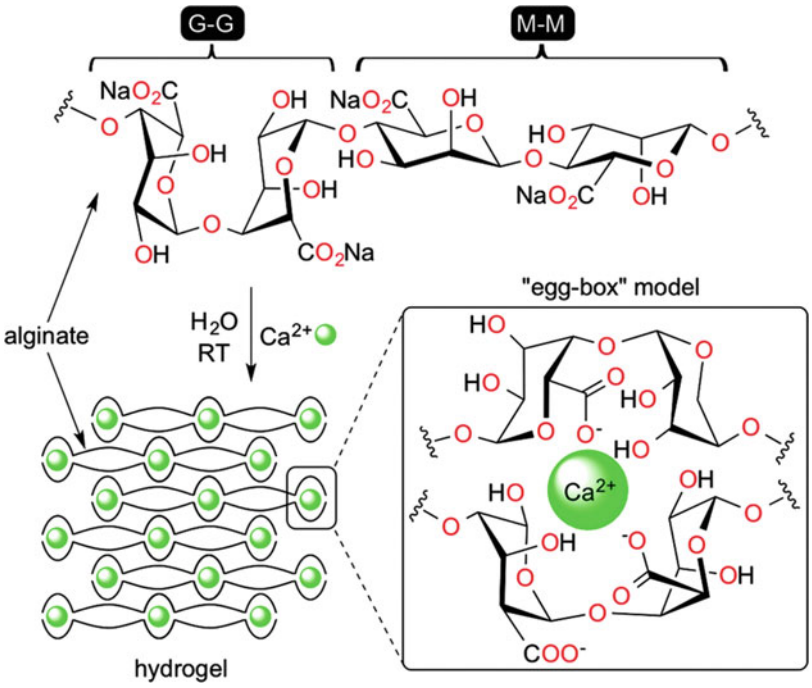

Fig. 2 Structure of the repeating units of sodium alginate (pK_a of carboxylic groups ~3 to 4) and formation of the hydrogel by coordination of Ca^{2+} cations between adjacent alginate chains as per the "egg-box" model. (Reproduced from [50]. Copyright © 2015, New Journal of Chemistry, Royal Society of Chemistry)

2.2.2 Chemically Crosslinked Hydrogels

Hydrogels that are chemically crosslinked are also known as thermosetting hydrogels. This type of networks has permanent junctions; that's why sometimes they are also called permanent gels [6]. Unless there is cleavage of the covalently crosslinked sites, they are not soluble in any solvents. Moreover, they cannot be successfully remolded or reheated after their initial heat-forming. Due to the lack of post-process modifications and process ability, benefit from chemically crosslinked hydrogels becomes limited [29].

Chemically crosslinked hydrogels can be formed by numerous techniques, like polymerization, radiation, small-molecule crosslinking, polymer-polymer crosslinking, etc. Radical polymerization of low-molecular-weight monomers like vinyl monomers in the presence of a crosslinking agent is a well-known method to generate chemically crosslinked gels (Fig. 3).

This type of hydrogels can also be attained by radical polymerization of polymers derivatized with polymerizable groups, i.e., macromonomer. UV-induced polymerization has been frequently used to prepare hydrogels in recent years. With this type of polymerization, patterned structures can be prepared. It should be kept in mind

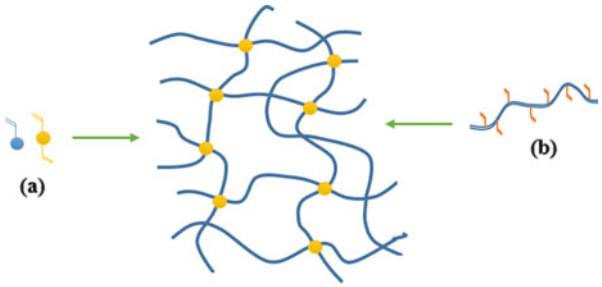

Fig. 3 Schematic diagram for formation of chemically crosslinked hydrogels by radical polymerization of (**a**) vinyl monomers and (**b**) macromonomers [30]

that the network structure might be affected if UV polymerization is carried out in presence of a drug [30].

2.3 Synthesis of Hydrogels

An enormous amount of works have been done and still going on to prepare novel hydrogels. Literature reveals that from preparation point of view hydrogels could be homopolymeric, copolymeric, and interpenetrating polymer networks (IPN). In this section, we will briefly discuss some of the preparation methods of these types of hydrogels.

2.3.1 Homopolymeric Hydrogels

Polymers that are derived from a single set of monomer/repeating unit are called homopolymers, which are the basic structural unit containing any polymer network [51]. Depending on the process of polymerization and the monomer nature, homopolymers may have crosslinked skeletal structure. Taking poly (2-hydroxyethyl methacrylate) (polyHEMA) as a monomer, polyethylene glycol dimethacrylate as crosslinking agent, and benzoin isobutyl ether as the UV-sensitive initiator is a one possible way to prepare homopolymeric hydrogel film. Prior to treating with UV radiation, the film was prepared in deionized water. In order to remove toxic and unreacted constituents that could harm living tissues, the film was immersed in water for 24 hours until it is fully saturated [37].

2.3.2 Copolymeric Hydrogels

When a polymer consists of more than one monomer/repeating unit, then they are called copolymer. Among these monomers, at least one must be hydrophilic in nature. These are arranged in random, block, or alternating configuration along the chain of the polymer network [52]. Using the mechanism of the ring-opening copolymerization of ε-caprolactone, the biodegradable triblock poly(ethylene glycol)-poly(ε-caprolactone)- poly(ethylene glycol) (PECE) copolymeric hydrogel can be synthesized for the improvement of drug delivery system. mPEG was used as initiator, stannous octoate as catalyst, and hexamethylene diisocyanate as coupling agent in the synthesis [53].

2.3.3 Interpenetrating Polymer Network (IPN) Hydrogels

This class of hydrogels is composed of two independent crosslinked synthetic and/or natural polymer components, contained in a network form. Generally, this type of hydrogels is synthesized by immersing a pre-polymerized hydrogel into a solution of monomers and a polymerization initiator. Thermodynamic incompatibility due to the perdurable interlocking of network segments is overcome, and limited phase separation can also be obtained in IPN method. It is believed that interlocked structure of the crosslinked IPN components can ensure the stability of the bulk and surface morphology [54]. To extend the functions of polyurethane (PU)-based hydrogels, Abraham et al. prepared IPN of PU and polyacrylamide (PAA) which could control water absorption. They mixed PU and PAA and then added the respective crosslinking agents, viz., vinylpyrrolidone and methylenebisacrylamide, followed by exposure of the mixture to UV radiation to obtain a hydrophilic hybrid IPN [55].

Because of hydrogels' extensive potential in wide range of applications, they have received much attention in the past 50 years [56]. Hydrogels' unique properties have made them ideal biomaterials for applications in cell encapsulation, drug delivery system, contact lenses, scaffolds for tissue engineering, biosensors, soft tissue replacement, intelligent cell culture substrates, wound dressing, and many more [57].

Hydrogels have been efficiently and effectively used in various biomedical applications because of its biodegradability and biocompatibility. As hydrogels mimic the natural tissues of the body, they have emerged as useful scaffolding biomaterials. For repairing tendon, ligament, cartilage, skin, blood vessels, and heart valves, both natural and synthetic hydrogels have been used as scaffolds in tissue engineering [58]. They are also used in wound dressings and as super-absorbent biomaterials due to their bio-adhesiveness [7, 21]. Synthetic hydrogels are suitable in the applications of contact lens, and some hydrogels have good transparency, high refractive index, and modulus which are essential for this product. PolyHEMA was the first hydrogel that has been used as a contact lens in 1960 [59].

The addition of the hydrogels to the surface of the soil can increase the water holding capacity of the soil and also can minimize the loss of nutrients from the soil [60]. The use of hydrogels as adsorbents for removing of heavy metal, recovering dyes, and removing of toxic components from various effluents has been studied. Adsorbents with carboxyl, sulfonic, phosphonic, and nitrogen groups on their surface favor metal ion adsorption [17].

3 Gelatin

Gelatin, a common, natural soluble functional protein compound having high interest and value, usually obtained by partial hydrolysis of the collagen which is the key fibrous protein element in the bones, cartilages, tendons, and skin, has the proficiency of forming transparent gels under certain conditions. Due to the variety

of the sources of collagen and extraction processes, gelatin illustrates a structure with changeable physical properties and chemical hybridism. Gelatin is exceptionally known for its unique gel-forming capability instead of some difference in the manufacturing techniques which ascertains it a worthy material for investigating the underlying functional properties in colloid studies [18].

3.1 Sources and Forms of Gelatin

There are different commercial sources of gelatin from which cattle bones, pig skin, hides, and fish are the primary ones. Special type of edible gelatin was extracted from edible Sudanese insects named melon sorghum bugs by Mariod et al. in 2011 [19]. However, mammalian gelatin is the primary contributor of the total world gelatin production, as well as fish gelatin provides a potential alternative. Plant sources are not available for gelatin, but gelatin can be obtained from seaweed extracts which is termed sometimes as vegetable gelatin.

3.1.1 Mammalian Gelatin

Connective tissues and bones of vertebrate animals are the primary element from where the mammalian gelatins are generated. Available sources of mammalian gelatin are pig skin (46%), bovine hide (30%), and pork and cattle bones (23.1%) [61].

However, researches on two different types of mammalian gelatins – bovine and pig sources – disclosed that both sources comprised of different components with wide distribution of molecular weight ranging from 10 to 400 kDa as well as the outcome established strong co-relationship between the average molecular weight and gel strength of the gelatin, with high isoelectric and melting points [62]. These two are usually required where health is concerned.

