Chapter 3
Emerging Trends in the Synthesis, Properties and Applications of Nanogels Derived from Pullulan, Collagen and Gelatin

Sarojini Balladka Kunhanna, Niveditha Nagappa Bailore, and Pushparekha

Abstract Biopolymers, namely polysaccharide pullulan and the two polyamides collagen and gelatin, offer themselves as versatile materials in modern drug delivery systems by forming nanogel networks. Pullulan is highly water soluble which on conjugation with cholesterol forms CHP with increased hydrophobicity and offers itself as a matrix for nanogel formation. Self-assembly of these hybrid materials is a well-established strategy to synthesize the nanogels. Another method to make these materials is physical and chemical grafting copolymerization technique. These nanogels are usually sensitive towards environmental conditions. The thermoresponsive CHP-PNIPAM nanogels exhibit quick deswelling and reswelling behaviour at stipulated temperatures. The uronic acid-grafted pullulan derivatives exhibit pH-responsive behaviour in drug delivery. Collagen and gelatin nanohydrogels prepared through physical and chemical cross-linking as well as irradiation techniques are mainly used for drug delivery, especially in wound healing. This chapter describes the synthesis of tailor-made functional nanogels for customized use as drug carriers as well as environmental sensors.

Keywords Nanogel · Pullulan · Collagen · Gelatin · Synthesis · Drug delivery · Stimuli response

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1 Introduction

Nanotechnology is the branch of technology which deals with the study of manipulating matter on an atomic scale. Hydrogels are a unique class of macromolecular/nanomolecular arrangements. It is a cross-linked network of hydrophilic polymers. Polymeric hydrogels can be classified into three groups based on their particle sizes; they are macrogels, microgels and nanogels. Nanogels are hydrogel networks at a nanoscale range between 1 and 100 nm [1].

Nanogels are defined as the nanosized particles synthesized through chemically or physically cross-linked hydrophilic polymer networks, which are capable of improving the swelling in the aqueous media [2]. One of the important advantages of nanogels is the faster swelling–deswelling properties. Due to its smaller size, it exhibits excellent permeation abilities and is also capable of improving the solubilization of many hydrophobic drugs and diagnostic substances [3]. Nanogels form different complexes with different drugs, proteins, peptides and DNA materials. Nanogels have great variety of applications in the field of drug delivery.

2 Classification of Nanogels

Nanogels are mainly classified as two major classes:

- Firstly on the basis of environment-responsive nature
- Secondly on the basis of type of cross-linking technology applied.

On the basis of responsive nature, nanogels can be categorized as stimuli-responsive or stimuli-nonresponsive gels [3]. Stimuli-responsive gels swell up due to environmental changes through electric and magnetic fields, pH and temperature. Nonresponsive gels just swell up when placed in an aqueous media.

Based on the second classification type, nanogels are classified as two types: chemical cross-linked gels and physical cross-linked gels [4].

Natural polymers such as polysaccharides (chitosan, hyaluronic acid (HA), heparin, chondroitin sulphate, agarose and alginate) and proteins (collagen, albumin and fibrin) are widely used for the preparation nanohydrogels. This is because of the attractive properties of natural polymers like biocompatibility, biodegradability and its lower cost [5, 6].

Polysaccharides from the natural source are most used materials in the form of food, cloth and other engineering materials since human civilization. Of late, they offer themselves as excellent materials for the preparation of hydrogel formulations [7]. The polysaccharide-based hydrogels either prepared by physical or chemical means offer versatility as materials of choice to use in medicinal fields as target drug transporters and in tissue engineering. Most of the personal care products and cosmetics preparations contain polysaccharide-based hydrogels as water replenishing agents in most of the cases [8]. They are used as water purifying

agents with superabsorbent nature [9] and used for controlled release of fertilizers and herbicides as well, along with paint removal in paper and textile industries [10–12]. The structure-specific properties of polysaccharides could be related to their primary structures and masses. The reactive pendent groups present on the monomeric units make these macromolecules available to various chemical and physical modifications. Hence, customized properties could be achieved [13].

A large number of polysaccharides form hydrogel under adequate conditions. Alginate, pectins and gellan are the typical polymers that form gels by ion complexation on interaction between carboxylic groups and divalent ions; locust bean gum and xanthan on mutual interfacing form gels [14, 15].

The hydrogels of consistency could be synthesized by the treatment of borax with some polysaccharides on reacting with hydroxyl groups [16]. Cross-linking reagents possessing multifunctional reaction sites are used to chemically cross-link the functional groups present in polysaccharides to form hydrogels [17, 18]. Sometimes, the hydrogels prepared from natural polysaccharides do not meet the properties needed for specific type of applications and also there are instances that they fail to form hydrogels itself. So, semi-interpenetrating polymeric networks (semi-IPNs) and interpenetrating polymeric networks (IPNs) are synthesized to have superior properties [19]. The IPNs exhibit different physical and chemical properties than the polymers from which they are derived. According to the customized demand, these materials could be synthesized [20].

