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REVIEW

# Preparation Methods of Hydrogel Microspheres and Recent Advances in Their Application for Treating Diabetic Wounds

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**Abstract:** Diabetic wounds are characterized by complex pathologies, such as vascular changes, nerve damage, and immune dysfunction, which make healing difficult. Hydrogel microspheres have shown great potential in the field of wound treatment due to their excellent biocompatibility, high water content, and soft physical properties. The review summarizes the preparation methods of hydrogel microspheres in detail, including microfluidic technology, spray method, electro spraying, emulsion method, phase separation, photomask method, and 3D printing technology. These methods have significant advantages in particle size control, morphological consistency, and functionalization. It then reviews the applications of hydrogel microspheres in the treatment of diabetic wounds, including the promotion of cell proliferation, migration, angiogenesis, and macrophage polarization, as well as precise treatment through controllable drug release and environmental responsiveness. In addition, the review explores the unique advantages of hydrogel microspheres in dealing with diabetic wounds complicated by infection and reviews the current challenges faced by these technologies in practical applications, such as preparation complexity, cost issues, and prospects for translation. Finally, the review looks forward to the future enhancement of hydrogel microsphere performance and application breadth through interdisciplinary research and new material development, providing more comprehensive and efficient solutions for the treatment of diabetic wounds.
**Keywords:** Hydrogel, microsphere, diabetic wounds, manufacturing

## Introduction

Wound healing is a dynamic and complex physiological process, mainly comprising four overlapping stages: hemostasis, inflammation, proliferation, and remodeling.[1] When any stage of wound healing encounters adverse factors that slow down or even halt the overall healing process, chronic wounds may develop.[2–4] Diabetes is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion or impaired biological function.[5] With the intensification of population aging, the incidence of diabetes is increasing annually both domestically and internationally.[6,7] The long-term hyperglycemic state can cause specific damage to the blood vessels, nerves, and immune system of diabetic patients.[8,9] Under such conditions, diabetic chronic wounds, represented by diabetic foot ulcers, are highly susceptible to occurrence. Once a wound forms, it is prone to enter a chronic inflammatory state.[2] Compared with ordinary wounds, the microenvironment of diabetic wounds is more complex, with a higher risk of infection, longer healing time, and a tendency to become chronic and refractory wounds. Even if treatment achieves certain effects, there is often a high recurrence rate.[5,10]

Appropriate and efficient therapeutic strategies to promote the healing of diabetic wounds have become a significant challenge faced by global healthcare systems. Current treatment methods are diverse but still face numerous challenges. In addition, biologics and tissue engineering technologies, such as growth factors, skin substitutes, and stem cell therapies, have also shown promise in diabetic wound repair.[11,12] However, their complex preparation processes, high

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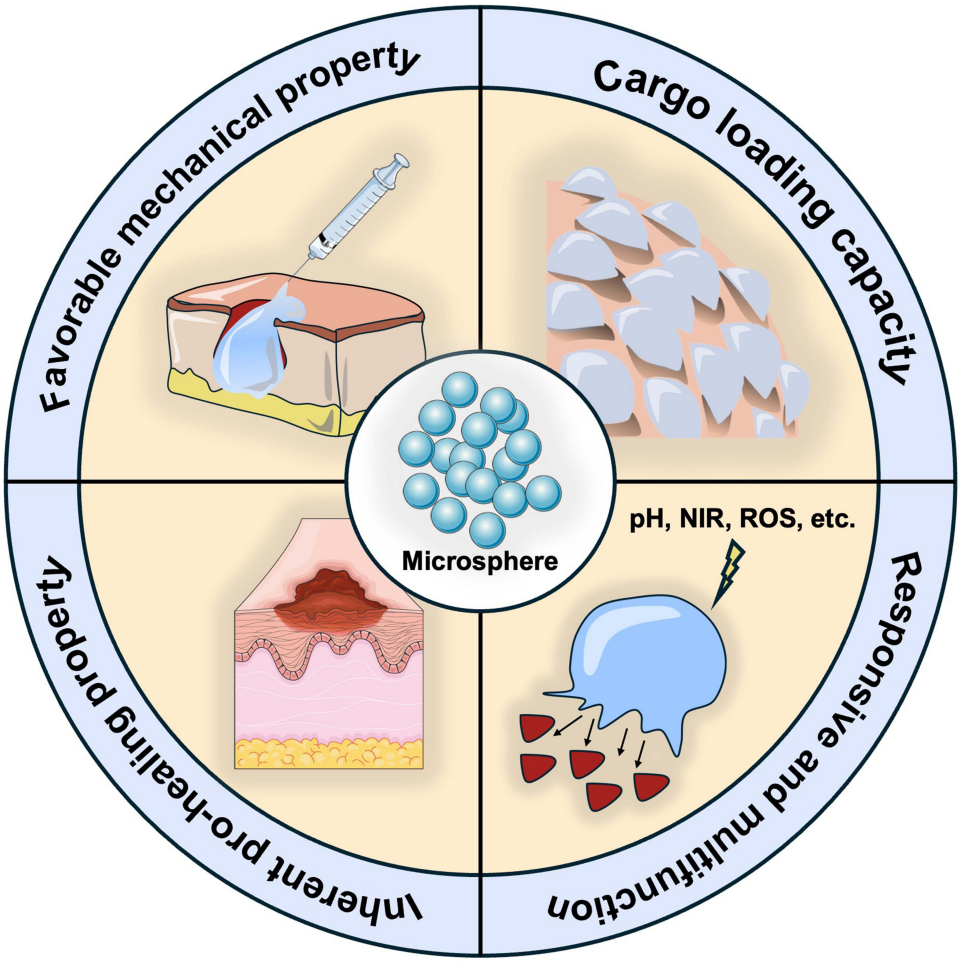
**Graphical Abstract**




