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Collagen-based micro/nanogel delivery systems: Manufacturing, release mechanisms, and biomedical applications

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Abstract

Collagen-based materials, renowned for their biocompatibility and minimal immunogenicity, serve as exemplary substrates in a myriad of biomedical applications. Collagen-based micro/nanogels, in particular, are valued for their increased surface area, tunable degradation rates, and ability to facilitate targeted drug delivery, making them instrumental in advanced therapeutics and tissue engineering endeavors. Although extensive reviews on micro/nanogels exist, they tend to cover a wide range of biomaterials and lack a specific focus on collagen-based materials. The current review offers an in-depth look into the manufacturing technologies, drug release mechanisms, and biomedical applications of collagen-based micro/nanogels to address this gap. First, we provide an overview of the synthetic strategies that allow the precise control of the size, shape, and mechanical strength of these collagen-based micro/nanogels by controlling the degree of cross-linking of the materials. These properties are crucial for their performance in biomedical applications. We then highlight the environmental responsiveness of these collagen-based micro/nanogels, particularly their sensitivity to enzymes and pH, which enables controlled drug release under various pathological conditions. The discussion then expands to include their applications in cancer therapy, antimicrobial treatments, bone tissue repair, and imaging diagnosis, emphasizing their versatility and potential in these critical areas. The challenges and future perspectives of collagen-based micro/nanogels in the field are discussed at the end of the review, with an emphasis on the translation to clinical practice. This comprehensive review serves as a valuable resource for researchers, clinicians, and scientists alike, providing insights into the current state and future directions of collagen-based micro/nanogel research and development.

Keywords: Collagen-based materials; Cross-linking; Delivery systems; Micro/nanogels; Responsiveness

Introduction

Hydrogels are three-dimensional cross-linked networks of hydrophilic polymers with a high moisture content that have emerged as attractive microenvironments or scaffolds for tissue engineering and biomedical applications because of their tunable porosities, controllable mechanical characteristics, and high permeability.[1] Depending on the size of the gel scaffold, hydrogels can be classified as macro-, micro-, or nanogels. When hydrogels are in the form of macroscopic networks with sizes greater than 1000 μm, they are generally considered macrohydrogels, also known as bulk gels.[2] Once the size of hydrogels is limited to 1–1000 μm, they are referred to as microgels. If the size of these microgels is further reduced to the submicrometer range (<1000 nm), they are classified as nanogels.[1] Compared with traditional bulk gels, micro/nanogels are inherently small in size with a large surface area, providing excellent drug-loading efficiency, high responsiveness to the surrounding environment, and greater diffusivity and mobility. Thus, increasing research has focused on encapsulating various bioactive substances (e.g., drugs, proteins, DNA, etc.) within micro/nanogels and their release behaviors *in vitro*/*in vivo* to explore their potential for tissue engineering, wound dressings, targeted drug delivery, and cancer prevention. Furthermore, their

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sufficiently small volume allows for injection through small needles and catheters, facilitating the minimally invasive delivery of biologics, or serving as bioinks for three-dimensional printing.

Generally, the hydrophilic polymers that form hydrogel scaffolds can be categorized as synthetic, natural, or hybrid polymers, which can be cross-linked through physical, chemical, or combined methods. Among the numerous polymers available, collagen, the principal natural component of the extracellular matrix in vertebrates, is recognized as a vital natural biomaterial for micro- and nanogel preparation. As a natural cell adhesion protein, collagen binds to various cell surface receptors (e.g., integrins), promoting the formation of a specific cell morphology and supporting cell adhesion, proliferation, and differentiation.[3] In addition, collagen hydrogels exhibit excellent biocompatibility, low antigenicity, mechanical flexibility, and controllable degradation rates, making them favorable for controlled drug release applications. However, pure collagen hydrogel networks still lack controllable mechanical strength and structural stability upon hydration. Hence, more researchers have focused on developing collagen derivatives or combining collagen with other materials to optimize the physicochemical properties of gel matrices. In the context of this review, we use the term "collagen-based micro/nanogels" to describe these materials.

Among all collagen derivatives, gelatin is the most well-known and widely used. When generated by thermal protein separation followed by acid or alkaline treatment, gelatin is a heterogeneous mixture of peptides featuring the Gly-Xaa-Yaa repeating sequence found in collagen. Furthermore, gelatin contains an arginine–glycine–aspartic acid sequence, providing adhesion sites for cells and resulting in a high level of biocompatibility with collagen. Lacking the highly ordered triple-helical structure, gelatin has better water solubility, greater structural modifiability, lower preparation costs, and unique thermosensitivity compared to collagen, allowing it to be more adaptable in drug delivery systems, tissue engineering, and other biomedical applications.[4] Thus, most current studies of the preparation of nanogels prefer gelatin hydrogel networks.

According to the Web of Science database (https://webofscience.clarivate.cn/), the number of annual publications on collagen-based micro/nanogels has steadily increased from 2001 to 2024 [Supplementary Figure 1, http://links.lww.com/CM9/C432]. This trend indicates a growing interest in collagen-based micro/nanogels. Comprehensive and systematic summaries of the preparation methods, material properties, potential for specific biomedical applications, and prospects of these newly developed micro/nanogels are still lacking. In this review, the dominant synthetic and manufacturing technologies related to collagen and collagen-derived micro/nanogels are summarized in detail. Subsequently, drug delivery systems based on collagen and collagen-derived micro/nanogels are presented and classified according to their release behavior. Finally, specific biomedical applications of these delivery vehicles, including bone tissue repair, wound healing, cancer therapy, and imaging, are outlined [Figure 1]. Therefore, the purpose of the present review is to systematically summarize the relationship between the preparation process and the structure–activity relationship, providing an up-to-date guide for collagen-based micro/nanogels based on the available data in the literature. This review provides valuable resources for researchers and clinicians and offers insights into the current landscape and future trajectories of collagen-based micro/nanogel research.

Techniques for Cross-Linking and Manufacturing Collagen-Derived Micro/Nanogels

Physical and chemical cross-linking strategies

Collagen-derived micro/nanogels are mainly formed by either physically or chemically cross-linked three-dimensional networks, as summarized in Table 1. Collagen-derived micro/nanogels formed through supramolecular interactions (e.g., noncovalent interactions such as hydrogen bonds, hydrophobic interactions, or electrostatic interactions) are typically reversible and spontaneous, without the need for toxic cross-linkers, contributing to their excellent biocompatibility and cell-friendliness. Compared with the harmless and straightforward process of supramolecular interactions, chemically cross-linked gels formed by covalent bonds generally display higher and more stable mechanical properties with faster reaction efficiency and shorter reaction times. However, the toxic reagents or free radicals generated during the chemical cross-linking process can pose potential risks to cells. For a deeper discussion of different hydrogel cross-linking strategies or formation strategies, we refer the readers to the review by Zheng *et al*.[5]

Both collagen and gelatin can form physically cross-linked hydrogels, but their gelation mechanisms differ [Figure 2]. Collagen self-assembles into a gel-like network as it warms from low temperatures to 37°C at a physiological pH of 7.4 or close to 7.4.[6] In contrast, as a reversible sol-gel material, gelatin dissolves above its melting point (27°C–34°C) and reverts to its helical structure when cooled below its melting point.[7] Importantly, all temperatures used during gelatin hydrogel preparation do not exceed 40°C to prevent excessive denaturation. In addition to the inherent physical cross-linking properties of gelatin, the abundant carboxylic acid groups in gelatin molecular chains can form ionic bonds in the presence of metal ions, creating a gel network through electrostatic interactions. A previous study revealed that Fe^{3+} enhances the elastic modulus and triple-helical fraction of gelatin hydrogels, whereas Ca^{2+} has the opposite effect.[8] Similarly, collagen-based materials can also be ionically cross-linked with polymers carrying opposite charges at the same pH value to induce pH responsiveness.

In addition to physical cross-linking, the abundant reactive groups in the molecular structure of collagen-based polymers, such as amines and carboxylic acids, provide numerous opportunities for chemical cross-linking, enabling the creation of stronger and more stable micro/