There are different methods developed to fabricate hydrogels; the number techniques are being developed to tailor the size of hydrogels. The bulk hydrogels are being used in many fields for specific applications, while the nanohydrogels are promising size-modified hydrogels which are looked upon for use in medical and engineering fields. Nanohydrogels can be generally synthesized by the polymerization of a monomer in homo- or nanoheterogeneous environment, chemical cross-linking lithography, template-assisted nanofabrication and self-assembly of polymers.

The most commonly used polysaccharides for hydrogel formation are hyaluronic acid, chitosan, gellan, scleroglucan, mannans which are prepared by varied techniques. But in this chapter, the focus will be on pullulan [PULN]–collagen-based nanohydrogels, their properties and applications.

3 Pullulan-Based Nanohydrogels

The polysaccharide-based nanohydrogels are usually prepared by the macromolecular self-assembly which result in nanostructured networks. Hence, hydrophobic and hydrophilic polymer chains are cross-linked carefully to use in drug delivery systems which is depicted in Fig. 1.

A nanohydrogel is ‘an aqueous dispersion of hydrogel particles formed by physically or chemically cross-linked polymer networks of nanoscale size’ as defined by

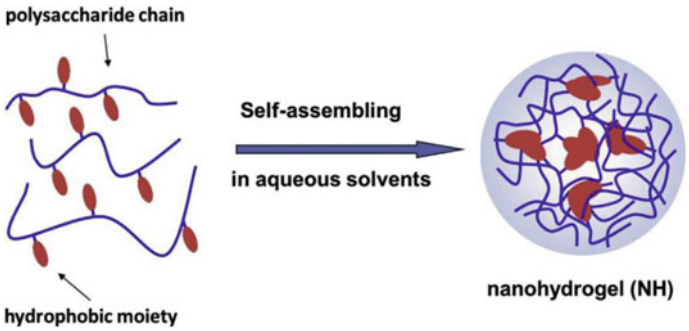

Fig. 1 Self-assembly technique for the preparation of nanogels, Ref. [13]

Kabanov and Vinogradov [21]. Sometimes during self-assembly, these nanostructured materials arrange themselves as self-assembled micelles and could be called as nanoparticles. The hydrophobized PULN was used for gelation; usually, it was identified as nanoparticles.

4 Synthesis of Nanohydrogels of Pullulan

4.1 Cholesterol-Modified Pullulan Nanohydrogels (CHP)

An exciting article by Whitesides et al. published in Science opened a new avenue to synthesize nanostructured materials by self-assembly (Fig. 2). The cholesterol-modified pullulan (CHP) was synthesized to coat liposomes [22]. The formation of polymer aggregates depended on degree of hydrophobicity. Hence, cholesterol-bearing pullulan could bind effectively on lipophilic guest molecules resulting in higher stability of colloids. In brief, first aminoethyl-carboxymethyl derivative of the polysaccharide was condensed with cholesteryl chloroformate to obtain CHP but later in an improved procedure cholesteryl *N*-(6-isocyanatohexyl) carbamate was synthesized and condensed with PULN which yielded stable nanogels [23]. The self-aggregated nanoparticles of CHP were obtained by sonication in water, but they were also prepared in water on dilution with DMSO [24].

4.2 Synthesis from Pullulan Acetates

The use of pullulan in drug delivery accounts for its film-forming capacity and high water solubility. So, PULN lipophilicity was increased by converting it into

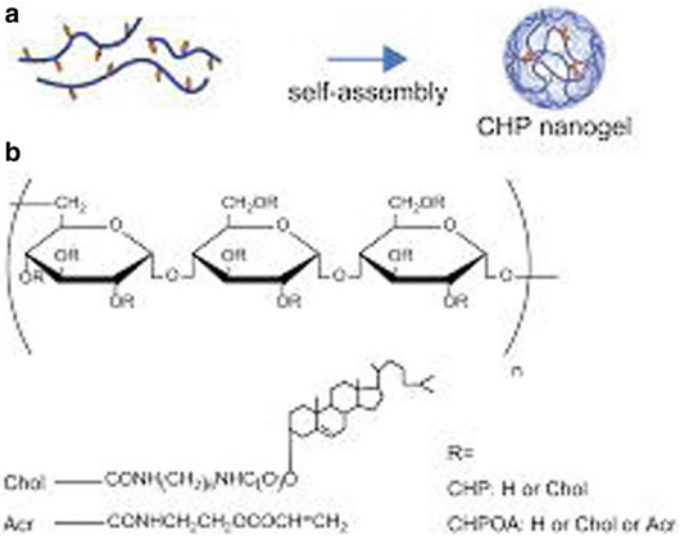

Fig. 2 Preparation of CHP, Ref. [25]

acetate which spontaneously aggregated into nanospherical particles [26]. A modified method accepted was to couple hydrophobized PULN with carboxymethylated poly(ethylene glycol). The incorporation of PEG increased craftiness of the nanoparticles for loading drugs [27].

4.3 Synthesis via Copolymerization to Hybrid Pullulan Nanogels

The hydrophobized PUL was mixed with modified poly(*N*-isopropylacrylamide) (HM-PNIPAM) to yield monodispersed nanogels via association of their hydrophobic groups which above 32 °C (i.e., the lower critical solution temperature of PNIPAM-C18Py) increased their diameter from 47 to 160 nm [28, 29]. The graft copolymerization of PNIPAM onto methacryloyl-substituted CHP nanogels in the presence of 2,2°-azobis[2-(2-imidazolin-2-yl)propane] as initiator resulted in different radii nanoparticles for customized use.