costs, and potential immune rejection issues still need to be further addressed.[13] Debridement combined with gauze and medication is one of the most widely used basic methods for treating diabetic wounds and other chronic wounds in clinical practice. This treatment method is favored in primary healthcare institutions and resource-limited areas due to its simplicity, low cost, and broad applicability.[14] However, it also has obvious limitations. Gauze, mainly made of cotton fibers, has certain liquid absorption and ventilation properties, but its adsorption capacity is limited and cannot maintain a stable moist environment, which is an important condition for wound healing.[15,16] In terms of drug use, the poor adsorption and release properties of gauze result in a short retention time of drugs at the wound site and difficulty in maintaining concentration, thereby reducing therapeutic efficacy.[16–18] Many drugs cannot effectively penetrate deep tissues or the wound base during use, leading to drug waste and increased treatment costs.[17,19] More importantly, this non-controlled release method can cause fluctuations in drug concentration, making it difficult to achieve sustained antibacterial or pro-healing effects.[18] Therefore, exploring new materials and intelligent drug delivery technologies targeting these issues is an important direction for future development.

Hydrogels, as three-dimensional cross-linked polymer network materials, have garnered widespread attention in the field of wound treatment due to their excellent biocompatibility, high water content, and soft physical properties.[20,21] Their high-water content can provide a moist environment for wounds, promote cell migration and the growth of new tissue, and effectively isolate external contamination to reduce the risk of infection.[16] Moreover, the porous structure of

hydrogels can carry drugs or bioactive factors to achieve controlled release, thereby improving the efficacy and stability of drugs.[22] Compared with ordinary hydrogels, hydrogel microspheres exhibit significant differences in morphology and function.[23] The micrometer-scale size of hydrogel microspheres gives them a higher specific surface area, enabling them to carry more drugs.[24] Meanwhile, due to their microsphere characteristics, they are convenient for filling irregular or deep wounds.[16] Their independent particulate structure endows them with more flexible application methods, such as precise delivery through injections.[24] Due to the fluidity between particles, microspheres can more evenly cover the wound area, avoiding the fitting insufficiency of traditional hydrogel dressings on complex wounds.[25] Hydrogel microspheres can be customized in terms of their internal and surface structures and chemical properties.[26] They can achieve multi-layered, controlled drug release, dynamically respond to wound environments (such as pH, temperature, and enzyme activity) to ensure sustained and precise drug efficacy,[27] and offer multiple therapeutic functions including pro-angiogenesis, antibacterial activity, pro-healing, and anti-inflammation.[28,29] Therefore, hydrogel microspheres, with their innovative structural and functional advantages, offer a revolutionary new choice in the field of wound treatment.