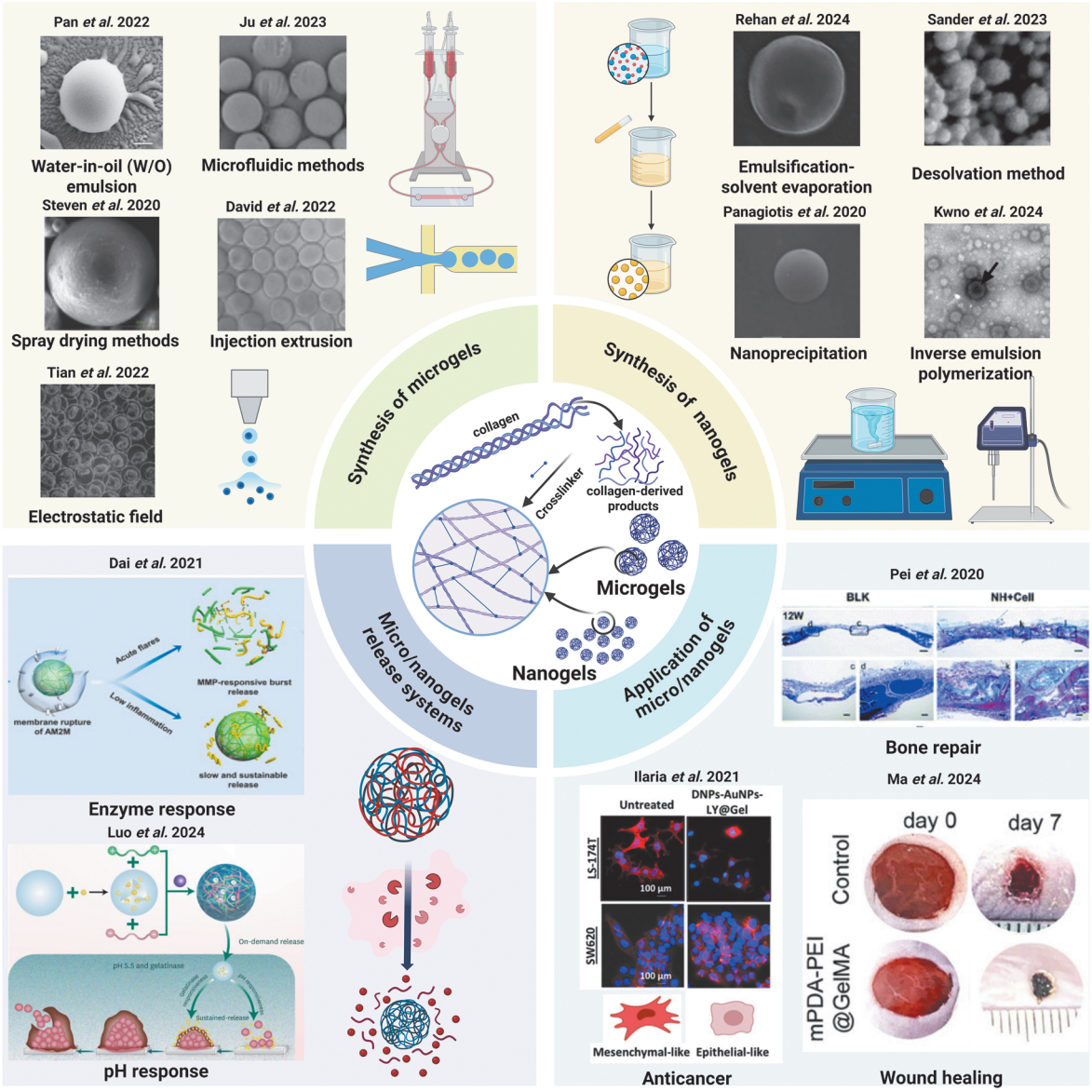

Figure 1: Outline of the synthesis, release systems, and applications of collagen and collagen-derived micro/nanogels. The structures of collagen-based micro/nanogels prepared by water-in-oil (W/O) emulsion, microfluidics, spray drying, injection extrusion, electrostatic field, emulsification–solvent evaporation, desolvation, inverse emulsion polymerization, and nanoprecipitation methods are displayed. Schematic diagrams of the reactions of pH-responsive and enzyme-responsive collagen-based micro/nanogels are shown. The anticancer, bone-repair, and wound-healing effects of the collagen-based micro/nanogel are shown. mPDA-PEI: Polyethyleneimine-functionalized mesoporous polydopamine. Created using *BioRender.com*.

Table 1: Merits and limitations of different cross-linking strategies for collagen-derived micro/nanogels.

Cross-linking types	Merits	Limitations
Physical cross-linking		
Self-assembly	Reversible and spontaneous; biocompatible; no toxic cross-linkers	Relatively weak mechanical strength
Electrostatic interaction	Simple cross-linking process; tunable bonding intensity; no toxic cross-linkers	Easy destruction of electrostatic interactions and unstable mechanical strength
Chemical cross-linking		
Schiff base reaction	Mechanically and structurally stable; fast and efficient	Toxic reagents; possible damage to cells
Photo-initiated radical polymerization	Mechanically and structurally stable; mild reaction conditions; short reaction time; irreversible process	Releases active free radicals; possible damage to cells

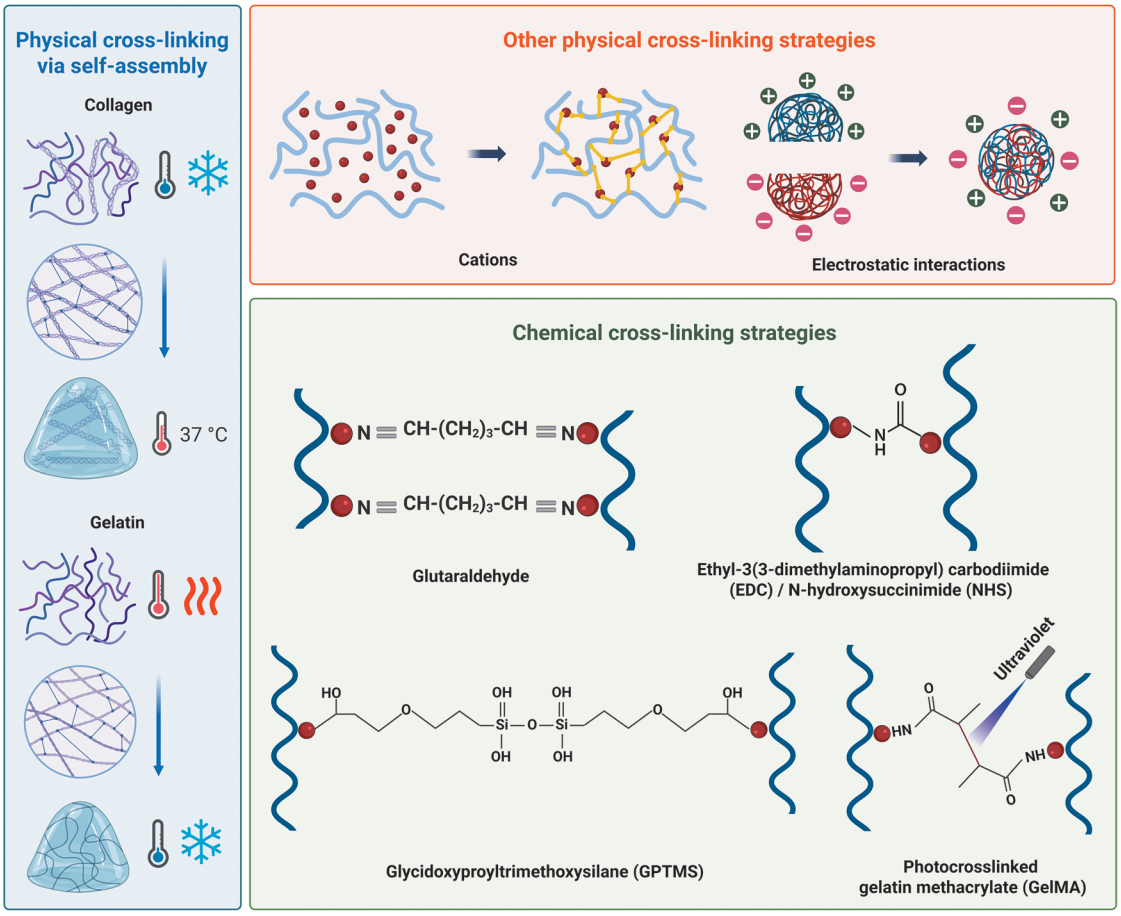

Figure 2: Physical cross-linking via self-assembly (intrinsic properties of the material), other physical cross-linking strategies (via material modifications), and chemical cross-linking strategies of collagen-based micro/nanogel materials. Created using *BioRender.com*.

nanogels. Schiff base reactions can be used to chemically cross-link collagen-based materials. A common method involves oxidizing polysaccharides (e.g., alginate aldehyde and gum Arabic aldehyde) in the presence of borax, allowing the aldehyde or ketone groups to undergo condensation reactions with the free amino groups in gelatin. This cross-linking method is reversible, as the imine bonds can break and reform under certain conditions, such as low pH values, thereby endowing the hydrogels with self-healing and stimulus-responsive properties.[9] Despite potential cytotoxicity concerns, glutaraldehyde, a common cross-linking agent, reacts with amine compounds to form Schiff bases. However, the multiple Schiff base bonds that form can undergo further condensation reactions to create a stable cross-linked structure, leading to irreversible reactions. Ethyl-3(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide and glycidoxypropyltrimethoxysilane have also been effectively used to induce cross-linking in collagen-based microgels. Another general strategy is the photocrosslinking of methacrylated gelatin by photoinitiators and ultraviolet (UV) light exposure.[10] Common choices for photoinitiators of methacrylated gelatin include lithium acylphosphinate salt and 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959). In addition, the concentrations of methacrylated gelatin and initiator, the degree of substitution, and the time of UV exposure are key parameters contributing to the photoinduced cross-linking density. The choice between physical and chemical cross-linking modification methods often depends on the requirements of the specific application, such as the desired hydrogel lifespan, mechanical strength, and biocompatibility. Both strategies provide valuable tools for tailoring the properties of collagen-based micro/nanogels for applications in tissue engineering and drug delivery.

Microgel fabrication

Through cross-linking strategies, collagen-derived hydrogels can be fabricated into microgels using various manufacturing methods. Since different applications require varying sizes, homogeneities, stabilities, drug-loading capacities, etc., an understanding of current manufacturing processes and how they affect the final properties of the hydrogels and the drugs loaded is essential. Typically, micron-sized hydrogels (microgels) are synthesized by fragmenting a polymer-containing aqueous phase into droplets within a different continuous phase under shear forces, followed by stabilization through cross-linking. Commonly used shear forces are generated between oil–water or gas–water interfaces. Based on this principle, a range of manufacturing technologies has been developed, allowing the formation of droplets of different sizes by

regulating the flow rate ratio and interfacial tension between immiscible phases. In this section, we focus on the dominant manufacturing strategies for collagen-based microgels, including conventional and microfluidic emulsion methods (shear at the oil–water interface) and spray drying and injection extrusion methods (shear at the gas–water interface). A comprehensive comparison of the above four preparation methods is summarized in Supplementary Table 1, http://links.lww.com/CM9/C432, with more detailed preparation information available in Supplementary Table 2, http://links.lww.com/CM9/C432. For more systematic insights into the fabrication methods of microgels, please refer to the review by Wei *et al*.[11]

Conventional and microfluidic emulsion methods

Both conventional and microfluidic emulsion methods use the shear force yielded at the water–oil interface to separate the collagen-based aqueous phase into small droplets.[12] The former has a longer application history with the advantage of batch preparation. As shown in Figure 3A, typical heterogeneous emulsification involves two consecutive steps: emulsification of the polymer aqueous phase in a continuous oil phase with an oil-soluble surfactant, followed by gelation with a water-soluble cross-linker.[13] Early studies used the mechanical force from homogenizers or high-speed mechanical stirrers to create customized collagen microgels of various sizes (5–400 μm).[14] Since then, various collagen-based hybrid microsphere systems with improved mechanical performance have been explored to overcome the mechanical instability of pure collagen microspheres. Examples of such hybrid hydrogel microspheres include chitosan/collagen, agarose/collagen, bacterial cellulose/collagen, hydroxyapatite/collagen, calcium phosphate/collagen, collagen/gelatin, gelatin/cassava starch and gelatin/chitosan/alginate [Figure 3A]. Nevertheless, this widely used emulsion technology still has several drawbacks,