3.1.2 Fish Gelatin

From the name it can be assumed that the gelatin is obtained from the bones and skins of the fishes. This type of gelatin accounted about 1.5% of the total gelatin production which is increasing day by day showcasing the fact that the production of gelatin from alternative nonmammalian sources had grown in importance [61].

Apart from the well-known sociocultural and sanitary aspects, the researchers have the ascendant interest to optimize the extraction of gelatin from the by-products of fish industries in the last decades as an alternative source of gelatin [63].

One of the vital problems of fish gelatin is that it is less stable and has worse rheological properties than any of the mammal gelatins, and this may cause their applications limited. However this is only true for the reported cold-water fish such as cod, lumpfish, megrim, salmon, Alaska pollock, etc. Recent studies on tropical and subtropical water fishes such as tilapia, niger perch, catfish, etc. reported that they have nearly similar thermostability and rheological properties like mammal gelatins depending on the raw materials, pH, temperature, time, and other processing conditions [63–67].

3.1.3 Insect Gelatin

The insects can act as an interesting alternative source of gelatin. Mainly in Sudan, some edible insects are found such as *Agonoscelis pubescens* (sorghum) and *Aspongopus viduatus* (melon bug), and the oils extracted from them are highly used in cooking and some medicinal purposes. Thus the extraction of gelatin from these also took a great interest. Different types of extractions provide different amounts of yield like extraction using hot water showed up to 3% yield whereas mild acid extraction provided 1.5% yield. However, distilled water extraction gave only 1% yields. Melon bug gave high yield (about 1.45%) rather than the sorghum bug (about 1.3%). FTIR spectra of the obtained gelatins were similar with the commercial one showing the amide II bands around at 1542–1537 cm^{-1} [68].

From the researches, it is evident that aquatic source gelatins are similar in functionality like land-based gelatins [69, 70]. Both pork and fish gelatin have similar properties like gel strengths and melting points that follow the same trend with increased maturation time. However, due to the lower melting point of fish gelatin, it takes more time to reach the steady values. Both pork and fish gelatin shows the same pH stabilities, but NaCl depresses more readily in fish gelatin. Sucrose also has similar effects on both types of gelatin by increasing the gel strengths and melting points.

3.2 Structure of Gelatin

Understanding the chemical composition of gelatin is needed in order to explain the mechanism of gelation and other functional properties of gelatin. Numerous studies were carried out in this regard [71, 72], and most feasible result was based on the data of gelatin and ox-hide collagen using a wide variety of analytical techniques.

From the documentation, it was accepted that gelatin contains about 18 amino acids with 3 of them predominating in the structure. All these amino acids are linked together in a partly ordered fashion. About 1/3 to 1/2 of the total amino acid residues are glycine or alanine where glycine is the predominant N-terminal residue for alkali-processed gelatin and alanine stands for the acid-processed gelatin. Proline or hydroxyproline stands for ¼ of the amino acid residues, and about ¼ remains acidic or basic [18]. However, it was noticed that aromatic acid residues and "tryptophan" an essential amino acid have been missing from all types of gelatin compositions [73]. Changes in composition have also been observed during the change of the sources of gelatin. For example, whale or fish gelatin has larger amount of hydroxyamino acid serine and threonine compared to other land mammals.

Gelatin may contain small amount (~ 1%) of sugar. Depending on the sources and method of extraction, the nature, type, and amount of sugars vary. Till date five types of sugars have been documented (galactose, glucose, lactose, mannose, and xylose). They actually arise from mucopolysaccharides, a cementing substance and converted as amino sugar.

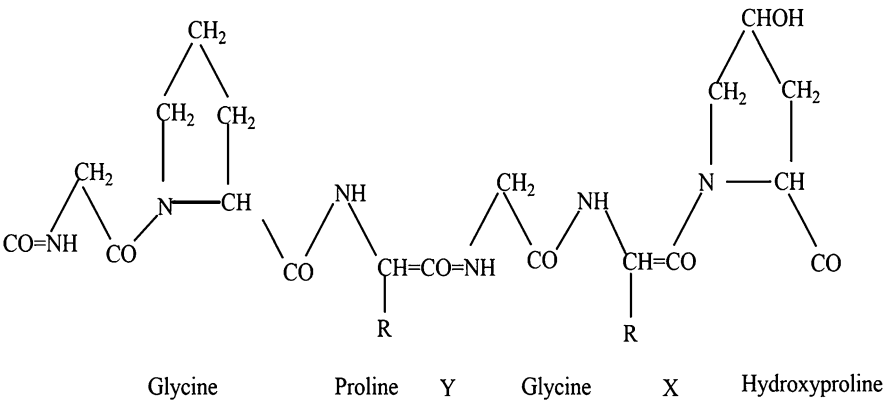

Fig. 4 Chemical configuration of gelatin

The real molecular structure of gelatin is still not clear till now, but arrangement of a small number of peptide fractions has been confirmed. A single-chain model consisting of repeating units of three amino acids in sequence -P-G-R where P is the proline/hydroxyl proline, G is glycine, and R is the side chain was first proposed by Astbury to elucidate a helical structure with the same sequences but that was omitted by Schroeder and Kroner as they speculated the sequence gly-pro-hypro-gly or gly-pro-hypro-gly-prohypro-gly which is the essential requirement of the structure of collagen. So, finally, gelatin is accepted as a linear chain with small branches as shown in Fig. 4 [18].

3.3 Properties of Gelatin

Gelatin is a biopolymer with keen interest based on mostly for its rheological and thermal properties. These properties diversify the application of gelatin.

3.3.1 Physical and Chemical Properties

Gelatin is very faint yellow to amber in color, and usually dry pure commercial gelatin is a tasteless, odorless, transparent, brittle, glass-like solid comprising a range of molecular weight about 40,000 to 90,000 Da.

It can establish the equilibrium both with acids and bases which can explain the nature of polypeptides and determine the amino acid composition due to the availability of amino and carboxylic groups on its backbone protein chain molecules and also help to unravel the structural stabilization and reaction nature toward other substances with it by using its ionization constant and electronically charged group.

Gelatin comprises several ionizable functional groups such as terminal α-amino and α-carboxylic groups, carboxylic and aspartic acid containing carboxylic groups,

the ε-amino group of lysine, the imidazolium group of histidine, and the guanidinium group of arginine. Isotonic point, which determines the dispersion and gelation as well as the use of gelatin in the food industry, is another most important property of gelatin, and it has been reported that the isotonic point of Type A gelatin is 7 to 9, whereas for Type B gelatin, it is about 4.8–5.1. Although gelatin is a water-soluble protein, the dispersion of gelatin must need some ample care. The dissolution process starts with the short time soaking of the granules in suitable amount of cold water and then increases the final temperature of the hydrated gelatin at about 35 °C either by continuous stirring and heating or by adding hot water to the system. Viscosity is another important property that varies extensively with the types of gelatin, temperature, time, and the concentration. However, the process of extraction also has an effect on it as acid-processed one generally possesses slightly higher intrinsic viscosity than the alkali-processed, but no difference had been found in the melting points.

Formation of gel, a vital process, does not have any clear concept. Several gelation processes of gelatin were recorded till date, but none of them can solely explain the mechanism clearly. A general idea being that the minute sections of gelatin undergo interactions and form crystallites which further turned into a ramified three-dimensional network susceptible to immobilize the liquid. This sol mixture finally resulted in the desired elastic solid or gel. The bindings in gel occurred by the implications of both hydrogen bonds and van der Waals forces as per the suggestions given by Ferry [20]. However another research concluded that the bindings were due to the peptide linkage [74]. Degree of acidity and speed of cooling time of the gelatin affects the properties of settled gels. Presence of acid lowers the liquefying temperature of a gel and increase the settling time. However, slow cooling speed permits the better orientation of the molecules within the gels. Gelatin is the rarest protein which is capable of forming good foams. Its sol can be cooled to 10 °C in order to obtain thick egg white consistency which is whipped to form foams.

Recently works has been focused on the properties of the film formed by gelatin as there are only a few experiments and results enlisted in the past on the film-forming ability of gelatin [75]. Gelatin, however, can form moisture impermeable edible films, and the properties like moisture content, water activity, and moisture barriers of these films have attracted the interest of the researchers. Due to the poor emulsifying properties of gelatin, it has a limited use in some sectors, but there is no certain method that has been worked out to improve this functionality till date. If this functional property can be improved, then it could be useful for multifunctional purposes in food and pharmaceutical industries. However, gelatin is capable to increase the viscosity of the continuous phase of an emulsion and thus can delay the flocculation and coalescence which improves the stability of O/W emulsions. The thermodynamic behavior of gelatin with acidic or neutral polysaccharides was difficult to understand because of the complex structure of individual polymers [76]. The pH and ionic strength of mixtures, ionogenic properties of gelatin, and gelatin-solvent interactions are responsible for the degree of interaction of gelatin with other biopolymer.

3.3.2 The Biological Activity of Gelatin

Although gelatin has some beneficial biological functions in food and pharmaceutical industries, the biological activity of gelatin has been reported as zero as per [73] because it lacks an essential amino acid "tryptophan" which is vital for a biologically completed protein, i.e., gelatin cannot provide a complete set of amino acid essentially required for the synthesis of protein.

Functional components can be entrapped by gelatin as a carrier and thus provides protection against oxidation or degradation in the time of storage as it can form complex with anionic polymer in the form of microcapsule. This is used when bioactive packaging is ingested in the body.

3.4 General Application

Every year a large amount of gelatin has been used in the food industry especially in making desserts, candies, jellied meat, ice creams, bakery goods, and dairy products as clarification agent, stabilizer, thickener, emulsifier, texturizer, and protective material [20, 74]. Due to the surface-active properties of gelatin, it has also find its use as a foaming, emulsifying, and wetting agent in pharmaceutical, medical, and technical applications.