With the scientific progress of hydrogel microspheres, an increasing number of novel preparation methods for laboratory hydrogel microspheres have come into the view of researchers. We searched for articles on the preparation of microsphere hydrogels and their applications in the treatment of chronic diabetic wounds over the past five years using databases such as PubMed and Google Scholar. This review provides a detailed summary of the preparation methods of hydrogel microspheres, covering the selection of different preparation methods to meet the needs of drug loading and precise delivery, and it combs through the therapeutic application progress of hydrogel microspheres in the treatment of diabetic wounds based on existing literature. Finally, we also discuss the current challenges faced by hydrogel microsphere technology in practical applications, such as complexity of preparation, cost issues, and prospects for applications.

# Preparation Methods of Hydrogel Microspheres

## Microfluidics and Microfluidic Chips

In recent years, microfluidics has gained significant attention and has been extensively developed for the fabrication of hydrogel microspheres. Microfluidics is an advanced processing method based on the control of fluid flow at the microscale and is widely applied in material science and biomedical fields.[30] It offers distinct advantages in the preparation of hydrogel microspheres. Microfluidic systems utilize precisely designed microchannel networks to control the flow of liquids within micrometer-sized channels, enabling the precise generation and manipulation of droplets.[31,32] One important characteristic of microfluidic systems is the unique fluid properties in the microscale environment, such as laminar flow and droplet formation.[33] By leveraging these unique fluid phenomena, microfluidics can perform a series of microfabrication and micromanipulation tasks that are difficult to achieve using traditional methods.[33] In the preparation of hydrogel microspheres, the hydrogel precursor solution and crosslinking agent are typically introduced separately into a microfluidic chip, where stable droplet structures are formed through shear forces or interfacial tension. Subsequently, these droplets undergo crosslinking reactions within the microfluidic device, solidifying into uniform hydrogel microspheres.[34] A core advantage of microfluidics is its exceptional ability to control particle size. By adjusting flow rates, channel dimensions, and the viscosity ratio of interfacial fluids, the size of droplets can be precisely regulated, resulting in hydrogel microspheres with uniform size and narrow size distribution.[35] Compared to traditional emulsification methods, microfluidics offers significant advantages in terms of size and morphological consistency of the microspheres. Additionally, microfluidics allows for flexible control over the composition and functionality of materials. For example, by introducing multiphase fluid systems, hydrogel microspheres with complex structures, such as core-shell,[36] hollow microspheres,[37] or multilayer functionalized microspheres,[38] can be fabricated. This structural precision provides greater flexibility and functionality for drug delivery and tissue engineering.[39] Another notable feature of microfluidics is its high efficiency and low waste during the preparation process.[40] Due to its minimal liquid consumption and controlled operating environment, microfluidics reduces material waste and provide gentle handling of sensitive active components (such as biomolecules or cells), preventing their deactivation during the fabrication process.[41] Moreover, microfluidics enables one-step continuous operation, reducing complex post-processing steps and further enhancing preparation

efficiency. Compared to traditional techniques, microfluidics offers high-throughput production lines and reproducibility between batches based on high flow rates.

Capillary microfluidic devices are the simplest and most common type of microfluidic apparatus, primarily consisting of an injection tube, a transition tube, and a collector.[42] These devices manipulate liquids through capillary action. When two immiscible liquids (eg, oil and water) flow through the microfluidic channel, one phase forms a highly uniform discontinuous flow under the influence of liquid/liquid interfacial tension and shear forces, which serves as the prototype for hydrogel microspheres.[43] Subsequently, the hydrogel network is crosslinked through UV irradiation, heating, cooling, or other activation methods to obtain the final hydrogel microspheres (Figure 1). However, the simple structure of capillary microfluidic devices limits their ability to fabricate more complex hydrogel microspheres.[44] Therefore, new microfluidic systems are being actively developed to produce hydrogel microspheres with richer functionalities, higher biocompatibility, and greater yield.