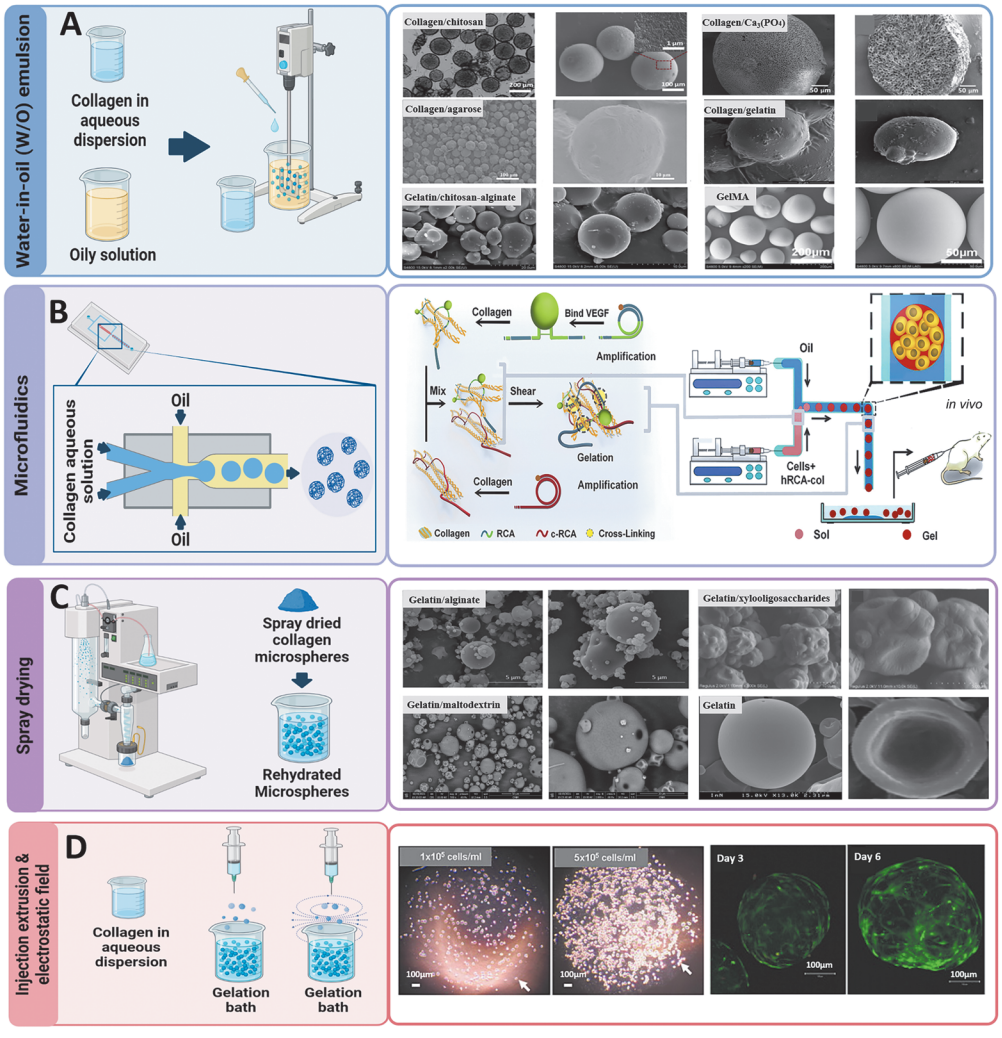

Figure 3: Schematic diagram of different microsphere fabrication technologies. (A) Operation of the emulsification process, along with SEM images of collagen/chitosan, collagen/agarose, collagen/$Ca_3(PO_4)_2$, collagen/gelatin, gelatin/chitosan-alginate, and methacrylated gelatin microspheres produced via emulsification. (B) Production process of microfluidic microspheres and the experimental diagram showing the promotion of collagen cross-linking within the microfluidic chip using interlocking DNA. (C) Spray drying equipment and SEM images of gelatin/alginate, gelatin/xylooligosaccharide gelatin/maltodextrin, and gelatin microspheres produced by spray drying. (D) Injection extrusion procedure and optical microscopy images of collagen microspheres encapsulating mesenchymal stem cells (1×10^5 cells/mL and 5×10^5 cells/mL) as well as confocal images captured after 3 and 6 days of culture. RCA: Rolling circle amplification; SEM: Scanning electron microscope.

including significant variability in the microsphere size and challenges in scaling up for industrial production. These limitations can be overcome by innovative oil-shearing fabrication technology, namely, microfluidics. Compared with emulsion methods, microfluidics operates at a scale comparable to that of biological cells, producing more uniform microgels while significantly reducing the consumption of costly biological reagents. Chips are the key to microfluidic technology. These chips consist of inlets for dispersed and continuous liquids, along with microchannels with tapered junctions, which partition pregel materials in the oil emulsion phase by fragmenting the dispersed liquid filaments into droplets [Figure 3B]. By changing the flow rate of the dispersed and continuous phases, microgels of diverse sizes and dispersities can be rapidly generated.[15] Moreover, droplet confinement and precise control of the cross-linking reaction time are also key parameters for producing monodisperse particles with a variety of shapes and morphologies.

Traditional emulsification and microfluidic techniques each have distinct advantages for the specific encapsulation of cargo in drug delivery and tissue engineering. Traditional emulsification methods are preferred for encapsulating hydrophilic compounds, such as small-molecule drugs, proteins, and oligonucleotides, which can be easily encapsulated into aqueous droplets through batch homogenization during emulsification. Chemical cross-linkers with high cross-linking rates and reactivities, including ethyl-3(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide, glutaraldehyde, and glycidoxypropyltrimethoxysilane, are commonly used to ensure the effective encapsulation of the above functional components during emulsification. Although conventional emulsification can also be used to encapsulate cells, only gentle physical cross-linking methods are used to avoid the cytotoxic effects of chemical cross-linkers and surfactants on the cells.

The precise operation of microfluidics at a cell-comparable scale has significant potential for live-cell encapsulation and tissue engineering. A variety of collagen-based microspheres have been investigated for the delivery of tendon-derived stem cells,[16] vascular endothelial cells,[17] and etc. Previous microfluidic studies have favored the self-assembly properties of collagen or gelatin to achieve mild gelation and ensure high cell viability in collagen-based microspheres.[18] Zhao *et al*[17] used the interlocking of ultralong DNA in combination with microfluidic shear forces to accelerate the self-assembly of collagen, reducing the gelation time of the collagen scaffold by more than 30-fold [Figure 3B]. In addition, numerous studies have selected methacrylated gelatin as the microsphere material, using photoinitiators and brief ultraviolet exposure to initiate the covalent cross-linking of the methacrylate functional groups on the methacrylated gelatin molecular chain for gelation.[16] The optically transparent microfluidic device allows UV light to penetrate briefly and promote photogelation while enabling the easy collection of gelled methacrylated gelatin droplets at the output without the need for complex microchannel designs. However, UV photocrosslinking may negatively affect particularly sensitive cells, thereby affecting the survival of the encapsulated cells.[19]

Gas-shearing fabrication technology

Similar to the principle of the oil-shearing method, gas-shearing fabrication technology requires airflow to initiate the formation of collagen-based droplets. In this process, the growing aqueous phase droplets are influenced by the competing forces of gas shear and liquid surface tension. Once the shear force from the airflow exceeds the resistance of surface tension, the droplets detach from the liquid flow, forming micron-sized gel precursor droplets. Compared with oil-shearing fabrication technology, gas-shearing strategies can overcome the limitations associated with the use of oils, photoinitiators, cross-linkers, surfactants, or UV irradiation. The gas-shearing techniques used in the synthesis of collagen-based microgels primarily include spray drying methods and injection extrusion. Although both methods rely on shear forces at the air–liquid interface, they differ significantly in terms of the cross-linking methods and application advantages.

As displayed in Figure 3C, during spray drying, the solution or suspension containing drugs, polymers, and particles is dispersed into fine droplets within a high-temperature airflow, where the solvent evaporates simultaneously, forming dry microspheres or microgels with diameters ranging from 1 to 10 μm. Compared with other strategies, this method can process large volumes of solution at once while maintaining low production costs, making it more suitable for industrial-scale batch production. However, based on the temperature-sensitive properties of proteins, spray drying technology is more suitable for collagen hydrolysate (e.g., gelatin) than for collagen.[20] A spray cooling method suitable for gelatin has been proposed to address the heating issue. In this case, the gelatin polymer solution is sprayed into a cold chamber, where the droplets solidify into microgels through self-aggregation by cooling rather than solvent evaporation.[21] To date, the preparation of alginate/gelatin,[22] guar gum/collagen hydrolysate,[23] maltodextrin/gelatin,[24] and gelatin/xylo-oligosaccharide[25] microspheres with the aforementioned two types of spray drying technology has been reported [Figure 3C]. In addition, spray drying technology can be used to encapsulate hydrophobic drugs, resulting in a high drug loading efficiency. Existing studies have used sodium dodecyl sulfate and ethanol to assist in the preparation of spray-dried gelatin microspheres, aiming to increase the solubility and oral bioavailability of hydrophobic drugs.[26] In addition, gelatin, when used as a conjugate combined with other polymers, enhances the properties of drug microcarriers. For example, hydrophobic luliconazole and crystalline methionine particles have been more effectively delivered using spray-dried alginate/gelatin microparticles.[27]

As another manufacturing method based on shear force at the gas–liquid interface, injection extrusion can produce collagen-based microspheres using an injection needle, a jet cutter, and a subsequent gelation bath [Figure 3D]. Compared with the abovementioned techniques, this strategy eliminates the need for special organic solvents

(e.g., oil or ethanol) and complex drying equipment or chips, suggesting its tissue engineering safety and low consumption. Thus, it is frequently used to encapsulate delicate mesenchymal stem cells.[28] Moreover, milder physical cross-linking methods are commonly used to ensure high biosafety with this technology, including the gentle self-aggregation of collagen at 37°C or the rapid Ca^{2+}-induced cross-linking of alginate/collagen-based hybrids.[29] However, this technology still has drawbacks, including the requirement of a gelling bath with a complex nature of formulation and its time-consuming nature with poor scale-up reproducibility.[30] Recent studies have used injection extrusion methods combined with pneumatic nozzle air atomization and high-voltage electrostatic field systems to increase the uniformity of the microspheres and overcome potential issues related to varying microsphere sizes and poor reproducibility.[29]