3.4.1 Food Industry

Due to its high content and protein, gelatin is widely used in the food additives or in healthy food. Gelatin possesses the unique hydrocolloidal property which makes it applicable in the numerous food industries. The major classical uses of gelatin are in clarification and stabilization. Gelatin removes turbidity of a solution by flocculation and sedimentation and thus brings the clarity. So, it is highly used in drinks and beverages containing tannins. Tannins react with gelatin to produce sediment as a form of tannin-gelatin complex. The amount of gelatin in clarification process should be accurate; otherwise over gluing or stabilization of colloidal matter may occur due to the excessive amounts or insufficient amounts of gelatin.

Around half of the edible gelatin in food industries is used for the preparation of desserts including food toppings, pastry toppings, etc. While using gelatin in desserts, the pH should be maintained between 3 and 3.5 for palatable tartness. Due to the foaming ability of gelatin, it is also used in the manufacture of marshmallows, a colloidal dispersion of gas within a liquid.

Gelatin is extensively used in pies, breads, and cakes as a setting agent, stabilizing substance, or foam-forming materials. It is also used in the meat industry especially in the preparation of bone-cooked hams, sausages, cheese, canned hams, and meat jellies as coating agents. Gelatin can also be used in frozen fruit puree and frozen turkey products. Due to the high swelling and water-binding capacity, gelatin impairs juiciness in frozen fish or meat product and thus reduces drip loss.

3.4.2 Pharmaceutical Industry

In developed countries, almost 10% of the edible gelatin is used in pharmaceutical industry especially in capsules and emulsions. Although the biological value of gelatin is zero, it has several surgical and serological characteristics and thus produces some oncotic effects and can be used in the preparation of plasma substitutes. Highly purified gelatin hydrolyzates are often co-administered with products to compensate calcium deficiency in pregnancy, adolescence, and lactation, or it can be used to treat calcium deficit associated with osteoporosis in the elderly [77]. Methionine and cystine from derived gelatin are carrier of sulfur, and that's why they are beneficial in treating connective tissue diseases like scleroderma, rheumatoid arthritis, etc.

Currently, gelatin hydrogels made progresses in the sector of bone regeneration and also emerge as a surgical tool for skull defect repair and reconstruction of skull base [78]. Gelatin is also used as injectable biomaterials for one surgery, oral capsules, breath freshener microcapsules, oral dissolvable medicaments, cosmetics, prosthetic heart valves for intravascular applications, and as raw materials for artificial skin [79–81].

3.4.3 Photographic Industry

For processing of exposed film material, gelatin emulsions have been thoroughly used as a component in a photographic developer as it enhances the capability of a developer to distinguish between exposed and unexposed crystals. It is the best medium for photographic emulsions and has been continuously improving the quality and speed [82].

3.4.4 Other Uses

Gelatin also finds some other uses which are summarized below:

- It is used as a carrier or separating agent of some other materials like β-carotene. It makes β-carotene water-soluble.
- It works as a binder between match heads and sandpapers.
- Hydrolyzed collagen is a non-gelling variant of gelatin which is broadly used in the cosmetics.
- It is also used as an external surface-sizing material for papers.
- Unrefined gelatin is used in manufacturing glue, i.E., hide glue.

4 Hydrogels from Gelatin

4.1 Synthesis of Gelatin-Based Hydrogels

In general, based on the various available forms (e.g., solid molded forms, pressed powder matrices, microparticles, coatings, membranes, etc.), hydrogel network can be fabricated by molecular entanglements and/or through secondary forces including ionic, H-bonding, or hydrophobic forces. Many natural and synthetic polymers are

capable of forming physical and chemical hydrogels. Physical homogeneous hydrogels are characterized by some physical interaction rather than chemical bonding, while chemical hydrogels mainly consist of covalent bond in presence of a crosslinker. But sometimes chemical crosslinker is not the major element to form chemical hydrogels. Gelatin is an amphipathic polymer, capable of forming chemical hydrogels in presence of crosslinkers. On the other hand, simple dissolving of gelatin in hot water and then cooling down to below the room temperature will form a physical gel of gelatin [21]. However, this type of physical hydrogel is not stable at body temperature and does not allow researchers to control and fine-tune its properties [83]. This is one of the drawbacks that have led to introduce chemical hydrogels of gelatin by crosslinking without prior modification and/or after functionalization of its side groups. Unmodified gelatin can be crosslinked in various ways to form a covalent network, such as by chemical or enzymatic crosslinking [26].

Considering the reaction techniques to form physically and chemically crosslinked hydrogels, several distinct techniques have been followed for decades. Radical polymerization, crosslinking with aldehydes, crosslinking by addition and condensation reaction, crosslinking by using radiation and enzymes, etc. are some of the most popular ways to form chemical gels. Of these, crosslinking with aldehydes and condensation reaction technique are the two most important pathways to form gelatin-based chemically crosslinked hydrogels [22].

For the last few years, many researchers have exhibited their interest in developing various gelatin-based hydrogels, because of its multifunctional prosperities and easygoing preparation methods. Some of the synthesis techniques of gelatin-based hydrogels are stated in the following subsection starting from 4.1.1.

4.1.1 Gelatin–Methacrylate Hydrogels

As a part of the chemical modification (or functionalization), methacrylic anhydride is widely chosen over other modifiers [26]. This reagent has been found to synthesize hydrogel with pure gelatin by Yue et al. By a direct reaction between gelatin and methacrylic anhydride in phosphate buffer, a substitute group gelatin methacryloyl is formed at 50 °C. This reaction introduces methacryloyl substitution groups on the reactive amine and hydroxyl groups of the amino acid residues. This gelatin methacryloyl undergoes photo-initiated radical polymerization (i.e., under UV light exposure with the presence of a photo initiator) to form covalently crosslinked hydrogels. This kind of hydrogels is suggested to be applied in biomedical field (Fig. 5) [84].

One recent example is the development of gelatin methacrylate (GelMA) hydrogels where the hydrogel is synthesized by adding methacrylate groups to the amine-containing side groups of gelatin, which becomes a photo-crosslinkable hydrogel [85]. The author has proposed the use of photo-crosslinkable GelMA hydrogel as a permissive biomaterial for the formation of functional vascular networks. A different study conducted by Jason et al. demonstrated that gelatin methacrylate hydrogel was fabricated from methacrylated gelatin macromer (by using methacrylic anhydride) which in turn crosslinked to get the final hydrogel.

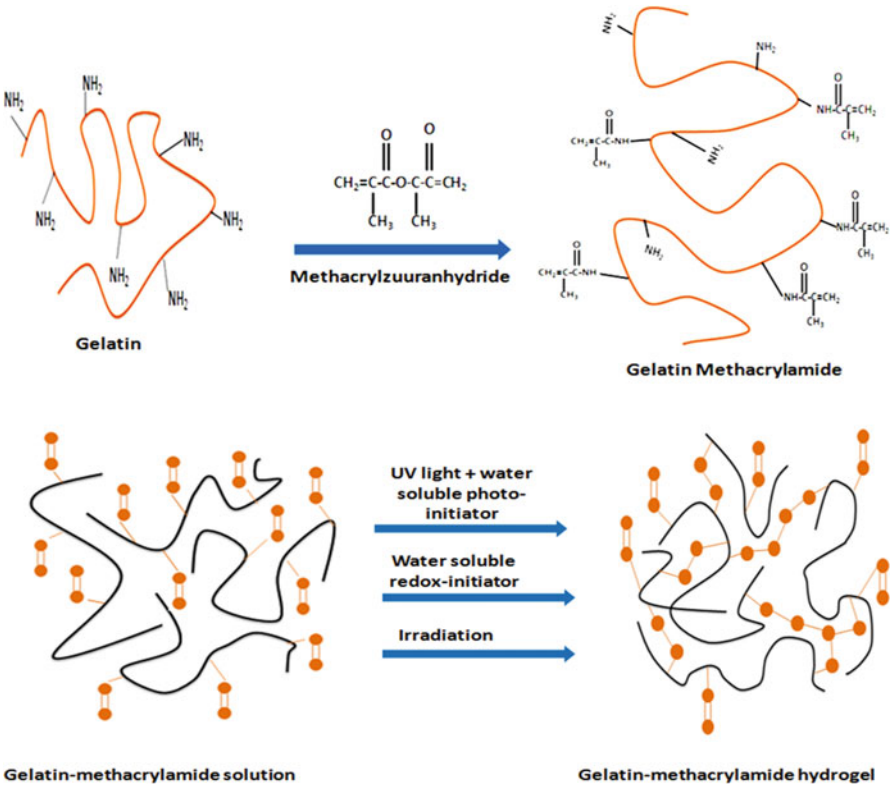

Fig. 5 Chemical modification of gelatin with methacrylamide side groups [91]

Rather than being applied as a functional vascular network, this type of hydrogel is highlighted for its application in microscale tissue engineering and as an attractive material for cell-laden microtissues [86]. The application of this hydrogel can also be selected by controlling some of the condition during its fabrication. For example, high degree of methacrylation (low biodegradability) during macromer preparation results its application to fabricate organized vasculature within engineered tissue constructs which is able to create platforms for drug and cytotoxity and basic biological studies [87]. However, immobilized inorganic-organic phase is also desirable for implant application. Focusing on this point, gelatin-methacrylated hydrogel-hydroxyapatite composites were synthesized by photochemical grafting which is followed by biomimetic mineralization and finally a covalent immobilization on titanium substrates. This kind of material is expected to be used for bone regeneration and repair not only on titanium substrate but also for many metallic, steel, and other polymeric substrates [88].