4.4 Pullulan Poly(Lactide) Nanogels

In a typical one-pot procedure, pure pullulan was dissolved in DMSO in N2 atmosphere, to this solution *L*-lactide was added slowly up to 10% (w/v) and stirring

continued at 70–75 °C for another 2 h. To this mixture, triethyl amine (TEA, 1.67 w/v) was added slowly to obtain nanogel [30].

5 Properties and Applications of Pullulan Nanogels

5.1 Physical Property of CHP Nanogels

The physical properties of CHPs self-assembled structures was determined by Dynamic Light Scattering (DLS) measurements. It showed spherical shape of the particles with average diameter of 25nm. The spherical shape of the particles indicated with negative stained electron microscopic observations. On keeping this hydrogel under different environmental conditions at room temperature, the properties of nanogel was unaltered indicating its stability. The tailor-made property for these nanogels could be obtained by appropriate changes in hydrophobicity, self-assembly and association of nanoparticles.

The complexation of protease enzyme chymotrypsin with CHP hydrogel resulted in stable nanoparticles. These conjugates exhibited increased thermal stability for structure and activity on bovine serine albumin (BSA). The chymotrypsin was found to be located deep inside nanogel network which was identified by the treatment of pullulanase enzyme [25].

Insulin loading in this nanohydrogel was the most investigated mechanism, as a supramolecular assembly was formed by just mixing of the two components. The assembly was very stable, and activity of the insulin was maintained without enzymatic degradations and aggregations. The insulin loaded in gel was active even after i.v. injection [31, 32]. Similarly, enzyme lipase was also mixed instantaneously into CHP nanogel, which increased its thermal stability. The enzyme was encapsulated in the nanogel matrix by lipophilic interactions [33] (Fig. 3).

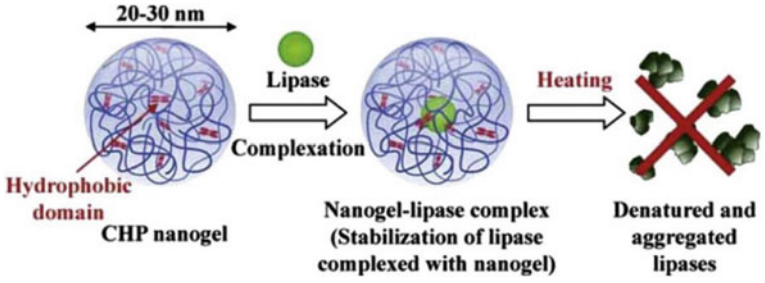

Fig. 3 Enzyme (lipase) thermal stabilization by loading within the CHP nanogel which holds the protein in the segregated nanomatrix by hydrophobic interaction (from Ref. [33] with permission)

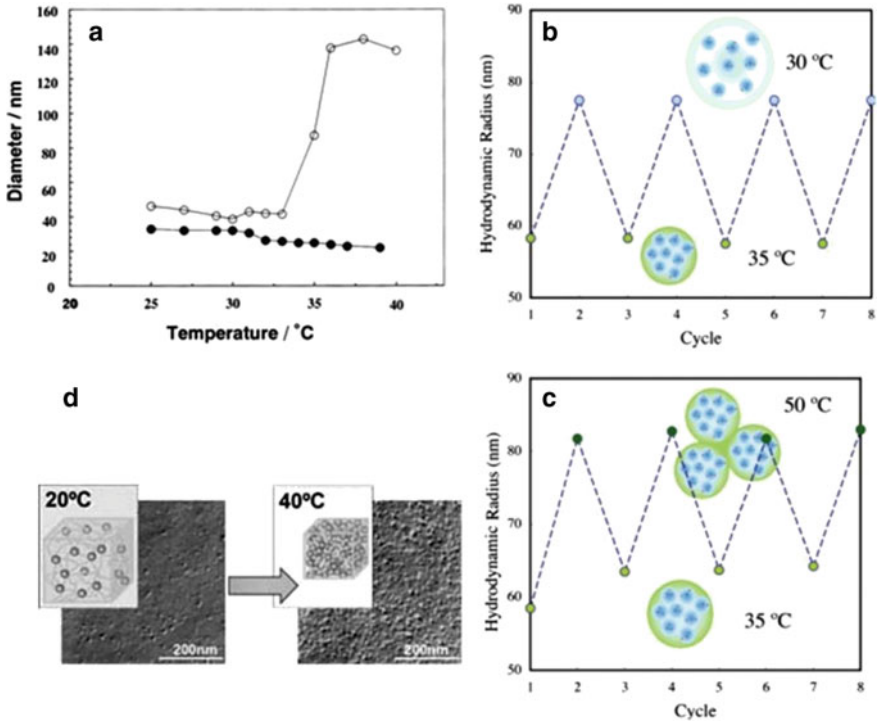

Fig. 4 Environmental-sensitive PUL nanogels. **a** Plot of the changes, as a function of temperature, of the average diameters of CHP nanogels in water (full circle) and of mixed CHP/PNIPAM-C18Py nanogels in water (open circle). **b** Changes in hydrodynamic radius of CHP nanogels with PNIPAM upon repeated changes in temperature from 35 to 30 °C and **c** from 35 to 50 °C with permission. **d** TEM image of the thermoresponsive hydrogel described in Ref. [13]