Nanogel fabrication

The fabrication of collagen-derived nanosized hydrogels (nanogels) involves different methodologies than those used for microgels. Typically, these methodologies generate colloidal nanoparticles, followed by physical or chemical cross-linking. Depending on how these nanoparticles are produced, nanogel fabrication techniques can be classified into two main types: (1) nanoemulsification, which creates nanoscale droplets for cross-linking, and (2) phase separation, which uses differences in solubility to form hydrogel particles. Recent studies have tended to apply gelatin rather than collagen for nanogel synthesis, which might be attributed to the better water solubility and easier molecular modification of gelatin, allowing its versatile application in nanogel formulations. Furthermore, due to the abundant Gly, Pro, and 4-Hyp residues in the gelatin polypeptide structure and its typical sequence of -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-, it is conducive to polymerization after the addition of free radical initiators for synthesizing colloidal particles.[31] Therefore, this section will focus on providing a detailed exploration of the synthesis methods and examples of gelatin nanogel preparations. For descriptions of more nanogel fabrication methods, we invite the readers to consult the review by Garg *et al*.[32]

Nanoemulsification

Similar to the emulsion methods used for microgel fabrication, nanoemulsification technology involves creating a stable emulsion of nanoscale droplets in the oil phase with the assistance of oil-soluble surfactants, followed by cross-linking. The cross-linked nanogels can be stably distributed in aqueous media after the removal of organic solvents and emulsifiers. The size and properties of the generated nanogels can be optimized by adjusting the concentration or feed ratio of the aqueous phase of the polymer and cross-linkers, the pH of the reaction medium, the choice of surfactants, the cross-linking time and temperature, etc.[32] Gelatin or modified gelatin nanogels synthesized through self-assembled gelation by using nanoemulsion methods have been developed to encapsulate curcumin, quercetin, nisin, and thymol. Furthermore, doxorubicin-loaded methacrylated gelatin nanoparticles rely on photoinduced gelation. In addition, gelatin microspheres produced via nanoemulsification through covalent gelation have been used to encapsulate methotrexate, irinotecan, hydroxyapatite, etc. Several previous studies have used oxidized polysaccharides to covalently cross-link with gelatin, forming hybrid gelatin nanogels via a Schiff base reaction to mitigate the potential toxicity of chemical cross-linkers.[33] In addition, one study explored an alternative approach by selecting tannic acid with hemostatic and antibacterial properties as cross-linkers, using dioctyl sodium sulfosuccinate as a surfactant and mixing tannic acid with a biopolymer emulsion under ultrasonication to generate a relatively safe chitosan/gelatin nanogel [Figure 4A].[34]

Phase separation technology

Phase separation for nanogel fabrication is characterized by polymer precipitation by altering conditions such as the temperature or solvent composition.[35] The preparation of collagen-derived nanogels by changing the solvent composition, that is, mixing aqueous polymer solutions with a miscible organic antisolvent (such as alcohol or acetone), is relatively simple.[36] The commonly used phase separation methods include desolvation and nanoprecipitation.

Desolvation refers to the addition of an antisolvent to a polymer solution to induce phase separation and aggregation of the hydrocolloid [Figure 4B]. Once the critical level of aggregation is reached, excessive aggregation is prevented by the addition of isopropanol, while the cross-linking of the gelatin nanospheres is accomplished through the introduction of glutaraldehyde. Following the optimization of the original desolvation strategy, a two-step desolvation method was developed to prepare gelatin nanoparticles with different molecular weights. Amjadi *et al*[37] used this method to eliminate high-molecular-weight gelatin, designing a novel nanocarrier with an increased betanin and doxorubicin loading efficiency by decorating gelatin nanoparticles with methoxy poly (ethylene glycol)-poly(2-dimethylaminoethyl methacrylate-co-itaconic acid) [Figure 4B]. Others have used the desolvation method to load different small-molecule drugs, such as linezolid, irinotecan hydrochloride, gliclazide, paracetamol, and pilocarpine hydrochloride, which are well documented in the literature. In addition, desolvation methods could be used to load proteins and small interfering RNAs into gelatin nanoparticles for controlled drug delivery, with applications spanning anticancer, anti-inflammatory, and antidiabetic therapies. Unlike the desolvation method, nanoprecipitation refers to slowly adding an aqueous gelatin solution to ethanol or acetone as the antisolvent, whereas poloxamer is often used as a stabilizer. Then, glutaraldehyde is added to cross-link the nanoparticles [Figure 4C]. The mechanism of nanoparticle formation in this process mainly relies on the interfacial turbulence generated during the solvent replacement process, which rapidly diffuses due to the mutual miscibility between the two solvents. During the turbulent diffusion process, nanoscale solvent droplets are disrupted at the interface, stabilized by surfactants,

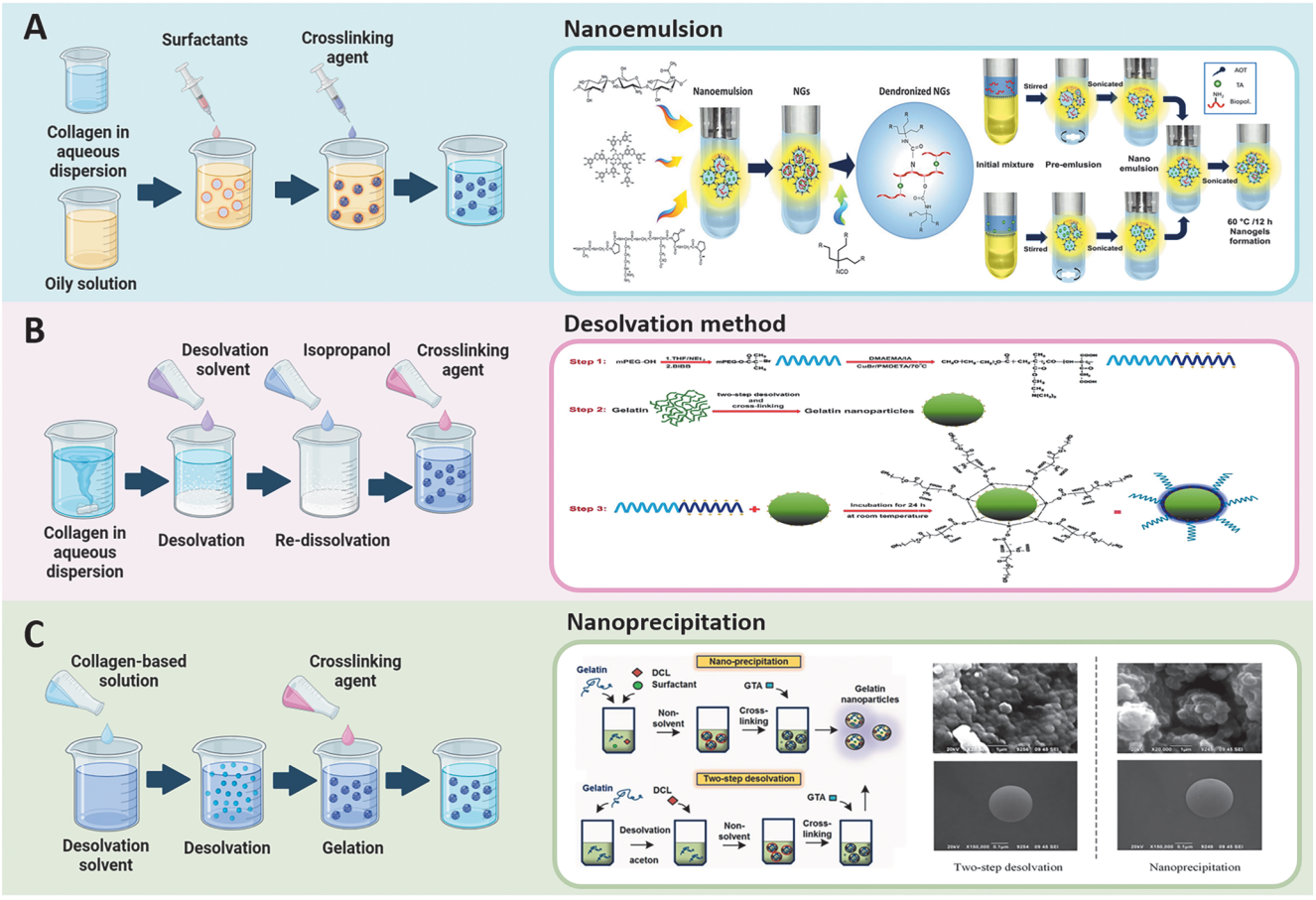

Figure 4: Schematic diagram illustrating various nanogel fabrication technologies. (A) Nanoemulsification operation and the process for producing chitosan/gelatin nanogels using tannic acid as a covalent cross-linker. (B) Desolvation method, with a detailed description of the steps involved in a two-step desolvation process to remove high-molecular-weight gelatin for the preparation of smaller nanogels. (C) Nanoprecipitation, highlighting the preparation and morphological comparison of gelatin nanoparticles encapsulating nonsteroidal anti-inflammatory drugs using nanoprecipitation and the two-step desolvation method. AOT: Dioctyl sulfosuccinate sodium salt; DCL: Diclofenac; GTA: Glutaraldehyde; NGs: Nanogels; TA: Tannic acid.

and gradually solidified as the solvent fully diffuses. A previous study revealed that at the same gelatin concentration, the nanoparticles produced by two-step desolvation are significantly smaller than those produced by nanoprecipitation.[38] Afterward, Koletti *et al*[39] conducted a comparative study of the preparation of gelatin nanoparticles encapsulating nonsteroidal anti-inflammatory drugs for systemic administration using nanoprecipitation and the two-step desolvation method [Figure 4C]. These results suggested that nanoprecipitation was the preferred method, as it could be accomplished in a single step and exhibited a slightly faster drug release profile. Compared with desolvation and nanoemulsion methods, nanoprecipitation is a simple, rapid, and straightforward approach for producing gelatin nanoparticles with a narrow unimodal distribution without requiring ultrasonic treatment, elevated temperatures, or water–oil interface processes.[40] Numerous drugs, including aescin, doxorubicin, 3-alkylpyridinium salt, metoprolol succinate, simvastatin, 5-aminosalicylic acid, and irinotecan, have been successfully encapsulated into gelatin nanoparticles prepared via nanoprecipitation. These gelatin nanoparticles have been used for tumor therapy and the inhibition of intestinal hepatitis and are administered through both oral and intravenous routes.