Similar crosslinkable hydrogels are fabricated by Salamon et al. without further photolytic crosslinking which is used for promoting in-vitro chondrogenic differentiation of human mesenchymal stem cells [89]. Another study conducted by

Loessner et al. stated the use of gelatin methacryloyl-based hydrogels as modular tissue culture platforms [83]. Again, gelatin was chemically modified with methacrylamide side groups which could be further polymerized by radical initiators or high energy irradiation. The methacrylamide side groups are introduced by reaction with methacrylic anhydride with the ϵ-amino groups of lysine residues [90]. Besides using this hydrogel solely, a hydrogel composite can be fabricated by embedding multipotent stromal cells (MSCs) and cartilage-derived matrix (CDM) particles into the prepared hydrogel. This kind of hydrogel composite was fabricated by Visser et al. which ultimately showed its application in the field of endochondral bone formation [91].

Besides using pure gelatin as a raw material for hydrogel fabrication, recombinant gelatin can also be used for sustained release of the proteins. This is also a pathway to make chemical gel through a chemical crosslinker. Here the recombinant gelatin is modified with methacrylic anhydride, and the product obtained upon methacrylation reaction acts as a crosslinker to form the desired hydrogels. After dissolving the modified gelatin, centrifugation was performed, and the final addition of potassium peroxodisulfate and N,N,N′,N′-tetramethylethylenediamine into the mixture helped to induce crosslinking of gelatin methacrylate residues to obtain the final hydrogels [92]. It is to be noted that to obviate the drawback of gelatin-based physical gel for being less stable at body temperature, gelatin has been chemically functionalized with unsaturated methacryloyl groups to result in gelatin methacryloyl, which forms covalently crosslinked hydrogels by photo-initiated polymerization under mild conditions [83].

4.1.2 Gelatin-Dextran Hydrogels

Several works have been published based on the aldehydes as the crosslinker in forming hydrogels from gelatin. For example, in a study of Draye et al., a hydrogel film was fabricated by using gelatin as a main raw material and dextran dialdehyde as a crosslinking agent. Here a 20% oxidized dextran dialdehyde was used as a crosslinker, and then gelatin is added to make the 1-mm-thick hydrogel film. Crosslinking between gelatin and dextran dialdehydes was executed by the production of Schiff base links between free ϵ-amino groups of the lysine and hydroxylysine residues present in gelatin and the aldehyde groups in case of oxidized dextran [93]. Vandenbulcke et al. have reported on the preparation of gelatin hydrogels crosslinked with partially oxidized dextrans (Fig. 6) [94].

4.1.3 Gelatin–Chitosan Hydrogels

Chitosan is considered as an excellent biopolymer due to their physicochemical and biological properties. Chitosan dissolves readily in acidic environment (pH below 6) which can be retained by crosslinking through various agents, e.g., glutaraldehyde, glyoxal, or epichlorohydrin [95]. However, a derivative of chitosan is carboxymethyl-chitosan (CM-chitosan) that showed better solubility profile as well as nontoxicity, biocompatibility, biodegradability, and low-immunogenicity [96]. CM-chitosan hydrogels were found mechanically weak. For this reason, gelatin

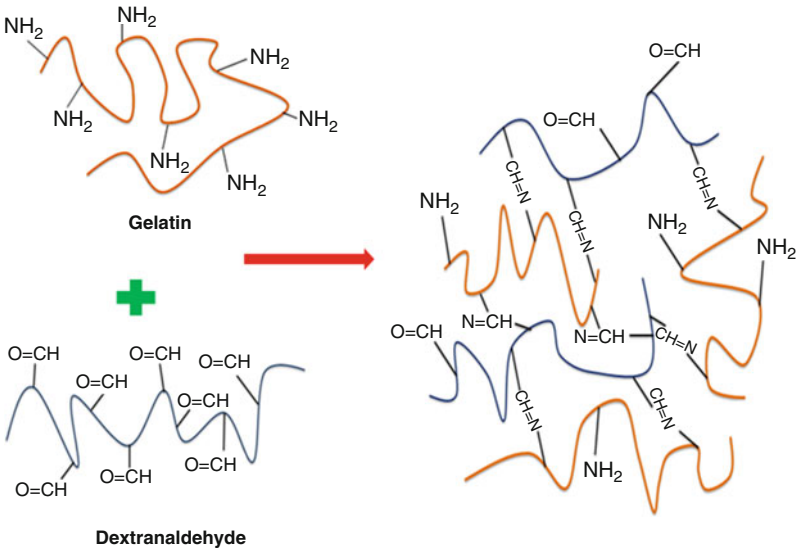

Fig. 6 Gelatin hydrogels crosslinked with partially oxidized dextrin [90]

was added to adjust the compressive modulus of the hydrogels. Huang et al. developed a CM-chitosan/gelatin hydrogels through radiation crosslinking approach which can be used as a promising wound healing material [97].

Besides using chitosan/gelatin hydrogels for wound healing material, sometimes natural phenolics are also added into the hydrogel system, and its application was observed as an antibacterial (antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus)* dressing for chronic wounded area. Under mild condition, the natural polyphenolic extract was collected from *H. virginiana which* was oxidized by laccase in a one-step process. Under the dual action, this laccase was also used to covalently crosslink chitosan and gelatin during hydrogel formation. Considering the synthesis technique, the natural phenolic compounds and tyrosine residue in proteins are oxidized by laccase which in turn converted into reactive quinones. This quinone further reacts with nucleophiles such as amino groups from chitosan and gelatin by 1,4-Michael addition or Schiff base formation [98].

Other than using natural product or chitosan derivative for the treatment of glaucoma, an injectable thermosensitive chitosan/gelatin/glycerol phosphate (C/G/GP) hydrogel as a sustained-release system of latanoprost was introduced by Cheng et al. [99].

In a very recent study conducted by Cheng et al., a thermosensitive hydrogel was fabricated by using chitosan and gelatin which was intended to be used in treating ischemic diseases through sustained release of adipose-derived stem cells. Among different techniques used for synthesizing chitosan/gelatin hydrogel, thermosensitive is considered advantageous since it does not need copolymerizing agents,

organic solvents, or an externally applied trigger for hydrogel formation. Considering chitosan-based thermosensitive hydrogel preparation, it needs only chitosan/glycerophosphate system. In this case the chitosan/gelatin hydrogel was fabricated by mixing chitosan-gelatin solution in glycerophosphate under controlled condition [100].

In Sect. 2.3.3 the formation of hydrogel through interpenetrating polymer network pathway was illustrated. The similar kind of technology was applied by Wang et al. who investigated the fabrication of in situ semi-IPN (interpenetrating polymer network) hydrogels by the free-radical polymerization and crosslinking reactions among chitosan, acrylic acid, gelatin, and N,N′-methylene-bis-acrylamide in an aqueous solution using gelatin as the interpenetrating component. Ammonium persulfate was used as an initiator of the free-radical polymerization. Deviating from the mainstream of biomedical and tissue engineering field, this hydrogel is solely applied in adsorbing the Cu^{2+} ion [101].

4.1.4 Gelatin-Polyvinyl Alcohol (PVA) Hydrogels

To fabricate chemical hydrogels from gelatin, condensation reaction is the second most popular way. Here a condensation reaction takes place between gelatin and other molecule, by eliminating a small molecule on being their combination. This kind of reaction was observed by Pal et al. during the formation of a hydrogel prepared from polyvinyl alcohol and gelatin. An esterification-condensation reaction occurred in presence of a catalyst (here, hydrochloric acid is used), between the two reacting sites of gelatin and polyvinyl alcohol (hydroxyl group of PVA with the carboxyl group of gelatin). The produced hydrogel membrane was proposed for biomedical application (e.g., contact lenses, artificial corneas, wound dressing, and coating for sutures, catheters, and electrode sensors) by the researchers [23]. In the field of tissue engineering, hydrogel from gelatin demands a chemical modification which could be achieved by covalent modification of proteins using crosslinkable functional groups and chemical grafting of synthetic polymers onto protein backbones. However, due to side chain disruption by chemical modification, protein degradation, denaturation, or loss of biological activity can be experienced. From this drawback, Lim et al. attempted to fabricate a gelatin-based hydrogel without performing any kind of modification to the protein molecule. As a result, a tyramine-functionalized poly (vinyl alcohol) (PVA) polymer (PVA-Tyr) was fabricated and finally turned into hydrogels with gelatin using a visible light-initiated crosslinking mechanism [102].

Most of the gelatin-based hydrogels are prepared for being used either in tissue engineering or biomedical implant field. However, Hui et al. explored a new hydrogel bead mainly prepared from PVA and gelatin through chemical crosslinking in the aqueous solution of saturated boric acid and $CaCl_2$ (curing agent) which is mainly applicable for adsorbing Pb(II). Besides PVA and gelatin, sodium alginate was added to provide the spherical skeleton for PVA hydrogel by curing. $CaCl_2$ reacted with carboxyl group present in the sodium alginate and gelatin, and as a result calcium ion can be induced into PVA crosslinking structure [103].

4.1.5 Gelatin–Alginate Hydrogels

Alginate is a linear anionic polysaccharide which is capable of forming ionic hydrogel in presence of divalent cation like calcium. Alginate has been extensively used in drug delivery and tissue engineering. However, non-biodegradability results from unmodified and photo-crosslinked alginate. Note that, photo-crosslinked alginate based hydrogel showed controlled mechanical properties [104, 105].

Wang et al. reported a hybrid hydrogel composed of alginate, gelatin, and nanocrystalline cellulose [106]. Here, gelatin provides the functional groups for chemical crosslinking. The novel hybrid hydrogels were synthesized through chemical bonding of alginate and gelatin. As well as ionic crosslinking of alginate with zinc ions and supramolecular interaction with nanocrystalline cellulose were also observed. The mechanical properties, crystallinity, and non-degradability of the hydrogel were improved by adding nanocrystalline cellulose; effective cell adhesion was obtained through gelatin molecule, whereas lower toxicity with controlled-ionic crosslinking was resulted from alginate and $ZnSO_4$. This injectable hydrogel was proved to be economically feasible for cell and growth factor delivery as well as healing bone defects [106].