The CHP nanogels efficiently detained the inactivation of proteins and enzymes usually caused by heat, guanidinium chloride and urea. Upon addition of β-cyclodextrins, the CHP nanogel networks rapidly unfolded releasing enzymes by hydrophobic interactions. This quality makes CHP gels suitable for targeted drug delivery in hydrophobic environments, Fig. 4. The CHP application in therapeutic use was studied by using mouse embryos, which were fed with growth factor receptor FGFR2 with S252W through nanogel to treat Apert syndrome a congential disorder [34].

5.2 Temperature- and pH-Responsive Properties

The graft copolymerized nanogels prepared from hydrophobized pullulan and PNIPAM and NIPAM showed thermosensitive properties. The different ratios of two

components resulted in different architectures of nanogels. The high concentration of NIPAM showed two-step temperature variances: one between 35–30 °C and 35–50 °C. This thermal response was attributed to the particular grape-like morphology of nanogel [35].

The nanogels prepared by cross-linking PNIPAM and cholesterol/methacryloyl PULN exhibited quick deswelling and reswelling behaviour at 20–40 °C, which could find use in releasing lipophilic and protein molecules [36] (Fig. 4).

The nanogels prepared from acid-labile cholesterol-modified pullulan with vinyl ether cholesterol substituents grafted on 100 kD pullulan backbone. The nanogels obtained showed radius 26.5 ± 5.1 nm at pH 7.0 and increased by ~135% upon acidification of the solution to pH 4.0. It was found that keeping the gel for 24 h at pH 4 degraded up to 80%. But acid-stable CHP remained undegraded at pH 4.0 as it is the case with acL-CHP at pH 4. So, these nanogels can release protein cargo at required pH [37].

The uronic acid and cholesteryl succinate conjugate grafted to pullulan showed a pH-sensitive response. These gel networks responded at pH 6.5. The doxorubicin, an antitumour drug, was physically loaded to UCPA nanogels, and drug release behaviour at a different pH was studied. The nanogel with degree of substitution of urocanyl and cholesterol in the composition of 6.8 and 3.5%, respectively, was found to be effective in vitro pH-induced drug release. The MTT assay and flow cytometry assay indicated the effective delivery of the drug to the site by these nanogels [38] (Fig. 5).

The above review has given a bird's eye view on the synthesis and their environmentally sensitive properties of pullulan-based nanogels. Pullulan offers as base material to engineer-customized nanohydrogels which respond to temperature and pH with high sensitivity. This property makes pullulan-based nanohydrogels to use in targeted drug delivery and functional-based applications.

6 Preparation of Protein-Based Gels from Collagen and Gelatin

Collagen is the principle protein of animal connective tissue and composed of approximately 30% of the total protein content of animal body [39]. The word 'collagen' is derived from the Greek words 'kolla' and 'genos' meaning glue and formation, respectively [40]. There are at least 29 different types of collagen so far identified; however, Types I to IV constitute 90% of them [41].

Collagen molecule is composed of three helical strands intertwined into one super-helix. Normally, collagen contains all essential amino acids except tryptophan. Glycine, proline and hydroxyproline are the major amino acids present in collagen at 10–20 folds the concentration found in other proteins. The most common

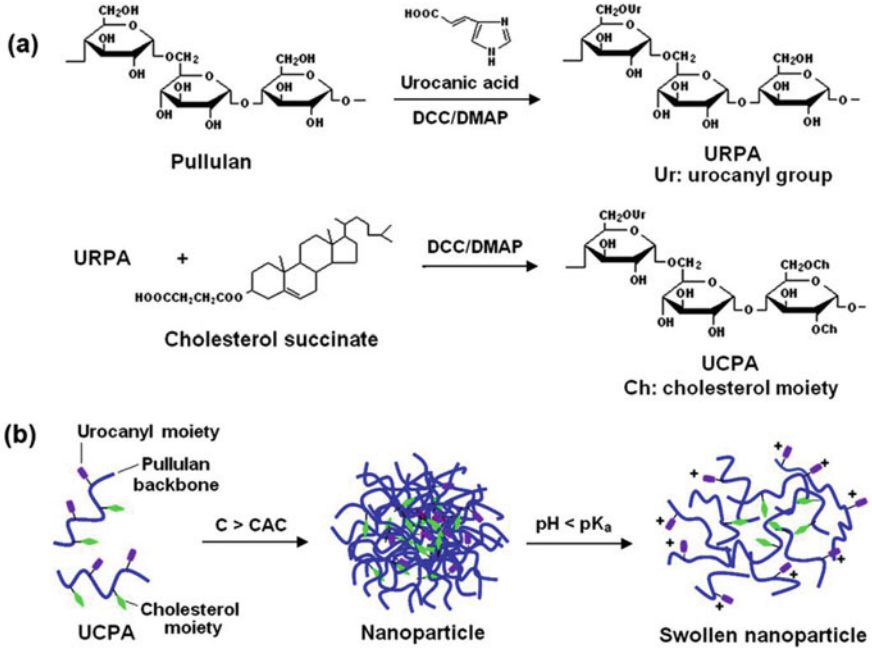

Fig. 5 pH-responsive urocanic acid-substituted nanogel, Ref. [37]

motifs in the amino acid sequence of collagen are glycine-proline-X and glycine-X-hydroxyproline, where X is any amino acid other than glycine, proline or hydroxyproline [42]. These three amino acids are very important for the formation of the specific triple helical structure and stabilization of collagen molecule [42, 43]. The hydroxyproline content is lower in fish collagen due to labile cross-links as compared to mammals [44, 45].