Collagen-Based Micro/Nanogel Release Systems

The properties of collagen-based micro/nanogels are not only directly determined by the structure and manufacturing process of the materials but also significantly influenced by the surrounding environment. These hydrogels typically engage in solvent and solute exchange with their surroundings, altering their cross-linked architecture and consequently their morphology, dimensions, and mechanical properties. This finding also implies that micro/nanogels may be sensitive to external stimuli, such as changes in temperature, pH, and physiological microenvironments [Figure 5]. Recently, Jin *et al*[41] summarized the work on various responsive hydrogels. This review reasonably explains the emergence of various environmentally responsive collagen micro/nanogels with different release behaviors, which will be introduced in detail in subsequent sections.

Sustained release

Sustained release systems are technologies that control the rate of drug release, aiming to maintain effective drug concentrations in the body, reduce the frequency of drug administration, and improve patient compliance. Collagen-based micro/nanogels exhibit sustained drug

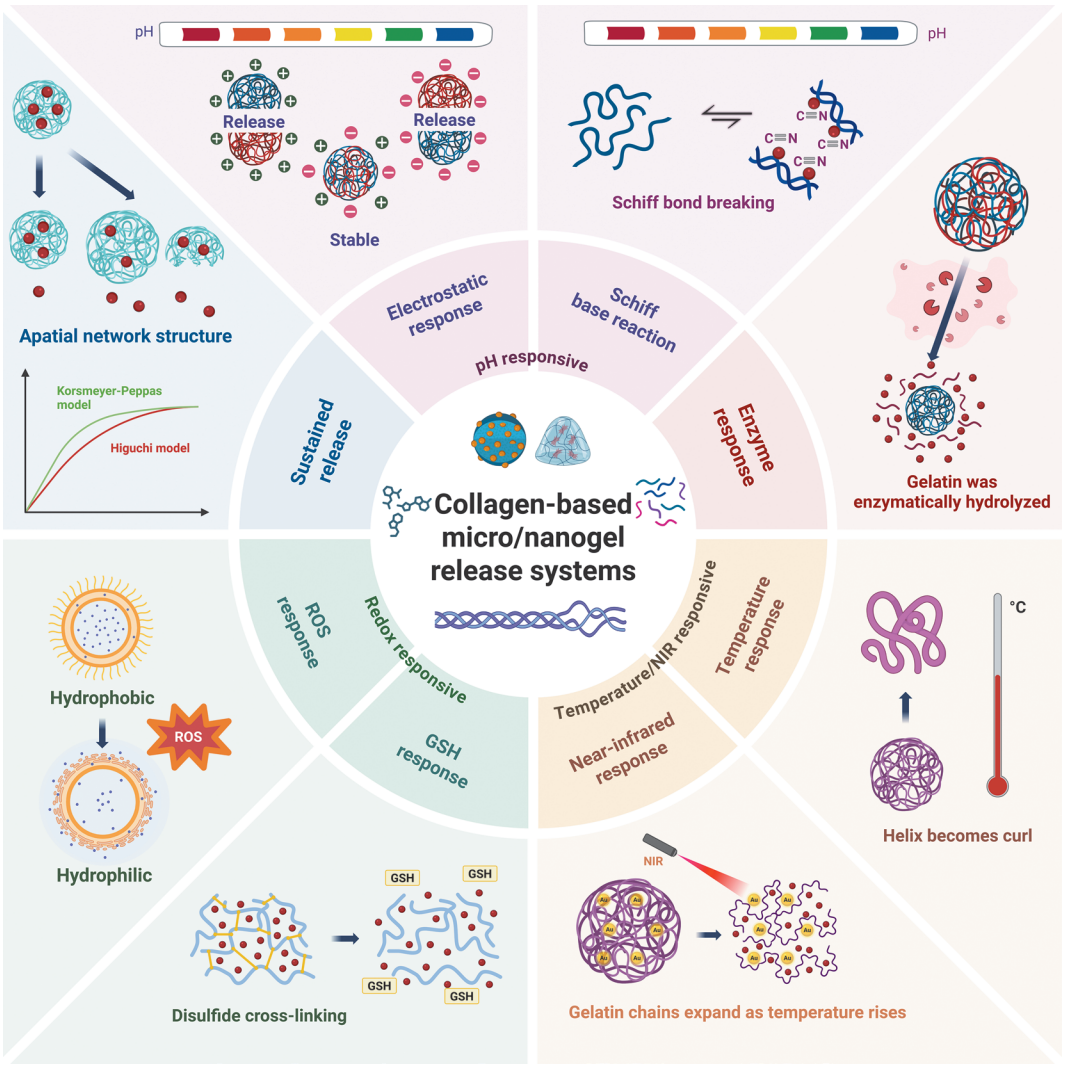

Figure 5: Collagen-based micro/nanogel release systems. Created using *BioRender.com*. GSH: Glutathione; NIR: Near-infrared; ROS: Reactive oxygen species.

release under physiological conditions due to their three-dimensional network structure and appropriate pore sizes, which facilitate drug encapsulation and retention. The gradual degradation of the hydrogel matrix further contributes to controlled release, making these systems suitable for applications in targeted drug delivery, wound healing, and regenerative medicine. The release of drugs from collagen-based hydrogels typically follows Fickian diffusion (e.g., the Higuchi model) or Case II diffusion.[42,43] Various release models have been proposed, among which the Korsmeyer–Peppas model is widely used because it can describe various drug release mechanisms, including Fickian diffusion, Case II transport, and release behavior between the two. The specific mechanism depends on the physicochemical properties of the hydrogel and the chemical properties of the drug. Based on recent publications, most collagen-based micro/nanogel drug delivery systems with relatively uniform substrates or high degrees of cross-linking tend to follow the Higuchi release model during the sustained release phase, mainly because of the stable structure of the collagen-based material in the physiological environment. The model assumes constant diffusion, which is directly related to the shape and surface of the hydrogel. Compounds slide through pores on the material's surface, avoiding changes in the initial dimensions and expansion of the material.[44] For example, a series of gelatin nanodrug release systems prepared by Susanta *et al*[42] mostly follows the Higuchi model, indicating that drug release is not governed by the swelling or erosion of the material. In this study, the structure of the highly cross-linked hydrogel release system barely expanded after water was absorbed, releasing only 30.41% of the drug after 24 h, whereas the control group without the cross-linking modification exhibited structural swelling and released most of the drug after 12 h.

On the contrary, systems with multicomponent substrates or loose structures tend to follow the Korsmeyer–Peppas model, primarily because of the swelling and erosion of the polymer matrix in the system. For example, the porous collagen and bacterial cellulose microspheres prepared by Zhang *et al*[43] exhibited the characteristics of an erosive swelling system, which showed rapid water absorption and swelling in drug-loading and drug-releasing environments. After implantation, collagen gradually

degrades over time, whereas bacterial cellulose does not degrade. This drug delivery system was able to release more than 30% of the drug within the first 10 h, and after 25 h, the cumulative drug release exceeded 40%, with the rate remaining stable. Owing to the large mesh structure inside the prepared porous microspheres, part of the drug is adsorbed within the microsphere wall pores, and part enters the internal bacterial cellulose mesh structure. The authors reported that the first-order, Higuchi, and Korsmeyer–Peppas models could better describe the drug release kinetics of porous collagen and bacterial cellulose porous microspheres, with the Korsmeyer–Peppas model having a better fit than the Higuchi model, indicating that drug release is not solely due to drug diffusion out of the hydrogel but is also influenced by the degradation of the hydrogel matrix.[45]

Enzyme-responsive release

Enzyme-responsive micro/nanogels can undergo phase transitions in response to various proteases in the environment and release drugs at predetermined times, locations, and dosages. They can also increase on-demand delivery to diseased sites, such as areas of infection or inflammation, thereby regulating the targeted delivery of drugs. Collagen and collagen-derived materials are prone to enzymatic degradation because of their composition. Thus, they are suitable candidates for designing enzyme-triggered drug release systems. For example, Dai *et al*[46] used a serine protease, trypsin, to study the *in vitro* hydrolytic behavior of gelatin-based nanogels. The gelatin/chondroitin sulfate-cross-linked nanogel showed adjustable drug release: burst release in the presence of trypsin, which is beneficial for acute conditions, and sustained release without enzymes, enhancing compliance and reducing side effects.

Collagen-derived micro/nanogels are highly susceptible to matrix metalloproteinases, which can be used as triggers to release drugs under specific pathological conditions. Matrix metalloproteinases are a class of zinc-dependent endopeptidases that can degrade various components of the extracellular matrix, such as collagen, elastin, and fibronectin. Under normal conditions, the activity of matrix metalloproteinases is strictly regulated by their endogenous inhibitors (such as tissue inhibitors of metalloproteinases-1) to maintain tissue homeostasis. However, in inflammatory and disease states, the expression and activity of matrix metalloproteinases can be abnormally increased, leading to the destruction of the tissue structure and loss of function. Zhao *et al*[47] took advantage of the high expression of matrix metalloproteinase-2 during tendon healing and designed a matrix metalloproteinase-responsive drug release system based on methacrylated gelatin. Methacrylated gelatin is a chemically modified gelatin with excellent matrix metalloproteinase sensitivity. By changing the concentration of the methacrylated gelatin solution, the researchers could control the cross-linking density and the network structure of nanogels, thereby achieving precise control of the drug release behavior. In the presence of matrix metalloproteinase-2, methacrylated gelatin nanogels degrade, thereby rapidly releasing the encapsulated drugs and providing a potential therapeutic strategy to promote tendon healing and reduce inflammatory responses. In subsequent studies, the enzymatic responsiveness of nanogels was further optimized by adjusting the structure of the gelatin molecules or introducing other enzyme-sensitive linkers. For example, the specificity of nanogels can be adjusted by changing the cross-linking density or introducing matrix metalloproteinase-specific substrate sequences, thereby achieving more precise control of drug release. In addition to matrix metalloproteinases, bacterial gelatinase is another common trigger for enzyme-responsive collagen-based micro/nanogels, which could be used to promote drug release to combat bacterial infection. Li *et al*[48] prepared xanthan gum–gelatin composite nanogels loaded with tedizolid phosphate with intracellular antibacterial effects, and these nanogels can enhance the ability of the nanogels to be delivered to host cells infected with bacteria.

pH-responsive release

The pH-responsive release mechanism is an intelligent drug delivery strategy that uses the chemical or physical changes in materials in environments with different pH values to control drug release. pH variations alter polymer chain charges, triggering swelling and drug release through matrix functional groups, hydrogen bonds, and electrostatic interactions between embedded nanoparticles. This mechanism is particularly suitable for tumor and antimicrobial treatments because tumor tissues and tissues infected with bacteria typically have a lower pH than normal tissues do, a characteristic that can be used to activate drug release.