Besides using pure alginate, some modified form of alginate was found to be observed in the field of biomedical and tissue engineering. In a study conducted by Yuan et al., sodium alginate and gelatin were used to synthesize double network hydrogels. A natural polysaccharide, sodium alginate, was modified by oxidizer to form aldehyde sodium alginate (ASA), and methacrylate groups were further grafted on the main chain of ASA forming aldehyde methacrylate sodium alginate. The second element gelatin was modified with ethylenediamine (ED) forming amino gelatin which can graft more amino groups. When aldehyde methacrylate sodium alginate and amino gelatin aqueous solutions were mixed, the Schiff base reaction occurred quickly to form the primary network between aldehyde groups in aldehyde methacrylate sodium alginate and amino groups in amino gelatin. After that to produce the secondary network, a 365 nm ultraviolet (UV) light was applied for initiating the radical reaction of methacrylate groups in aldehyde methacrylate sodium alginate. This double network hydrogels may have great potential application in the field of therapeutic materials and regenerative medicines [107].

In a recent study, Balakrishnan et al. reported the use of oxidized alginate in forming hydrogel with gelatin. Rather than using toxic crosslinking agents, the authors preferred to use self-crosslinking technique [105]. Thus periodate-oxidized alginate and gelatin undergo self-crosslinking in the presence of borax, to form in situ gelling hydrogels. The hydrogelation happens between periodate-oxidized alginate and ϵ-amino groups of lysine residues of gelatin through en route of borate-diol complexation followed by Schiff reaction. This self-crosslinked hydrogel may be predicted to be a promising agent for neo-cartilage formation as well as in treating osteoarthritis [105].

4.1.6 Gelatin–Carbon Nanotube Hydrogels

The addition of carbon nanotube imparts good electrical conductivity to the gelatin-based hydrogel [108]. In general, a hybrid gelatin hydrogel with carbon nanotubes

was prepared by physically mixing the ingredients [25]. A modified grafting technique and emulsion polymerization method are involved in presence of sodium methacrylate and N,-ethylenebisacrylamide to fabricate the hybrid hydrogel from gelatin and multiwalled carbon nanotubes. In addition, different amounts of nanotubes were covalently incorporated into the polymeric hydrogel network in order to determine the percentage bestowing the maximum electric sensitivity to the hybrid hydrogel composite microspheres. A modified grafting from approach was used which involves a direct one step polymerization of the unmodified multiwalled carbon nanotubes. The prepared hydrogel was mentioned to be used as a drug delivery microsphere which can turn on the electro-responsive release of Diclofenac sodium salt investigated by Spizirri et al. [109].

In a different study, multiwalled carbon nanotubes (MWNTs)/gelatin composites were synthesized by dispersion of MWNTs through ultrasonication in an aqueous medium. An anionic surfactant, e.g., sodium dodecyl sulfate, was used for the hydrogel fabrication [108]. Similarly within the gelatin hydrogels, functionalized single-walled carbon nanotube has been incorporated and dispersed to make hybrid hydrogel better suited in terms of stability, elasticity, and conductivity compared to native gelatin-based gel [110].

It is also possible to fabricate the hybrid hydrogel with carbon nanotube by using methacrylate gelatin (GelMA). This type of study was conducted by Shin et al. where the methacrylate gel coating was applied on the carbon nanotube (CNT). GelMA is photopatternable that allows the easy fabrication of microscale structure. The coating is possible due to the hydrophobic interaction between the polypeptide chains of the GelMA and the sidewalls of the nanotubes. The authors indicated the possible use of this CNT reinforced methacrylate gelatin hydrogel in fabricating complex 3D biomimetic tissue-like structures or can be used in in-vitro cell studies [111].

Other than these much classified sections of gelatin-based hydrogels, some hydrogels can be fabricated by using renowned crosslinker or crosslinking techniques. Some of them are stated below:

The most common crosslinking agent used for gelatin-based hydrogels is glutaraldehyde. In a study of Tabata et al., fibroblast growth factor was incorporated both in acidic and basic gelatin-based hydrogels, differing in isoelectric point. However, both of the gelatins use glutaraldehyde as the crosslinking agent, and a chemical crosslinking reaction takes place between gelatin and glutaraldehyde [112].

From the data obtained by the researchers, it is well understood that aldehyde is considered as a good crosslinker for covalent crosslinking of gelatin without chemical modification. Howsoever, aldehyde is avoided for simultaneous cell encapsulation application due to its immunogenicity, cytotoxicity, and inflammatory effects of their degradation products [113]. In a replacement of aldehyde, genipin, a natural crosslinking agent, is used, which is deliberated less cytotoxic compared with aldehydes. But it must be used at a low dose when the hydrogel is used to encapsulate cells [114]. Over and above, major disadvantages of direct crosslinking methods (without prior modification of gelatin) include poor control over the crosslinking density and the resulting stiffness of the hydrogel. For these reasons,

using functionalized gelatin has become a favorable way over the direct crosslinking of gelatin [26].

Compared with direct crosslinking techniques, introduction of functional groups to the gelatin backbone is a crosslinking strategy, with a high degree of control over hydrogel design and properties. Two frequently used techniques for hydrogels preparation after functionalization are (photo)-radical-initiating systems and enzymatic crosslinking of functionalized gelatin. Photoinitiation provides good temporal and spatial control on the crosslinking process over direct crosslinking techniques, which is essential for constructing an architecturally complex tissue analog. For photoinitiation, both ultraviolet light (UV) and visible light (VIS) are used [26]. However, enzymatic crosslinking of gelatin under physiological conditions by means of transglutaminases or tyrosinases ensures a better cell friendly approach [115].

4.2 Properties (Physical and Chemical)

Native gelatin has found to show low level of immunogenicity and cytotoxicity, biodegradability, clotting property, etc. Specifically, scientists have explored modification of the gelatin backbone with PEG-dialdehyde and/or ethylene-diamine-tetraacetic dianhydride (EDTAD) to alter the physicochemical properties of the gelatin and to affect the subsequent release, degradation, and solubility of model drugs from and within the hydrogel. Modification of gelatin with EDTAD introduces polyanionic molecules into the gelatin chain, increasing the hydrophilicity of the gelatin backbone with the addition of charged groups and thereby potentially improving the swelling capability of the resulting hydrogel. Additionally, modulation of the crosslinking modality (i.e., percent glutaraldehyde or self-crosslinking via exposure to dry heat) of unmodified and modified gelatin are introduced to affect the solubility and density of the resulting matrix, which contribute to the swelling/degradation and the release mechanism of therapeutic agents [24].

Earlier it was discussed that the gelatin gels are nearly unstable at body temperature because of their low melting point. So, it is necessary to stabilize the gelatin gels before they can be used for wound healing purposes in contact with the body. This is usually done by crosslinking between the protein chains by treating the gelatin with either formaldehyde or glutaraldehyde as crosslinkers. Alternatively, this can be achieved by crosslinking of gelatin with polyaldehydes produced by partial oxidation of polysaccharides such as dextran. Alone or in combination, gelatin and dextran are widely used for drug delivery systems [93].

Because of the acidic property of gelatin-based hydrogels, sometimes they are used for storing the basic fibroblast growth factor (bFGF). bFGF is known to be stored in the body through ionic complexation with acidic polysaccharides of the extracellular matrix such as heparan sulfate and heparin. This poly-ion complexation property of gelatin protects bFGF from in-vivo denaturation and enzymatic degradation [112].

Some recent studies have shown a possible way to fabricate recombinant gelatin which exhibited well-defined sequence, molecular weight, and isoelectric point compared to nonrecombinant gelatin. This property also enables the gelatin to become crosslinked with methacrylate residue by radical polymerization. Particularly, compared to other hydrogels, methacrylated recombinant gelatin-based hydrogels are better suited for the protein delivery [92].

4.3 Applications in Variegated Fields

4.3.1 Tissue Engineering

Tissue engineering is a modern technique to treat the imperfection due to injury, disease, and failure both in the tissue and the body. A peroxidase-catalyzed enzymatic crosslinking of the gelatin chain results to a gel that does not melt at 37 °C. In this case the gelatin was modified by incorporating a phenolic hydroxyl group. The control in phenolic group imparts an effect on gelation time, mechanical properties, and proteolytic degradability. The application of this kind of gel was found in both tissue engineering and drug delivery fields. In addition, in case of practical application of this gel, researchers have injected a mixture of produced gel (gelatin-phenolic hydroxyl), horseradish peroxidase, and H_2O_2 which have successfully gelled at the injected site in-vivo and remained intact for 1 week without inducing necrosis in the surrounding tissues [92]. However, various types of enzyme enable the formation of hydrogels from gelatin through many different ways (Table 1).

In the field of tissue engineering, a hydrogel can be composed of mainly gelatin methacrylamide and polyethylene glycol (PEG). Both the crosslinking reaction of PEG and the incorporation of gelatin methacrylamide were formed by two distinct types of coupling reaction. This kind of hydrogels showed enhanced mechanical integrity and cytocompatibility and thus is accepted for tissue engineering scaffold application [119].

The following table (Table 2) has enlisted some of the recent developments in the field of tissue engineering by gelatin-based hydrogels.