Collagen has widespread applications in numerous fields such as pharmaceutical, medical, biomedical, food industry and cosmetics (Fig. 6).

Soluble collagen is the starting materials used in collagen research. It can be extracted by acid-soluble collagen or pepsin-soluble collagen. In pepsin-soluble collagen, the enzyme pepsin is used to cleave the telopeptide region of collagen [47].

6.1 Properties of Collagen Nanogel

Physically formed collagen gels are thermo-reversible in nature which exhibits poor physical and chemical properties when compared to the covalently cross-linked collagen gels. The pH and temperature are the important factors involved in the gel formation. At lower temperature, collagen gels are formed which exhibits larger

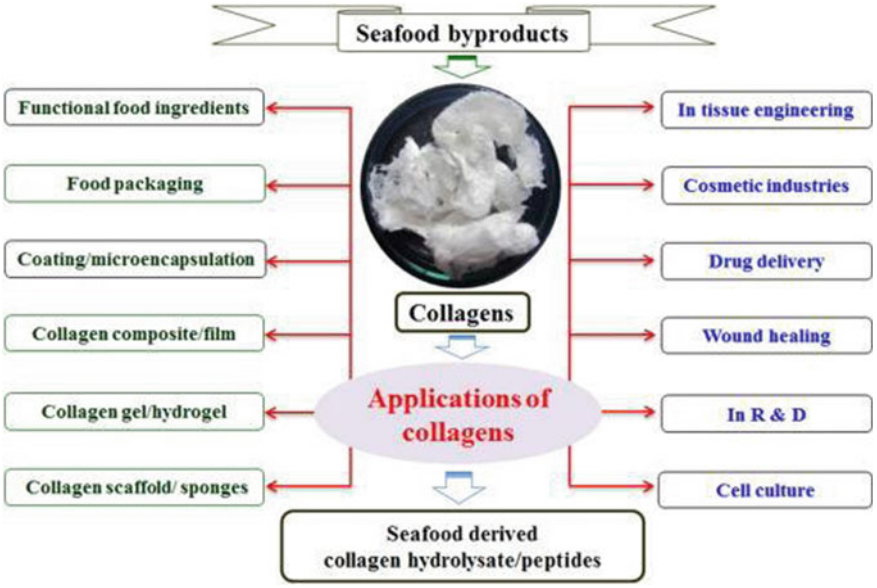

Fig. 6 Schematic representation of collagen applications [46]

pore size with enhanced cellular response [48]. On the contrary, higher temperature and higher pH accelerate fibrillogenesis but result in the formation of the gels with small diameter fibre and pore sizes [47].

For reducing the biodegradation rate of protein, numerous chemical cross-linking techniques were developed. The individual protein chains are linked with a cross-linker by covalent bond, thereby stabilizing the protein.

6.2 Preparation of Collagen Nanogels

6.2.1 Preparation of Collagen Gel with Gold Nanoparticle via EDC

The skin elasticity decreases during the ageing process, by 3-way connection through pyridinoline crosslinking of three side groups of collagen. Collagen gel was prepared by using 'multiple-way' linker as gold nanoparticles which is chemically cross-linking via 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). The surface of gold nanoparticles contains multiple carboxyl groups; these are capable of forming multiple cross-links with the collagen. For improving properties like porosity, it used new types of cross-linking agent called as tiopronin (*N*-(2-mercaptopropionyl) glycine) which modified gold nanoparticles and form multiple cross-links with collagen molecules via EDC. There was a formation of eight bonds between each nanoparticle and the collagen molecules resulting in the reduction of the pore size,

depending on the concentration of nanoclusters. This prepared new material has potential for the delivery of small molecule drugs as well as Au nanoparticles for photothermal therapies, imaging and cell targeting [49].

6.2.2 Preparation of Curcumin-Loaded Fish Scale Collagen (FSC)–Hydroxypropyl Methyl Cellulose (HPMC K100) Nanogel (CNG)

Nanoemulsion was prepared by aqueous titration method by using curcumin, oleic acid, Tween 80 and ethanol. Prepared curcumin nanoemulsion was loaded with hydroxypropyl methyl cellulose (HPMC K100) and fish scale collagen isolated from Catla fish. The prepared nanogel was evaluated for ex vivo permeation, and in vivo skin irritation and stability study. Ex vivo permeation study demonstrated that CNG prolonged release and exhibited higher per cent contraction value of wound compared to other formulations. In vivo study of CNG demonstrated higher wound contraction value compared to other formulations. Skin irritation study revealed that prepared nanogel was safe for dermatological (wound healing) applications [50].