Collagen hydrolysate, as a zwitterionic compound, inherently possesses pH-sensitive groups, such as hydroxyl and carboxyl groups, and collagen-based hydrogels can form composite micro/nanogels with other pH-sensitive polymers through electrostatic interactions. Wei *et al*[49] prepared a pH-responsive nanogel using gelatin and carboxymethyl chitosan. At neutral pH, type A gelatin has a positive charge, and carboxymethyl chitosan has a negative charge, ensuring the stability of the material. At the site of *Staphylococcus epidermidis* infection, the environment is weakly acidic (pH 5.5), and gelatinase[50] is released, leading to the partial hydrolysis of gelatin; both carboxymethyl chitosan and gelatin carry positive charges, and the nanogel can respond to the pH and release drugs. Furthermore, this material can also respond to pH and release drugs in an alkaline environment, as the alkaline environment causes both gelatin and carboxymethyl chitosan to carry negative charges, which can also release drugs.

In addition to electrostatic interactions, the pH-responsive drug release from the collagen-derived micro/nanogels could be attributed to the pH-dependent Schiff base. For example, at neutral pH, the amine groups from gelatin can react with aldehyde-containing polysaccharides, forming hydrogels via Schiff bases. The acidic environment of the tumor microenvironment can destroy the Schiff base bonding network of the hydrogel, thereby releasing drugs from the nanoparticle carriers and delivering them to

tumor cells. Nisha *et al*[9]prepared bio/nanocomposite hydrogels embedded with oxidized Catalpa gum/gelatin/Fe_3O_4 nanoparticles carrying the anticancer drug doxorubicin hydrochloride and the antibiotic gentamicin sulfate through an *in situ* method. They synthesized oxidized Catalpa gum by partial oxidation with sodium periodate and further modified it by cross-linking it with amine groups on gelatin through Schiff base reactions, forming a polysaccharide-crosslinked protein mixed hydrogel network. At lower pH values, both the protonated carboxyl groups and Schiff bases on the gel matrix exhibit repulsive forces with the phenolic hydroxyl and amino groups of doxorubicin hydrochloride, leading to pH-responsive drug release.

Other responsive systems

In addition to their inherent enzyme and pH responsiveness, gelatin and collagen are thermoresponsive materials that have been effectively used to develop temperature-sensitive micro/nanogels. In a precisely chemically cross-linked state, gelatin micro/nanogels can form semiflexible, relatively rigid "worm-like" helical structures at low temperatures. These helical structures may transform into disordered random coils with temperature changes, thereby endowing the polymer chains with greater flexibility and leading to the swelling or shrinkage of the nanoparticles. Chanjoong *et al*[51] prepared temperature-sensitive gelatin nanogels that exhibit a volume transitions at 32°C. This transition process may be due to the reduction in the end-to-end distance between two cross-links during the transition from the helix to the coil. Furthermore, temperature-responsive drug release can be triggered by photothermal effects and near-infrared light. In clinical applications, near-infrared light can be applied to photothermal materials, such as gold nanoparticles, to achieve local heating and a higher temperature, which further triggers drug release. Song *et al*[52] incorporated gold nanorods into gelatin/hydroxyapatite composites, leveraging the photothermal effects of gold nanorods under near-infrared light for controlled drug release. Gelatin plays a multifaceted role in achieving a near-infrared response, not only by increasing the stability of the composite material but also by helping to improve the efficiency of the photothermal effect, providing an effective platform for the biomedical application of this delivery system.

Collagen-based micro/nanogels can be responsive to redox or reactive oxygen species (ROS) triggers if cross-linked by redox- or ROS-sensitive linkers. For example, Zhao *et al*[53] modified gelatin, which is rich in amino and carboxyl groups, to prepare redox-responsive gelatin/silica nanogels and graft small interfering RNA molecules onto the nanogels through disulfide bonds. This process leads to the reversible release of functional small interfering RNA molecules in the presence of glutathione via disulfide bond cleavage. In addition, Abhay *et al*[54] developed ROS-sensitive collagen-poly(thioether) microgels, where poly(propylene sulfone) was used to cross-link with collagen. Upon ROS exposure, poly(propylene sulfone) is oxidized, and its sulfur bonds transform into hydrophilic sulfones, causing the material to swell and release drugs.[55] This redox-responsive release system provides an effective strategy for on-demand drug delivery.

Applications of Collagen-Based Micro/Nanogel Delivery Systems

The structural versatility of collagen-based micro/nanogels, combined with environmentally responsive drug release profiles, results in a wide range of therapeutic applications, from tissue regeneration to cancer treatment and diagnosis. Here, we present selected applications in different therapeutic categories [Figure 6]. In Table 2, we summarize the sizes, therapeutic agents, and release mechanisms of collagen-based micro/nanogels in highlighted applications.

Bone repair

The incidence of skeletal diseases is increasing, making bone repair a hot topic in the medical field. Currently, bone defects often require sustained drug release for repair purposes.[56] Due to the presence of collagenase in the bone environment, collagen can be digested enzymatically, enabling the sustained release of the drug. Therefore, collagen-based micro/nanogels can continuously release active substances following an *in situ* injection at the injured site to achieve bone tissue repair and regeneration. Among these materials, collagen-based microspheres are mainly used for transplanting stem cells or large cytokines into the body and provide a platform for stem cell growth, proliferation, and osteogenic differentiation.

Stem cell-loaded collagen microspheres can promote local bone formation and minimize adverse reactions. Chan *et al*[57] fabricated self-assembled collagen–mesenchymal

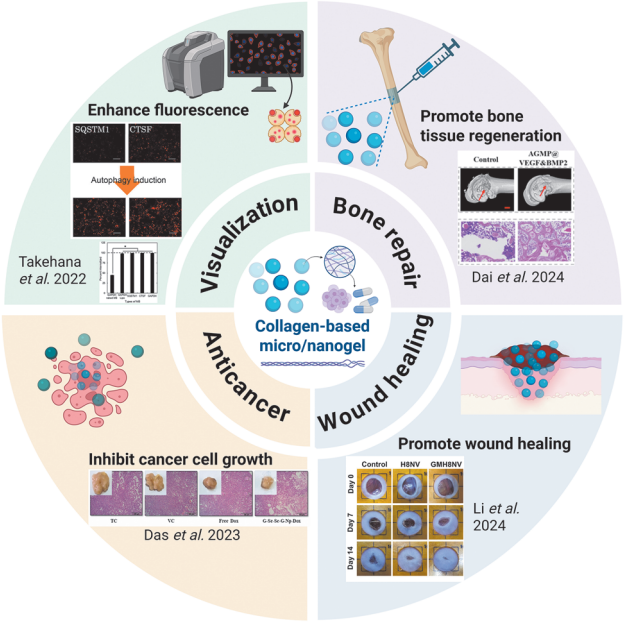

Figure 6: Applications of collagen-based micro/nanogel delivery systems, including bone repair, wound healing, anticancer treatment, and visualization. Created using *BioRender.com*.

Table 2: Comparison of the common sizes and release systems of the collagen-based micro/nanogel delivery systems used in different applications.

Applications	Size	Cross-linking and manufacturing technique	Release system	References
Bone repair				
Stem cells	Micro	Microfluidic emulsion methods	Enzyme response/cell migration	[57,58]
Cytokines	Micro	Microfluidic emulsion methods	Sustained release	[59]
Drugs	Nano	Phase separation technology/ ultraviolet photoccross-linking	Enzyme response/sustained release	[60,61]
Wound healing				
Cellular active substances	Micro	Conventional emulsion methods	Redox response	[63]
Drugs	Micro/Nano	Conventional emulsion methods	Redox response/sustained release	[64–66]
Zn^{2+}	Micro	–	Sustained release	[68]
Anticancer				
Doxorubicin	Nano	Phase separation technology	pH response/glutathione response	[72]
Doxorubicin	Nano	Phase separation technology	Target response	[75]
Visualization				
Molecular beacons	Nano	Phase separation technology	–	[77,78]
Contrast agents	Nano	γ-ray irradiation method	–	[80]

–: Not applicable.

stem cell microspheres, within which mesenchymal stem cells migrate out and proliferate after 72 h while retaining their cellular characteristics. These collagen microspheres provide novel cell delivery devices with optimal biological and functional properties that are suitable for clinical applications in bone regenerative medicine. Wu *et al*[58] used a microfluidic device to prepare methacrylated gelatin microspheres of different sizes loaded with bone marrow mesenchymal stem cells. The study revealed that freeze-dried cell-laden microspheres with a diameter of 300 μm and a pore size of 50 μm presented a high stem cell loading capacity. After 4 weeks, calcium deposition in freeze-dried cell-laden microspheres loaded with bone marrow mesenchymal stem cells reached 57%, and the expression of osteogenic differentiation-related genes such as collagen type I, osteocalcin, osterix, runt-related transcription factor 2, and osteopontin increased. An experiment in which femoral defects were treated in mice [Figure 7B] revealed that after three weeks of treatment with freeze-dried cell-laden microspheres loaded with bone marrow mesenchymal stem cells, the femoral defect site was significantly recovered, with no significant difference from the normal tissue. The bone tissue volume/total tissue volume ratio and bone mineral density were both significantly increased.

In addition to loading stem cells that promote bone regeneration, cytokines have also been proven to be deliverable through hydrogels into the body, regulating cells for bone tissue repair. Dai *et al*[59] incorporated bone morphogenetic protein 2 and vascular endothelial growth factor into Cu/alginate/gelatin methacrylate double cross-linked microspheres, which showed good biocompatibility. Vascular endothelial growth factor encapsulated in the microspheres was rapidly released, increasing the length of the vasculature by 200% and accelerating vascular regeneration, whereas bone morphogenetic protein 2 was slowly released, with alkaline phosphatase activity and the mineralization rate in bone marrow mesenchymal stem cells increasing after 14 days, promoting bone tissue regeneration [Figure 7C].