4.3.2 Drug Carrying Vehicles

Gelatin, the biological macromolecule, due to its biodegradable, biocompatible, non-antigenicity, and low cost with easy availability, it is considered as a versatile drug/vaccine delivery carrier in pharmaceutical field. The main reason behind this drug

Table 1 List of enzymes helping in the formation of gelatin-based hydrogels

Sl. No.	Material	Enzyme	Application	Reference
1.	Gelatin	Microbial transglutaminase	Tissue engineering scaffolds	[116, 117]
2.	Gelatin-chitosan conjugates	Tyrosinase	Tissue glue Wound dressings	[118]

Table 2 Gelatin-based hydrogels in tissue engineering application

Sl. No.	Hydrogel fabrication main materials	Purpose	Reference
1.	Gelatin-methacrylate	Bone replacement materials	[120]
2.	Gelatin-methacrylamide û Hyaluronic acid methacrylate	Tissue engineering	[121]
3.	Gelatin/hyaluronan	Treatment of brain injury	[122]
4.	Collagen-chitosan-hydroxyapatite û Gelatin	Biodegradable scaffold	[123]
5.	Gelatin/alginate/fibrinogen	Hybrid cell/hydrogel construct	[124]
6.	Gelatin, sodium alginate	Tissue engineering	[125]
7.	Gelatin/chitosan and type I collagen	Tissue engineering scaffolds	[126]

attachment is the primary structure of gelatin that offers chemical modification and covalent drug attachment with gelatin. This kind of drug attachment can be done either within the matrix of the particles or on the particle surface only [127]. Moreover, gelatin-based hydrogels are considered to boost up the drug attachment/loading due to its high porosity that can easily be adjusted by controlling the affinity to water and the density of crosslinks in their matrix. The advantages offered by hydrogels for drug delivery applications include the possibility for sustained release [127]. The hydrogel carrying drug can be applied to various tissues of the human body, e.g., ocular, nasal, oral cavity, stomach, small intestine, colon, rectum, transdermal, etc. [128].

An injectable physically crosslinked gel-like structure was fabricated from several blends of natural polymers. One of the blends is gelatin-agar combination. The other similar types of blends are starch-carboxymethyl cellulose, hyaluronic acid-methylcellulose, etc. Due to the absence of chemical crosslinker and as the formulations are resembled to extracellular matrix polymers, gelatin-agar hydrogel exhibits excellent biocompatibility. However, hydrogen-bonded networks can dilute and disperse over a few hours in-vivo due to influx of water. This restricts the use of hydrogel to relatively short-acting drug release systems unless some other form of crosslinking is also used [129].

In a different study, a quick gel-forming hydrogel was fabricated from oxidized konjac glucomannan and gelatin. Oxidized konjac glucomannan was added as a crosslinker. The composite hydrogels were sensitive to the pH value of the medium. The results of in-vitro drug (ketoprofen) release experiments showed that all the hydrogels showed sustained release properties and the dependence of release rate on the equilibrium swelling ratio of hydrogels and pH value of medium [130].

Instead of using physical crosslinker, chemical crosslinker can also be used to fabricate gelatin-based hydrogels. An example of this type is the fabrication of magnetic hydrogels by chemically crosslinking of gelatin hydrogels and Fe_3O_4 nanoparticles through genipin (GP) as crosslinking agent. Smart magnetic hydrogels based on gelatin-ferrite composites were investigated and can be applied for the development of a new magnetically induced drug delivery system. Furthermore, the drug release profile of the resulting hydrogels is controllable by switching “on” or “off” mode of a given magnetic field [131].

Table 3 Gelatin-based hydrogels as a drug carrying media

Sl. No.	Hydrogel fabrication main materials	Purpose	Reference
1.	Gelatin/monomethoxy poly(ethylene glycol)-poly(D,L-lactide)	Antibacterial drug delivery	[133]
2.	Gelatin-poly(ethylene Oxide) semi-interpenetrating polymer network	Oral drug delivery	[134]

A plant-originated polysaccharide (κ-carrageenan) has been used to prepare one component and blend hydrogels, with two natural polymers, agar and gelatin, for the release of a drug theophylline. In the mixed system, the additional crosslinking between the chains of the different molecules slowed down the diffusion rate of the guest drug molecule through matrix [132]. Some other drug delivering hydrogel matrices are listed below in Table 3.

4.3.3 Wound Dressing Agent

Modern dressings are designed to facilitate wound healing rather than just to cover it. A novel membrane of the hydrogel was prepared by Kunal et al. from gelatin and polyvinyl alcohol. The prepared hydrogel was observed to show hemocompatibility and moisture retentive property that enabled its possible use in moist wound care. To keep the wounded area moist is really very helpful as it speeds the healing process by absorbing exudates while maintaining the products of tissue repair, including lysosomes and growth factor in contact with the wounded area [135]. Blood is the pathway to work for most of the drugs. Adrenochrome is a blood coagulating agent which has been added to a hydrogel comprising of gelatin and polyvinyl alcohol along with some other materials. This hydrogel is used in wound healing function in different forms like patch, gel, ointment, etc. The gelatin with adrenochrome in hydrogel has a synergistic effect in wound healing [136].

A wound dressing hydrogel material was described by Balakrishnan et al. that is capable of treating the wound within 10 days without any side effect. This hydrogel was fabricated in situ from gelatin and oxidized alginate containing dibutyryl cyclic adenosine monophosphate [136].

Another gelatin-based hydrogel pad was described by Rattanaruengsrikul et al. This pad was fabricated from gelatin and $AgNO_3$ which turned into nano silver particle upon mechanical stirring in presence of acetic acid. However, for crosslinking purpose glutaraldehyde was added into the mixture of $AgNO_3$ and gelatin. This pad was found effective against both the gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria and therefore suggested to be used as an antibacterial wound dressing pad [137].

A specialized hydrogel dressing for patients with burn was fabricated from gelatin, chitosan, and honey (Fig. 7). Chitosan has been widely used for wound dressing in the form of hydrogel. In fact, honey has been commercially used for

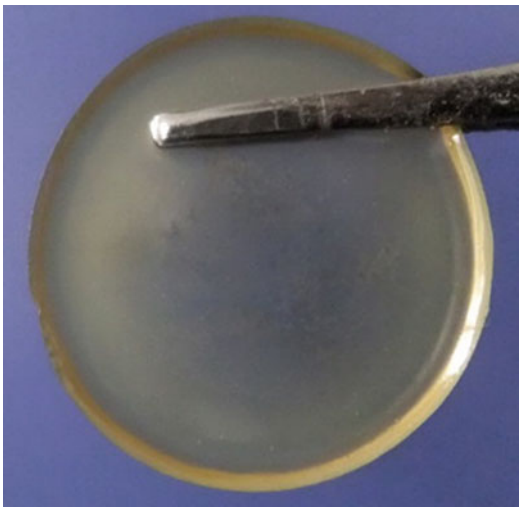

Fig. 7 Photographic appearance of the hydrogel sheet composed of gelatin, honey, and chitosan [138]. (Copyright © 2017, Elsevier)

wound dressings due to its suitability for all stages of wound healing. Gelatin is a biocompatible protein with excellent water absorbing power. Especially for these reasons, the three materials were chosen for preparing hydrogel dressing sheets, which showed better performance in wound healing better than typical antibiotics ointment (MEBO® ointment, Shantou MEBO Pharmaceuticals Co., Ltd., Guangdong, China) or without any treatment. The sheet took 12 days to treat the wounded area (Fig. 8). In addition, it had powerful antibacterial efficacy to *S. aureus* and *E. coli* and significantly promoted burns healing. Moreover, upon swelling in water from 40% to 130%, the prepared hydrogel was discovered with microporous cross-section from smoother one. Further increase in water content (200%) tends to give a collapsed surface with increase in pore size compared to hydrogel containing 130% water. However, the opposite phenomenon was espied in case of pore numbers. More pores were observed in case of hydrogel containing 130% water compared to hydrogel with 200% water (Fig. 9) [138].

Besides chitosan/gelatin hydrogel, it is possible to fabricate pectin/gelatin hydrogel, which is available in the form of membrane and can be used as a wound dressing material. This material was developed by Mishra et al. The hydrogel membrane showed enhanced thermal stability, tensile strengths, and elongation at break up to a certain percentage of gelatin in the hydrogel. An intermolecular interaction was observed between the two naturally occurring polymers that also showed better moisture retentive property which enabled this membrane to be applied as moist wound care element [139].

A very recent paper deals with a new composite material. As an antitumor agent, gelatin-based hydrogel is a promising candidate that is capable to restore water as well as desired drug in proper amount with appropriate way to release in the body stream. Such kind of hydrogel was prepared by Konishi et al. by chemical

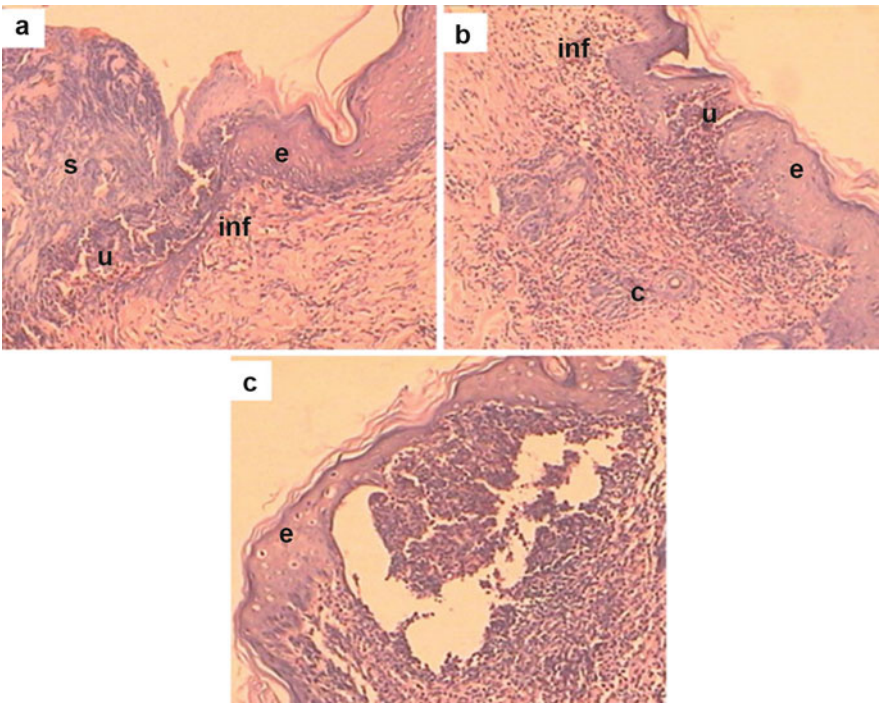

Fig. 8 Photomicrographs of burn wound tissues at day 12 post-burned: (**a**) untreated wound, (**b**) MEBO treated wound, (**c**) gelatin-, honey-, and chitosan-based hydrogel-treated wound, *s* scab, *u* ulcer, *inf* inflammatory cells, *c* cyst, *e* epidermis (100×) [138]. (Copyright © 2017, Elsevier)

crosslinking of gelatin with different concentrations of glutaraldehyde containing "cisplatin" as the drug with control release.