7 Preparation of Gelatin-Based Nanogels

Gelatin is a biopolymer of animal origin (i.e., the skin and bones of bovine, porcine and fish sources) that is derived from the heat-induced hydrolytic degradation of collagen. Gelatin shows excellent biocompatibility, biodegradability, nontoxicity and high hydrophilicity in nature, and its multiple functionality (with –COOH and $–NH_2$ groups) is easily accessible for modification; due to this reason, it has been used for nanogel preparation for different applications. Gelatin nanogels can penetrate through the pores of the endothelial junctions found in tumour cells. Gelatin formed gels that are thermo-reversible in nature, and its gel-to-sol transition takes place at 30–35 °C [51–53].

Gelatin-based nanogels have been obtained via several methods as precipitation polymerization [54] and inverse miniemulsion polymerization [55].

Gelatin-based nanogels prepared without cross-linking were found to be unstable and tended to aggregate upon ageing [56, 57]. Therefore, a number of cross-linkers used are aldehydes, genipin, carbodiimide/*N*-hydroxysuccinimide, $CaCl_2$ [58] or enzymatic cross-linker as transglutaminase [59].

7.1 UV-Cross-Linked Gelatin Nanogel

Thermoresponsive behaviours of gelatin nanogel were investigated by using the preparation of UV-cross-linked gelatin nanogel without using any chemical cross-linking agents. The particle size of the nanogels decreased on heating, which is attributed to the helix-to-coil transition of gelatin [60].

7.2 Gelatin Nanogel by Desolvation Method

Gelatin nanogels were prepared via a one-step desolvation method to study the thermoresponsive property. Desolvating agents such as sodium sulphate or acetone were added to the gelatin solution. It leads to the coacervation of gelatin chains. When reaching the critical level of coacervation, the coacervate was redissolved by the addition of isopropanol. Glutaraldehyde was added to initiate the cross-links. Gelatin nanogel was prepared at various cross-linking conditions such as time, temperature and cross-linker concentration to optimize the helical structure of gelatin over the course of preparation. Thermoresponsive study showed a volume transition at 32 °C. This study shows how the temperature changes affect the particle size, the molecular configuration and factors influencing the thermoresponsive properties. It was studied by using dynamic light scattering (DLS), transmission electron microscopy (TEM) and polarimetry [61].

7.3 Stimuli-Responsive Gelatin Nanogel by Quantum Ray (60 Co Gamma) Irradiation

Akiyama et al. have studied to control the particle diameter and volume phase transition point of stimuli-responsive gelatin nanogel by quantum ray (^{60}Co gamma) irradiation at room temperature [62]. They succeeded to control the particle diameter in the range of 20–70 nm. Irradiated gelatin nanogel showed pH- and temperature-dependent response. They reversibly swelled and shrunk by stimuli of pH and temperature change. The changes in the volume phase transition point and swelling ratio are depending on the absorbed dose and the concentration of gelatin.

7.4 Polyethyleneimine-Based Core–Shell Nanogels

The core–shell nanogels were synthesized by two-stage reaction. The preparation of gelatin nanogel involves conjugation of polyethyleneimine (PEI) to the gelatin nanoparticles by desolvation and drying of the gelatin–PEI nanogels in ethanol/water

mixture. The resulting nanogels show a well-defined nanostructure that contains a gelatin core and a PEI shell. They have an average diameter of 200 ± 40 nm with high uniformity. The nanogel particles possess positive zeta potential values of up to +40 mV at neutral pH, indicating that they are highly positive and very stable in aqueous media. The gelatin–PEI nanogels were able to completely condense siRNA and effectively protected siRNA against enzymatic degradation. The study shows nanogels were four times less toxic than native PEI. Due to less toxicity, the nanogels were able to effectively deliver siRNA into HeLa cells. It was studied by using confocal laser scanning microscope. This concluded that the gelatin nanogel protects siRNA against enzymatic degradation with lower toxicity and enhances cellular uptake up to 84% [63].

7.5 *Preparation of Gelatin Nanogel by Gamma Ray Irradiation*

Gelatin nanogels were prepared from aqueous gelatin solution by gamma ray irradiation generated from 60 °C source at room temperature around 20 °C without using any chemicals at a dose rate of 10 kGy/h.

The hydrodynamic radius of prepared nanoparticles was studied by using static and dynamic light scattering, and it was found to be 10 nm. The size of the nanoparticle could be controlled by adjusting the preparation condition of irradiation dose and concentration of gelatin. The ordered conformation of the original gelatin could be replaced by random conformation in the nanogel revealed by CD measurements. It was found to be the highly and randomly packed structure given a very high stability against the temperature change to the nanogel [64].

7.6 *Curcumin-Loaded Alignate Aldehyde Gelatin Nanogels by Miniemulsion Technique*

Gum arabic (GA) was oxidized to gum arabic aldehyde, and then it was cross-linked with gelatin to obtain nanogels. Physiochemical properties were studied by dynamic light scattering, NMR spectroscopy and scanning electron microscopy. Hemocompatibility and cytocompatibility of the nanogels evaluated the anticancer activity towards MCF-7 cells studied by using confocal laser scanning microscopy [65] (Fig. 7).