Small-molecule drugs, such as dexamethasone, can also play a role similar to that of cytokines, driving the differentiation of progenitor cells to repair bone tissue. Qi *et al*[60] investigated the osteogenic effects of dexamethasone-loaded gelatin nanoparticles, which initially release a burst of dexamethasone for anti-inflammatory purposes, followed by the sustained release of dexamethasone at a low concentration of 20 ng per day for 28 days. On the 14th day of the action of the gelatin nanoparticles on osteoblasts, the expression levels of the osteogenic-related proteins Runt-related transcription factor 2, type I collagen, and osteocalcin were significantly increased, indicating optimal effects on promoting osteogenic differentiation. In addition, gelatin microspheres can also be used as biomimetic lubricants and injected into bone joints to release drugs and alleviate inflammation, achieving the goal of treating osteoarthritis.[61] This gelatin microsphere lubricant alleviated the inflammation induced by interleukin 1β by significantly reducing the levels of the tumor necrosis factor-α and interleukin 6 proteins. In addition, animal experiments revealed that the width of the bone joint gap had returned to normal and that the joint surface had become smooth.

Wound healing

Wound healing is a lengthy and continuous process that includes four phases: hemostasis, inflammation, proliferation, and remodeling. These stages are often accompanied

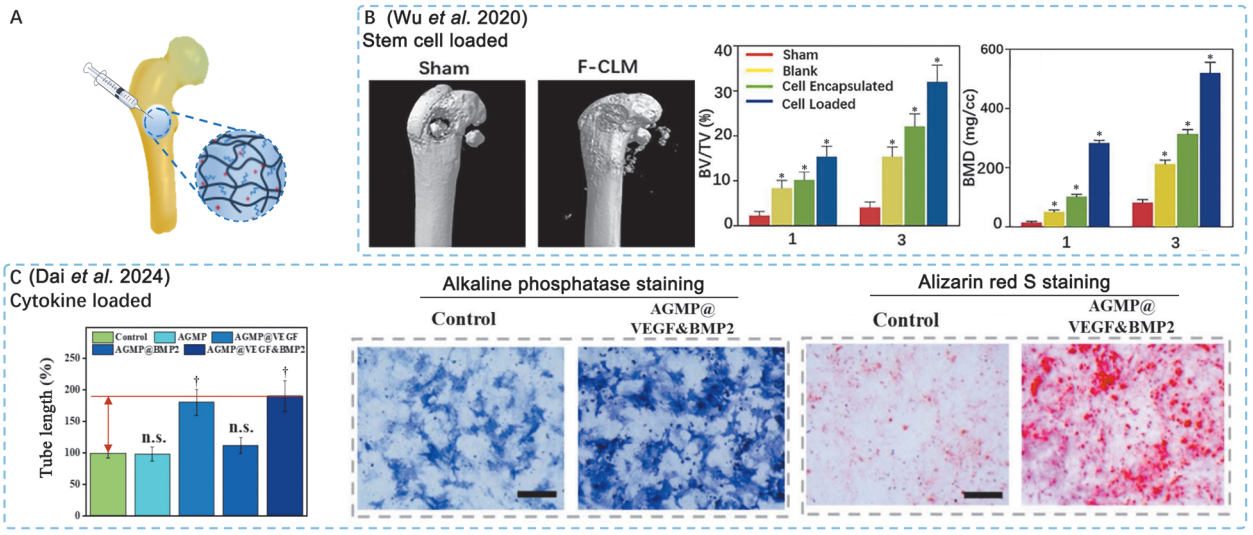

Figure 7: Representative bone repair applications of the collagen-based micro/nanogel delivery systems. (A) Schematic diagram of the *in situ* injection of a collagen-based micro/nanogel with osteogenic activity. (B) *In vivo* evaluation of the effects of injecting gelatin microspheres loaded with bone marrow mesenchymal stem cells at the site of femoral injury in mice after 3 weeks, including micro-CT images, bone tissue volume/total tissue volume, and bone mineral density. (C) *In vitro* evaluation of the effects of vascular endothelial growth factor- and bone morphogenetic protein 2-loaded gelatin microspheres on bone tissue, including the tube length and osteogenic differentiation of bone marrow mesenchymal stem cells. Sham: saline; blank: blank microspheres; F-CLM: freeze-dried cell-laden microspheres, Bars: 200 μm. $^{*}P$ <0.001, $^{\dagger}P$ <0.01, n.s.: Not significant.

by oxidative stress or bacterial infection, requiring drugs to rapidly exert antibacterial effects and then continuously released for a long time to promote tissue regeneration.[62] The delivery system of collagen-based micro/nanogels can meet this demand by fine-tuning the structure and release mechanisms. Below, we briefly introduce several designs of collagen-based micro/nanogels for wound healing applications and highlight the loaded active substances.

Cellularly active substances have been proven to accelerate wound healing by regulating oxidative stress and matrix metalloproteinases. Li *et al*[63] prepared gelatin microspheres and loaded H8 macrophage membrane-derived nanovesicles to play a role in the healing of diabetic wounds. These gelatin microspheres could stimulate the release of H8 macrophage membrane-derived nanovesicles due to the excess matrix metalloproteinase 9 in the wound area. The released H8 macrophage membrane-derived nanovesicles reduced local reactive oxygen species levels and protected cells from oxidative stress-induced injury and inflammation. Wound closure in the H8 macrophage membrane-derived nanovesicle-loaded gelatin microsphere group reached 92.08% on the 14th day, whereas the wound closure rate in the control group was only 73.44%.

Collagen-derived micro/nanogels loaded with small-molecule drugs, synthetic polymers and inorganic materials as active components also promote wound repair. Pathan *et al*[64] prepared fish scale collagen/hydroxypropyl methylcellulose nanogels loaded with curcumin to evaluate their wound healing effects. The study revealed that collagen/hydroxypropyl methylcellulose nanogels loaded with curcumin could facilitate nearly complete wound healing within a 20-day period, with a wound contraction value of 95.42±12.20%, which was greater than that of the control group and free curcumin. Yuan *et al*[65] prepared gelatin microspheres loaded with ε-polylysine by emulsification, which could kill more than 75% of *Staphylococcus. aureus* or *Escherichia coli*. On the contrary, these gelatin microspheres promoted the adhesion of fibroblasts, which highlights the potential application of gelatin microgels as wound dressings. Chen *et al*[66] fabricated a gelatin/chitosan composite microsphere loaded with MnO_2 nanosheets to improve the microenvironment of chronic wounds. This type of microsphere exerted an effective reparative effect on oxidative damage in an L929 cell model injured by H_2O_2 and $^{\cdot}O_2^-$, with cell viability increasing from 5.4% to 46.0% and from 16.4% to 67.2% after 5 days, respectively. In addition, these microspheres exhibited outstanding bacteriostatic performance against bacteria such as *S. aureus*.

As a safe material, Zn^{2+} can increase the expression of anti-inflammatory cytokines, thereby improving cell growth and differentiation and accelerating tissue repair.[67] Furthermore, zinc-containing materials have antibacterial properties, which are beneficial for wound healing. Malathi *et al*[68] prepared collagen microgels embedded with ZnO nanoparticles to repair wounds caused by scratches *in vitro*. The results revealed that collagen microgels containing ZnO had greater antibacterial activity than those containing ZnO, and the diameter of the inhibition zones for *Bacillus subtilis* and *Pseudomonas aeruginosa* increased. After the collagen microgel was applied to the injured area for a certain period, more cells migrated to the injured area, and the wound was completely repaired. The wound healing rate of the scratch wound area in the control group was only 27.35% at 72 h.

Anticancer treatment

Cancer remains a significant global disease burden. Due to the enhanced permeability and retention effect of tumor cells, coupled with a microenvironment characterized by

weak acidity and high levels of reactive oxygen species, nanodelivery systems with pH-responsive and redox-responsive properties exhibit greater therapeutic efficacy in cancer treatment.[69] Collagen and its hydrolysates, with properties such as controllable cross-linking and amphiphilic behavior, can be formulated into multiple responsive collagen-based nanogels.[70]

Collagen-based nanogels can be loaded with a variety of substances for cancer treatment. For example, doxorubicin hydrochloride is a commonly used anticancer drug. Anandhakumar *et al*[71] prepared collagen peptide–chitosan nanogels using the ionic gelation method and loaded them with doxorubicin hydrochloride for advanced cancer treatment. Compared with those in the free doxorubicin hydrochloride group, the apoptosis rate of the HeLa cells treated with the collagen peptide nanogels loaded with 0.85 μg/mL doxorubicin hydrochloride was as high as 46% after 24 h, confirming the significant anticancer characteristics of the doxorubicin hydrochloride-loaded collagen peptide nanogels. Moreover, this system also had good anticancer effects on animal models. DAS *et al*[72] used selenide-functionalized gelatin to fabricate a pH- and glutathione-responsive nanodelivery system for the release of doxorubicin hydrochloride. Compared with free doxorubicin hydrochloride, this system exhibited reduced cytotoxicity and significantly inhibited the growth of lung cancer tumors in a mouse xenograft tumor model. Moreover, these collagen nanogels have no significant toxicity or side effects on normal cells. In addition to common drugs, the controlled release of cancer-related RNA[73] has become a new idea for collagen-based nanogels in the anticancer field.

In recent years, active targeted therapies for cancer have been extensively studied. Nanoparticles modified with biomimetic cell membranes have shown great potential as a new type of drug delivery system. This method can prolong the blood circulation time, reduce reticuloendothelial system clearance, and promote drug accumulation in the tumor by preserving the surface antigens of the source cells.[74] Wang *et al*[75] prepared a doxorubicin hydrochloride-loaded cancer cell-mimicking nanoplatform. When this platform was intravenously injected into a 4T1 xenograft mouse tumor model, the fluorescence intensity in the tumor was approximately 1.47 times higher than that of the tumors injected with the pure nanogel after 12 h, significantly enhancing the targeting performance. After 14 days, the platform displayed a stronger tumor-suppressive effect than free doxorubicin hydrochloride did, with a tumor growth inhibition rate of up to 70.36%. This approach greatly enhances the targeting ability of the delivery system.

Visualization

Visualization through imaging can be used to assess cell-based tissue regeneration outcomes, thereby observing the treatment of tissue defects, and has broad applications in the field of regenerative medicine.[76] On the contrary, visualization through imaging can also be used for the detection and examination of pathological tissues. As delivery carriers, nanometer-sized gelatin hydrogels can deliver fluorescent agents, contrast agents, etc., to the treatment site and can be visualized in real-time.