4.3.4 Protein Releasing Media

A crosslinked hydrophilic polymer is considered as a media for absorbing large amount of compatible penetrant (e.g., water). This polymer gets swelled, thus forming a gel and called as hydrogel. During the swelling process if there exists any solute, the material releases that solute to the environment. In many cases the solute is a drug material. This property of hydrogel material has been practiced for delivering drug for more than a decade. However, it is now trying to use the hydrogel material to deliver protein due to its ability to release drugs at a zero order rate and to target proteins to specific sites, such as the upper small intestine, which extends their biological activity [140]. Gelatin is a well-known polymer to fabricate hydrogel in many ways with or without the presence of a supporting material other than crosslinker. Some recent developments in the protein releasing field by gelatin-based hydrogel have inspired the researchers to explore more and more ways of its fabrication, modification, and application in the protein releasing field.

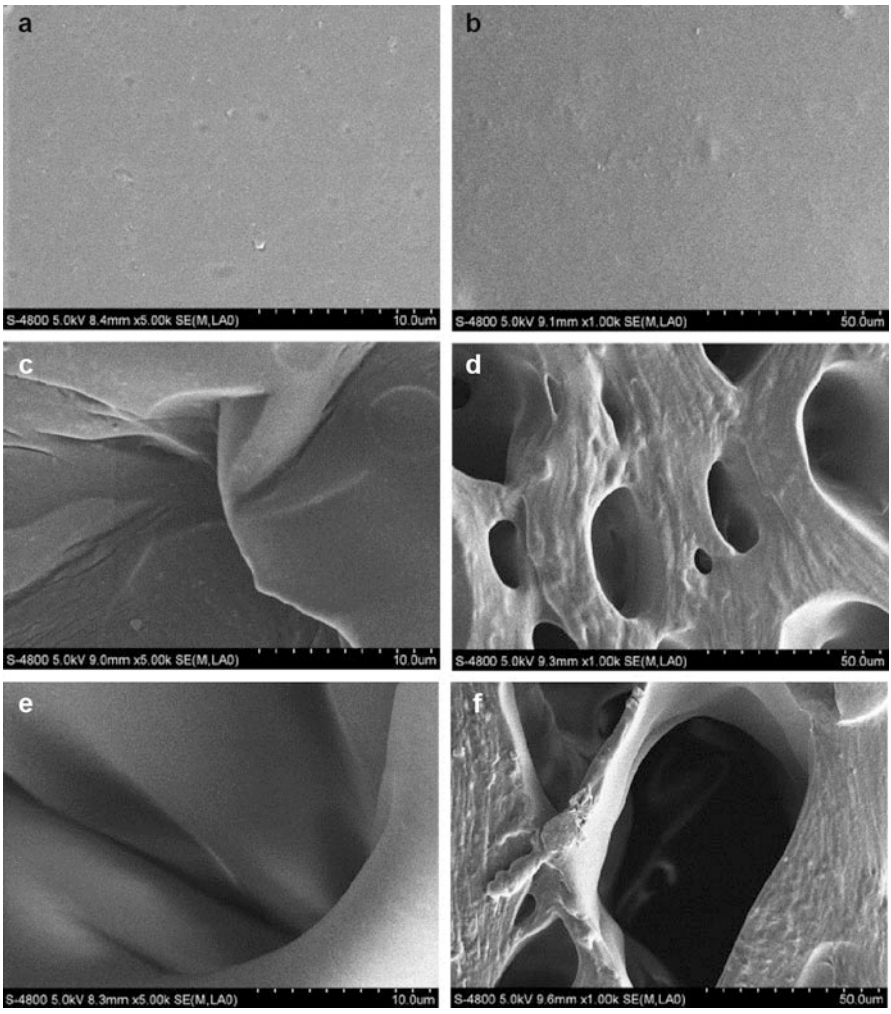

Fig. 9 SEM images of the lyophilized hydrogel sheet HS with different water content. Surface morphology of HS with 40% water (**a**), 130% water (**c**), 200% water (**e**), and (**b**), (**d**), and (**f**) the corresponding cross-section morphology [138]. (Copyright © 2017, Elsevier)

Various antibacterial proteins are discovered by the researchers to apply in various antibacterial purposes through incorporating with hydrogel originated from gelatin. A study conducted by Kujipers et al. describes that during the cardiac valve replacement a serious complication was observed due to the infection caused by the adherence of bacteria to the prosthetic valve or to tissue at the site of implantation. The release of an antibacterial protein through crosslinked gelatin hydrogel can reduce or diminish the possibility of infection. In a release system, the antibacterial proteins incorporated in the Dacron sewing ring of the prosthetic heart valve would

cut down the incidence of prosthetic valve implantation complication due to the infection. This hydrogel was tested for uptake and in-vitro release of lysozyme, a small antibacterial cationic protein [141].

Using the same incorporation base (i.e., Dacron) but different fabrication materials other than gelatin, it is also possible to prepare an antibacterial protein releasing hydrogel. A chemically crosslinked gelatin-chondroitin sulfate (ChS) hydrogel was prepared, impregnated in Dacron, and crosslinked with a water-soluble carbodiimide (EDC) and *N*-hydroxysuccinimide. This hydrogel is capable of releasing lysozyme and recombinant thrombocidin. As a part of the practical application of this hydrogel, it is implanted in the subcutaneous pockets in rats which showed a mild tissue reaction and almost complete degradation within 18 weeks of implantation [142].

Besides antibacterial protein bone morphogenetic proteins can also be loaded in the gelatin-based hydrogel system. One of these studies was conducted by Chen et al. They investigated the formation of bone morphogenetic proteins by radical crosslinking and low dose ϒ-irradiation from glycidyl methacrylated dextran and gelatin. The researchers also found that BMP release from microsphere temperature-sensitive hydrogel compounds could be accordingly controlled and the release period could be varied from 18 to more than 28 days with biodegradation quality [143].

In a different study of protein release, amino acid modified gelatin hydrogels slow down the release of lysozyme and trypsin inhibitor protein. To an extent the release rate is directly proportional to the strength of the charge interactions between the amino acid chain and the entrapped proteins [92]. Besides using pure, non-modified gelatin recombinant, gelatin can also be used in fabrication of hydrogel material. For hydrogel fabrication, recombinant gelatin is preferred because of its well-defined molecular weights, amino acid sequences, and isoelectric points. Sutter et al. used this kind of recombinant gelatin modified with methacrylate residues for chemical crosslinking and gel formation which is used for sustained release of the protein. For experimental purpose, release of the incorporated model proteins lysozyme and trypsin inhibitor occurred by diffusion. Recombinant gelatin derived hydrogels were enzymatically degradable by human matrix metalloproteinase which indicates in-vivo biodegradability [92].

So far we discussed the release of a single protein by gelatin-based hydrogel. However, a significant one is the release of multiple proteins through combination of their mechanism and rates of the hydrogel materials. An example of this class is the fabrication of hydrogel from glycidyl methacrylate and polyethylene glycol with gelatin [144]. The growth factor(s) (mainly protein) can be attached to the hydrogel matrix in many ways. Then the growth factors are delivered to the cell that results in tissue regeneration phenomena. A general representation of this process is shown in Fig. 10 [144].

It is also possible to fabricate two protein based hydrogel. A study conducted by Gil et al. suggested that a thermo responsive gel can be prepared by blending gelatin and silk fibroin which was stabilized at 37 °C by the presence of crystals of silk fibroin. The swelling profile of this hydrogel material below and above the temperature allows it to release protein from the matrices. The gel showed a higher swelling

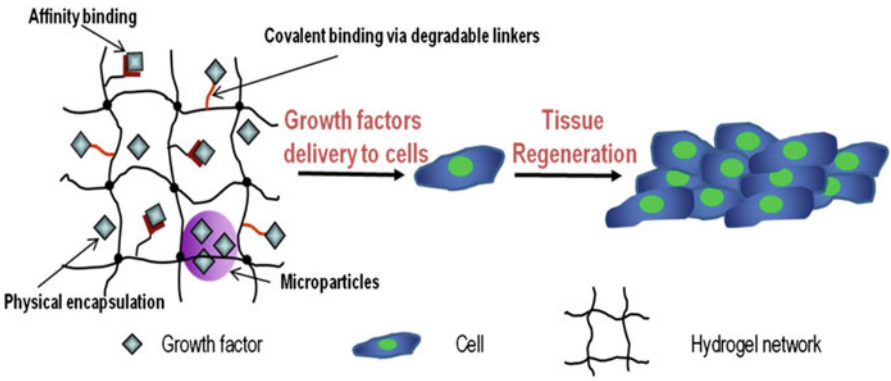

Fig. 10 Hydrogel for protein delivery and tissue regeneration [144]. (Copyright © 2017, Elsevier)

at physiological temperatures as compared to 20 °C. However, a higher mass loss was also observed due to dissolution and release of gelatin [145].