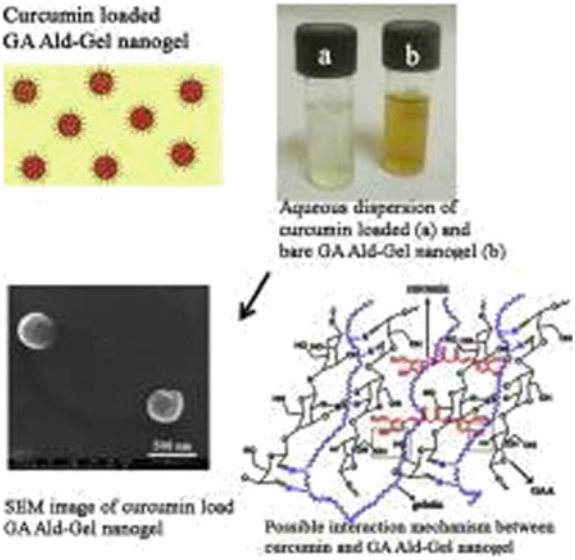

Fig. 7 Curcumin-loaded alignate aldehyde gelatin nanogels by miniemulsion technique [66]

7.7 Gelatin Methacryloyl Nanogels by Inverse Emulsion Method

Generally, polymeric nanoparticles have been developed mainly to deliver hydrophobic drugs to the dermis layer researchers such as Kim et al. [66] who have studied the suitability of gelatin methacryloyl (GelMA) nanogels for transdermal delivery of macromolecules and established the potency of the nanogels as a transdermal delivery carrier for hydrophilic macromolecules [51, 66]. This method involved the labelling of fluorescein isothiocyanate to bovine serum albumin (FITC-BSA) which further loaded to GelMA nanogels (FGNs) by water-in-oil emulsion droplets. The nanogel was formed by photo polymerization of methacryloyl substituents. Both GNs and FGNs existed as fine particles in aqueous condition (pH 7.4) for 7 days. High percentage of cell viability is studied by the MTT assay. The skin penetration study results showed that FGNs permeated across the epidermis and into the dermis of a porcine model when compared to the FITC-BSA dissolved in PBS. Possible penetration routes of FITC-BSA through the stratum corneum (SC) were illustrated by visualizing the SC structure with fluorescent signals of FITC-BSA. The penetration mechanism of FGNs across the SC layer was successfully demonstrated by intercellular, follicular and transcellular route. This results suggested that GNs have a potential as a transdermal delivery carrier for hydrophilic macromolecules.

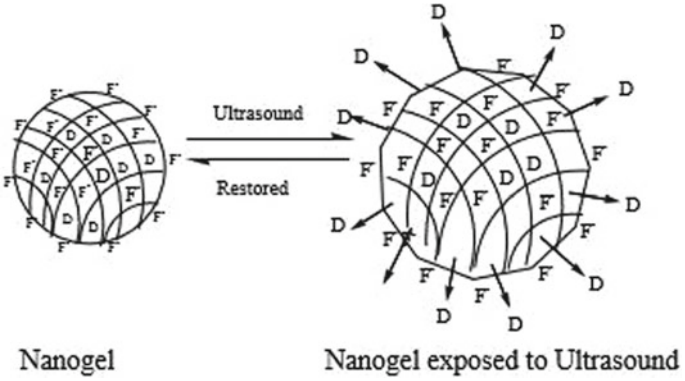

Fig. 8 Drug release of nanogel by ultrasound, Ref. [54]

7.8 Adriamycin Gelatin Nanogel Modified with Fluoride Anion (ADM-GNMF)

Gelatin nanogel was prepared by a modified co-precipitation method with fluoride anion and sodium sulphate for ultrasound-triggered drug release. Cross-linked gelatin solution was prepared by using ox-dextran. Adriamycin was introduced to cross-linked gelatin solution with Tween 20 and span 80. The size and shape of ADM-GNMF were determined by electron microscopy and photo-correlation spectroscopy. The ADM-GNMF was stable in solution with an average diameter found to be 46 $\pm$ 12 nm. This prepared nanogel releases the drug in response to ultrasound and is used as promising controlled drug release system for targeted therapy for cancer or other diseases [54] (Fig. 8).

7.9 Gelatin Nanogel by Inverse Miniemulsion Technique

Interpenetrating polymer network (IPN) nanogels composed of poly(acrylic acid) and gelatin were synthesized by one-pot inverse miniemulsion (IME) technique. Acrylic acid (AA) monomer stabilized around the gelatin macromolecules in each droplet was polymerized using ammonium persulfate (APS) and tetramethyl ethylene diamine (TEMED) and cross-linked with *N*,*N*-methylenebisacrylamide (BIS) to form semi-IPN (sIPN) nanogels, which were sequentially cross-linked by using glutaraldehyde (Glu) to form IPNs. Dynamic light scattering (DLS) and scanning electron microscopy (SEM) studies of purified nanogels showed small, spherical IPN nanogels. Interpenetration of the two networks was confirmed by FTIR, SEM, DLS, X-ray, XPS (photoelectron spectroscopy) and zeta potential studies. These nanogels have showed tailoring properties in order to use them as high-potential drug delivery vehicles for cancer targeting [67].