Molecular beacons are imaging probes that can convert gene expression into fluorescent signals, thereby visualizing the expression of target genes in living cells. However, negative electrostatic repulsion has been observed between molecular beacons and the cell membrane. Therefore, gelatin nanogels with cations, which are helpful for molecular beacon internalization, have become one of the best choices for molecular beacon probe carriers. Takehana *et al*[77] prepared cationic gelatin nanoparticles with positive charges by introducing spermine and incorporating molecular beacons into them to visualize autophagy activity in cancer cells. Compared with molecular beacons without cationic gelatin nanoparticle encapsulation, cationic gelatin nanoparticles/molecular beacons can be internalized into cells with an efficiency of 100%, whereas the internalization efficiency of naked molecular beacons is only 44.7%. In addition, this cationic gelatin nanoparticle/molecular beacon system revealed an increase in fluorescence intensity with the induction of autophagy, providing a new method for visualizing autophagy in cancer cells over time. Murata *et al*[78] incorporated a cell proliferation target, the Ki67 molecular beacon, into cationic gelatin nanoparticles to study the proliferative capacity of cells and to increase the internalization of molecular beacons into cells. The experiment revealed that after a coincubation with cells for 24 h, the fluorescence intensity of cationic gelatin nanoparticles/Ki67 molecular beacons with growth factors was much higher than that of the group without growth factors, indicating that the cationic gelatin nanoparticle/molecular beacon system has the potential for the chronological visualization of the cellular proliferative capacity, providing a means for the visual monitoring of the tissue repair process. Overall, the cationic gelatin nanoparticle/molecular beacon system has made significant contributions to the application of drugs and postoperative recovery. In the development of antitumor drugs, the cationic gelatin nanoparticle/molecular beacon system can be used to monitor the proliferation of tumor cells and surrounding normal cells, which can be used to determine drug efficacy and side effects. In the field of tissue engineering, the ability of cells to proliferate on scaffolds can be visualized through cationic gelatin nanoparticles/molecular beacons without damaging the cells or scaffolds. In addition, growth factors can be added to promote cell proliferation.

Contrast agents have been widely used in clinical screens for pathological sites, but the toxic substance gadolinium tends to deposit in the brain and exerts toxic effects, thereby posing physiological risks.[79] However, 1–20 nm nanoparticles can be quickly excreted by the body without entering the brain through the blood–cerebrospinal fluid barrier or the leaky blood–brain barrier. Therefore, safe, nontoxic, and biodegradable gelatin nanogels have become an ideal matrix for contrast agents. Kimura *et al*[80] chelated gadolinium from contrast agents into radiation-crosslinked gelatin nanogels, resulting in Gd/gelatin nanogels with an average particle size of 6 nm. The Gd/gelatin nanogels demonstrated rapid renal excretion

in less than 1 hour, indicating a low risk of the Gd/gelatin nanogels releasing Gd ions and their deposition in the brain.

Challenges and Perspectives

Collagen-based micro/nanogels are characterized by their high water content and tunable chemical and physical properties. In particular, their size can be precisely manipulated from the submicron level to several tens of nanometers, which endows them with a substantial surface area. These hydrogels possess an intricate internal network that facilitates drug loading. They are also rich in reactive functional groups, enabling reactions with various cross-linking agents and allowing for conjugation with other materials and cells. The high biocompatibility and biodegradability of collagen-based materials ensure favorable biosafety profiles, which are crucial for their application. In this review of collagen-based micro/nanogel drug delivery systems, we delved into their fabrication techniques and drug release mechanisms and highlighted their biomedical applications in tissue engineering and cancer therapy.

Despite notable advancements in this field, some challenges remain to be overcome (highlighted in Figure 8, blue section). First, some confusion persists about the concept of collagen and its hydrolysates, such as gelatin. This confusion is reflected in the descriptions of the materials, which often mistakenly refer to collagen hydrolysates as collagen. Furthermore, collagen that is subjected to high temperatures or acid–base conditions during the preparation of micro/nanogels is highly susceptible to hydrolysis. As a result, the final product may have compromised structures, which should be considered in the material design and interpretation of the results. Second, the source of collagen and potential immunogenicity could be concerns, which are not well recognized. Although collagen has inherent biocompatibility due to its widespread presence in the human body, the original animal source of collagen may affect immune responses.[81] Collagen-based materials must be optimized by removing telopeptide regions and using cross-linking strategies to ensure the stability of the materials within the body and reduce immune responses.[82,83] Furthermore, owing to their size and surface charge, these micro/nanogels are often recognized by the body's immune system as foreign entities, leading to a high clearance rate by the reticuloendothelial

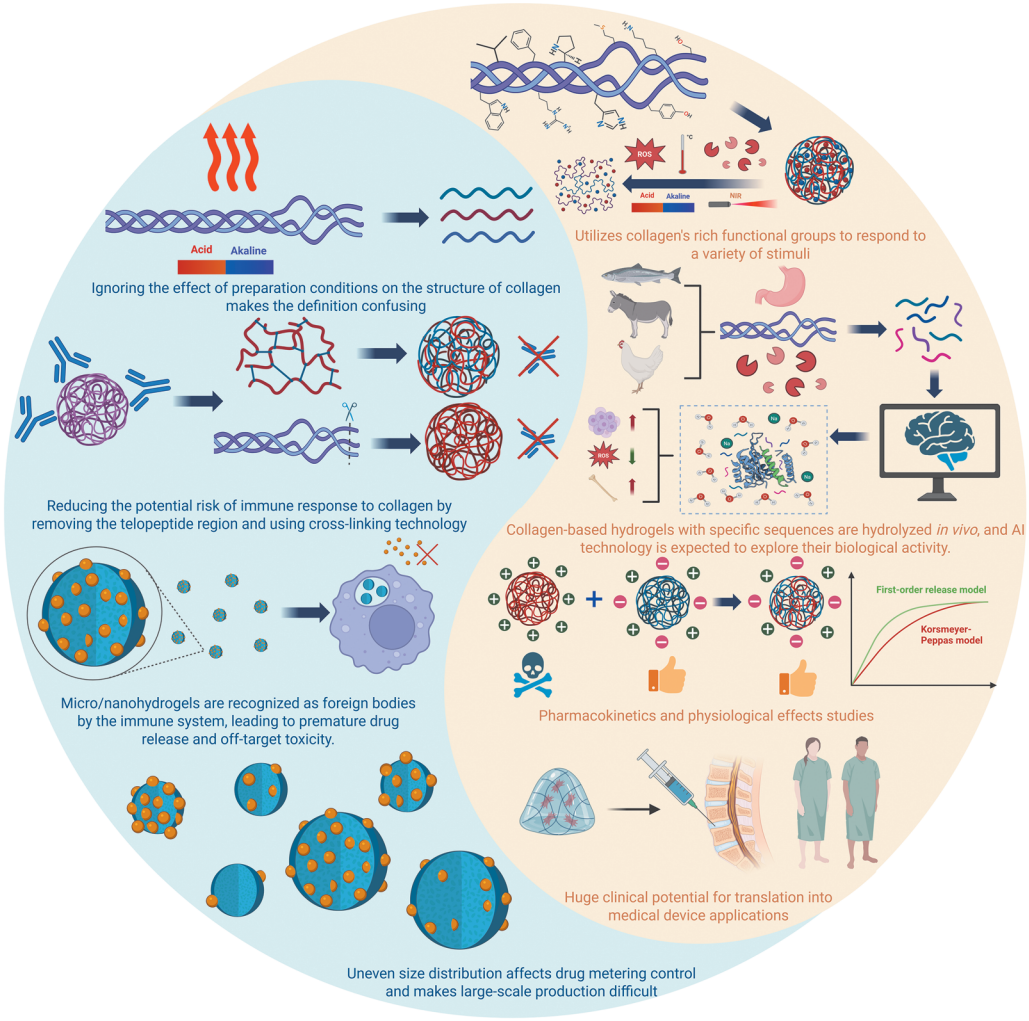

Figure 8: Challenges and perspectives of collagen-based micro/nanogel delivery systems. Created using *BioRender.com*. AI: Artificial intelligence; NIR: Near-infrared.

system, immune cells, and kidneys. Immune clearance and premature drug release are common issues associated with micro/nanoscale entities, and improved fabrication processes are needed to ensure the stability of hydrogels under various physiological conditions and to precisely control the targeting and release of drugs. Finally, achieving the large-scale production of collagen-based micro/nanogels while reducing costs is essential for advancing their clinical application. In addition, few studies have investigated the structural characteristics of hydrogels after drug release, which complicates their release profiles and further impacts their biodistribution and cellular interactions. Addressing these challenges requires a deeper understanding of the biological properties of the materials, as well as innovative production techniques to meet the demands of clinical applications.

The future research and application prospects for collagen-based micro/nanogel drug delivery systems are vast [Figure 8, yellow section]. Collagen-based materials possess many functional groups, endowing them with the potential to form composites with a diverse array of materials. This property enables the development of hydrogels that integrate a multitude of functions and are responsive to various stimuli. These controllable systems are anticipated to provide real-time monitoring of disease progression and to dynamically adjust drug release, thereby optimizing therapeutic outcomes. Second, the combination of artificial intelligence with collagen-based materials provides unprecedented opportunities to unlock the potential of these materials. For example, artificial intelligence can analyze the structural differences in collagen derived from various sources (such as donkey skin gelatin), which exhibit distinct properties. By leveraging artificial intelligence, we can optimize micro/nanogel delivery systems crafted from specially sequenced collagen-based materials. These systems, after discharging their drug cargo, are designed to be safely degraded by the human body into bioactive small-molecule collagen peptides. This degradation process may contribute to disease treatment by providing bioactive peptides. Furthermore, artificial intelligence can be instrumental in elucidating the untapped functionalities of these collagen hydrolysates, thereby increasing their effectiveness and applicability in medical therapies. Furthermore, collagen-based micron-scale hydrogels are already extensively used in medical devices as embolic agents and fillers.[84] Given their relatively straightforward application compared with pharmaceuticals, the clinical potential for the translation of collagen-based microparticles and nanogels in medical device applications is considerable. However, further research is necessary to ensure their safety and potential in various applications, including pharmacokinetics and assessments of their physiological effects. Through these efforts, we envision that collagen-based micro/nanogel drug delivery systems will play an increasingly vital role in the future of health care.

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Conflicts of interest

None.

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