4.3.5 Extracellular Matrix and Growth Factor Release

A new type of covalent synthetic extracellular matrix (ECM) was developed through a new disulfide crosslinking method. For preparing ECM, blended hyaluronan (HA)-gelatin hydrogels are formed initially. Both the HA and gelatin were chemically modified using 3,3′-dithiobis(propionic hydrazide) (DTP). After reduction with dithiothreitol (DTT), the thiol derivatives of HA and gelatin were obtained. Both the modified HA and gelatin were blended in different concentration and prepared in 1% NaCl solution. The mixture was crosslinked by disulfide bond in air, and a second crosslinking was performed in presence of hydrogen peroxide. The degradation of the hydrogels by the enzymes was governed by the ratio of modified HA and gelatin and the type of enzyme responsible for degradation [146].

For control releasing of a biologically active growth factors, Yamamoto et al. developed biodegradable hydrogels carrier through glutaraldehyde crosslinking of gelatin with isoelectric points (IEP) of 5.0 and 9.0, i.e., "acidic" and "basic" gelatins, respectively. Basic fibroblast growth factor (bFGF) and transforming growth factor-β 1 (TGF-β1) are basic in nature and that were found well sorbed with time to the acidic gelatin hydrogel, while less sorption was observed for the basic gelatin hydrogel. Nevertheless, bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) were sorbed to the acidic gelatin hydrogel to a smaller extent than the two other growth factors though their IEPs are higher than 7.0. Both in-vivo and in-vitro analyses indicate that the growth factor immobilized to the acidic gelatin hydrogel through ionic interaction was released in-vivo as a result of hydrogel degradation [147].

In another study the release of fibroblast growth factor was found to be more efficient when impregnated in a hydrogel compared to free growth factor. This is possible due to the in-vivo degradation of the hydrogel. The hydrogel material was fabricated by crosslinking of acidic gelatin with the isoelectric point of 4.9. It was

concluded that, the fibroblast growth factor impregnated in a hydrogel leads to more efficient induction of neovascularization and tissue granulation than free fibroblast growth factor without impregnation in the hydrogel [148]. In a different investigation, the release of growth factor by thiolated gelatin/thiolated hyaluronic acid/polyethylene glycol diacrylate-based networks was described by Peattie et al. [149].

4.3.6 Bioadhesive

The major goal of bioadhesive controlled drug delivery is to enhance the drug absorption process in a site specific manner through localizing a delivery device within the body. Hydrogel materials from various sources can be used as a bioadhesive agent. Other than direct biomedical uses, gelatin-based hydrogels can be used as biological glue or bioadhesive materials.

A hydrogel fabricated from gelatin-poly (γ-glutamic acid) could be applied as soft tissue mixed adhesives. For a faster mixed adhesive fabrication, a crosslinker is necessary. The molecular weight of gelatin and poly (γ-glutamic acid) increases the bonding strength but lessens the time required for gelation. The investigation of the mixed adhesives result no cytotoxicity and no inflammatory response [150].

A supramolecular hydrogel macromer was fabricated from aromatic residue of gelatin and photo-crosslinkable acrylated β-cyclodextrin (Ac-β-CD) monomers. The subsequent crosslinking of the macromers produces highly resilient supramolecular gelatin hydrogels that are solely crosslinked by the weak host-guest interactions between the gelatinous aromatic residues and β-cyclodextrin (β-CD). This host-guest supramolecular macromer (HGM) was used to fabricate the final mechanically robust gelatin-based hydrogel (Fig. 11). The excess β-CDs in the hydrogels enable the tissue adhesion and enhance the loading and sustained delivery of hydrophobic drugs. Besides tissue regeneration application, the hydrogels are also considered as a bioadhesive and are able to retain and release hydrophobic drugs, thereby enabling the delivery and long-term release of drugs at the targeted locations. Moreover, it was also observed that the prepared hydrogel is favorable to glue two fractured femoral swine bones together. The adhesion property of the HGM hydrogel was compared to the adhesive power of methacrylated gelatin macromer-based hydrogel, and the HGM hydrogel showed better adhesion power in case of swine femoral bone even after the addition of 100 g weight in excess to the lower part of the bone [151].

The use of different crosslinker materials has a noticeable effect on the properties of the hydrogel material, e.g., adhesion power, gel formation time, cytotoxicity, etc. Gelatin was crosslinked with different materials, and their properties as a bioadhesive were evaluated in a study conducted by Wen Sung et al. to select the right adhesive for a particular application. Gelatin and resorcinol were crosslinked with formaldehyde (GRF glue), glutaraldehyde (GRG glue) and epoxy (GRE glue), carbodiimide (GAC glue), and genipin (GG glue). It was found that GRE, GRF, and GRG are more cytotoxic compared to GAC and GG. However, GRF and GRG showed maximum adhesion in minimum time and therefore suggested to be used

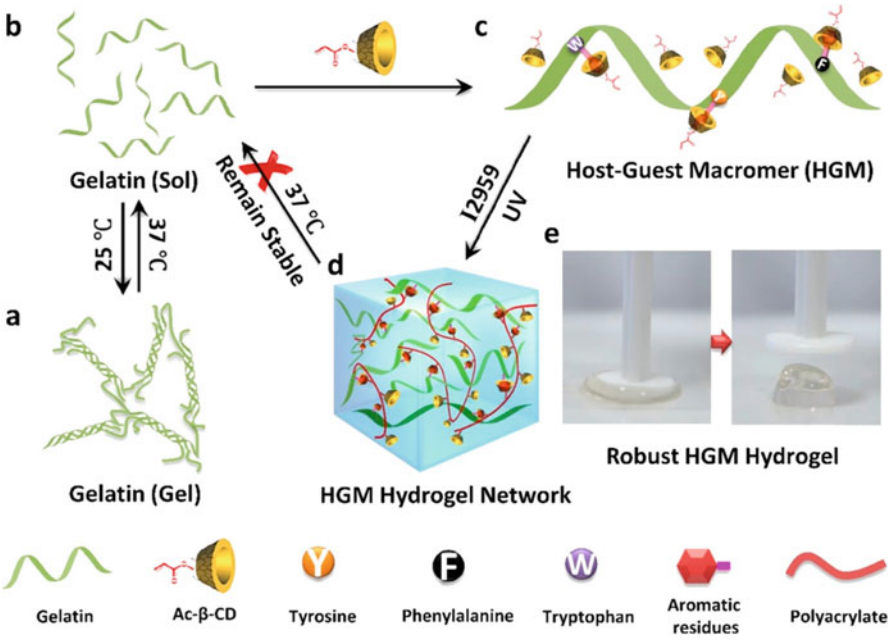

Fig. 11 (**a**) Pure gelatin solutions form hydrogel below 30 °C. (**b**) The gelatin hydrogels dissolve at 37 °C. (**c**) Formation of host-guest macromere (HGM) through complexation between the free diffusing monofunctional Ac-β-CDs (with one single acrylate group per β-CD) and the aromatic residues of gelatin. (**d**) The acrylate groups in the gelatin HGM leads to the formation of the HGM supramolecular hydrogels by UV-initiated radical polymerization, which are stable at 37 °C. (**e**) The HGM hydrogels can withstand cyclic excessive compression that represent its excellent compressibility [151]. (Copyright © 2017, Elsevier)

when a rapid and tight bonding is required. GAC and GG exhibited comparable adhesion power, while GRE showed no binding strength [43].

The practical application of some of the hydrogels stated above was described by Wen Sung et al. in another study. It was suggested that the cytotoxicity of the GRF glue can be reduced by changing the pathway of crosslinking operation. As a part of the change in the pathway, an alternative crosslinker water soluble carbodiimide or genipin had been introduced which formed GAC glue and GG glue. These GAC and GG were applied to close skin wound lesions in a rat model.

A very common crosslinking agent is polyacrylic acid. A gelatin-based hydrogel was fabricated by Ghavamzadeh et al. by using polyacrylic acid. This hydrogel was applied in-vitro as a bioadhesive material for soft tissues. To crosslink the mixture of gelatin and polyacrylic acid (PAA), water-soluble carbodiimide (WSC) was used. The cured hydrogel showed sufficient adhesion to mouse skin with a higher bonding strength compared to fibrin glue [152].

However, most of the gelatin-based hydrogels are focused on mainly biomedical and tissue engineering application; some gelatin-derived hydrogels are fabricated for adsorbing metals like lead and copper [103]. Besides these, sometimes carbon

nanotubes are found to be incorporated in the gelatin-based hydrogel forming a hybrid suitable for drug delivery, encapsulation with tuned mechanical strength and biocompatibility [109, 111].

5 Conclusions

Hydrogel based networks have been considered as important tools to meet the needs of different applications because of their physical and chemical characteristics and technical feasibility of utilization. Although, during the last two decades, natural hydrogels were gradually replaced by synthetic hydrogels due to long service life, high capacity of water absorption, and high gel strength of the latter, still natural polymers are the key components of most of the natural and synthetic hydrogels due to their numerous availabilities. Researchers, over the years, have developed a well variety of gelatin and modified gelatin-based hydrogels. Because of their well-controllable network, they become attractive materials for numerous fields of application like tissue engineering, drug delivery, wound dressing, protein releasing media, growth factor matrix, etc. A wide range of smart gels, combining gelatin with other natural/synthetic polymers, with different mechanical properties and potential applications in different fields can be produced through the correct control of the different experimental parameters and the addition of well-compatible crosslinker. We hope that the current rate of advancements in this field will yield the next generation efficient materials with availability for different biomedical applications.

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