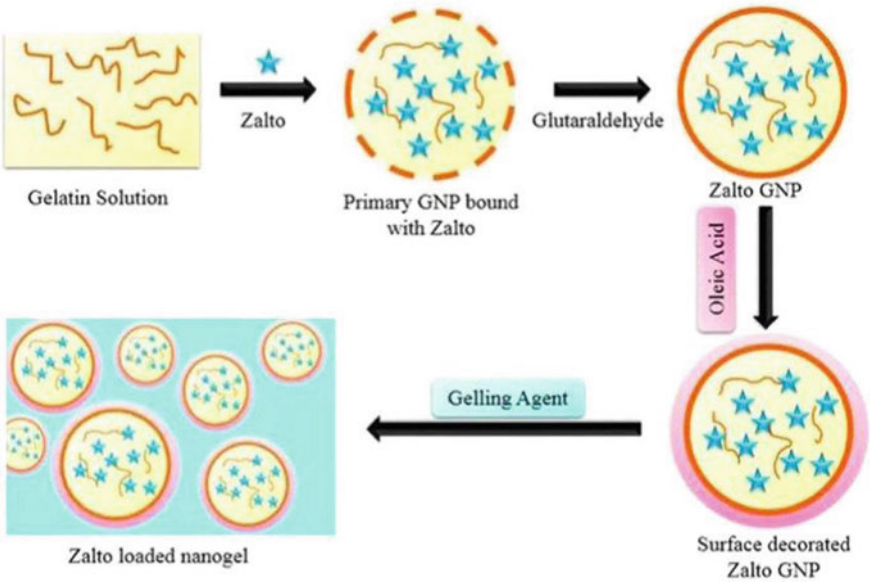

Fig. 9 Surface modification on zaltoprofen-loaded gelatin nanoparticles, Ref. [68]

7.10 Oleic Acid-Coated Gelatin Nanoparticle Impregnated Gel

Gelatin nanoparticles were first prepared by two-step desolvation method by using desolvating agent as ethanol to precipitate high molecular weight (HMW) gelatin. The supernatant was discarded, and the HMW gelatin was redissolved by adding distilled water containing zalto–cyclodextrin complex containing zaltoprofen as a result of formation of gelatin nanoparticle by the drop-wise addition of ethanol. Glutaraldehyde was added to cross-link the nanoparticles. The surface modification was done on zaltoprofen-loaded gelatin nanoparticles by adding oleic acid for preparing nanoparticulate suspension and for topical drug delivery. The smooth and spherical shape of GNP was confirmed by SEM. In vitro and ex vivo drug release showed that there was 69.47 and 78.59% drug released within 48 h. The good texture properties of nanogel were observed from texture analysis graphs. Stability data revealed stability of nanogel formulation up to 3 months [68] (Fig. 9).

7.11 Gelatin Nanogels by Sol–gel Technique

Enoxaparin-immobilized gelatin/poly(ε-caprolactone) (PCL) or Eudragit RS230D nanogels were prepared in the presence of tetraethyl orthosilicate (TEOS) that acts

as polycondensation reagent by sol–gel technique for drug delivery ability. The sol–gel process was carried out under a dry nitrogen atmosphere with HCl as catalyst. For cross-linking of the organic matrix, the drug-loaded nanogel samples were exposed to ultraviolet (UV) light (150 W, XBO xenon lamp, light spectrum 200–1100 nm) for 15 min. Sol–gel formation was conducted based on hydrolysis and condensation mechanisms. Eudragit was used as model drug at different concentrations. The prepared nanogel was analysed by thermal analysis (DSC and TGA), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning and transmitting electron microscopes (SEM and TEM). The particle size, zeta potential and in vitro release profiles were investigated. The prepared nanogels exhibited amorphous nature with stable colloidal particles (9.3 nm) and high surface charge density (negative zeta potential). The immobilization of enoxaparin into the gel network led to the formation of stable nanogels with ionic functional groups, which enable the efficient loading and sustainable release [69].

8 Conclusion

Biopolymers, namely polysaccharide pullulan and the two polyamides collagen and gelatin, offer themselves as versatile materials in modern drug delivery systems by forming nanogel networks. Pullulan is highly water soluble which on conjugation with cholesterol forms CHP with increased hydrophobicity and offers itself as a matrix for nanogel formation. Self-assembly of these hybrid materials is a well-established strategy to synthesize the nanogels. Another method to make these materials is physical and chemical grafting copolymerization techniques. These nanogels are usually sensitive towards environmental conditions. The thermoresponsive CHP-PNIPAM nanogels exhibit quick deswelling and reswelling behaviour at stipulated temperatures. The uronic acid-grafted derivatives exhibit pH-responsive behaviour in drug delivery. Collagen and gelatin nanohydrogels prepared through physical and chemical cross-linking as well as irradiation techniques are mainly used for drug delivery, especially in wound healing. This chapter describes the synthesis of tailor-made functional nanogels for customized use as drug carriers as well as environmental sensors.

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