Microfluidic chips are an important development in microfluidics, integrating microfluidic systems into a chip platform based on microscale processing techniques to achieve precise liquid manipulation and reaction operations.[45] Microfluidic chips represent a more advanced and efficient microfluidic technology compared to capillary microfluidic devices.[46] The main materials used for microfluidic chips include silicon wafers,[47] glass,[48] polydimethylsiloxane (PDMS),[49] polymethylmethacrylate (PMMA),[50] polytetrafluoroethylene (PTFE),[50] and paper-based substrates,[51] which are manufactured through microfabrication techniques such as photolithography, etching, or soft lithography.[52,53] Among these, PDMS is the most widely used due to its ease of processing, optical transparency, elasticity, and low cost.[54] The shift in production materials has enhanced its applicability in both research and industrial settings. The chip's internal microchannel network is precisely designed to control droplet generation and manipulation, with common microchannel structures including T-shaped and psi-shaped designs.[55] Compared to traditional microfluidic devices, microfluidic chips are smaller in size, more integrated in structure, and offer the advantages of high throughput and high precision. By adjusting chip design parameters, such as channel width, branching ratio, and injection pressure, hydrogel microspheres with uniform size and stable morphology can be fabricated.[56] Moreover, microfluidic chips support multiphase flow operations, allowing the simultaneous processing of multiple liquids within a single device to achieve complex structural designs. This further expands the functionality and application scope of hydrogel microspheres. Another significant advantage of microfluidic chips is their scalability and ease of customization. By integrating multiple microreactor units, the chips can achieve high-throughput fabrication while saving material and time costs.[57]

However, there are still many challenges to be addressed in the future development of microfluidic chips. For example, microchannels are prone to clogging, which may reduce the lifespan of the device.[58] The yield of hydrogel microspheres produced by microfluidics is lower than that of traditional emulsion stirring methods.[59] These factors limit

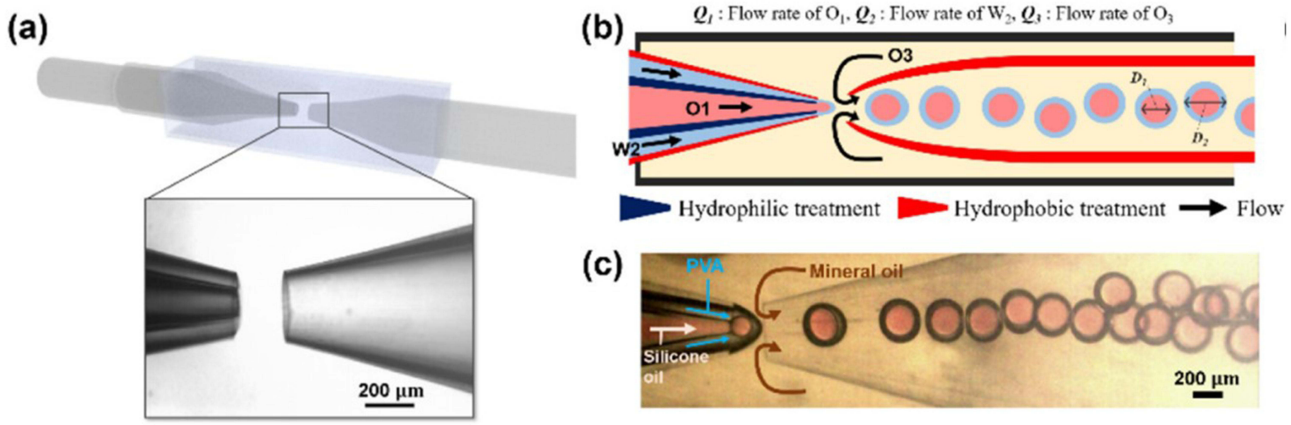



**Figure 1** Schematic and optical microscope (OM) image of a glass capillary microfluidic device with double concentric channels. (**a** and **b**) Schematic illustration and still-shot OM image (**c**) of the microfluidic device for the generation of O1/W2/O3 double-emulsion droplets in a dripping mode at the tip-to-tip junction. Reproduced with permission from Oh Y, Kim SH. Concentric capillary microfluidic devices designed for robust production of multiple-emulsion droplets. *Langmuir.* 2024;40(36):19166–19175. Copyright © 2024 American Chemical Society.[44]

the industrialization of microfluidic hydrogel microspheres. Additionally, microfluidic chips rely on droplet generation and manipulation within microchannels, and their processing capacity is limited by the number of channels and fluid flow rate in the chip.[56] Although throughput can be increased by parallelizing the design to add more channels, this significantly increases the complexity and manufacturing difficulty of the chip and may lead to uneven droplet generation and system instability. Furthermore, microfluidic systems have high requirements for operating environments and equipment. Large-scale industrial production typically demands devices with high durability and the ability to adapt to complex production environments. However, microfluidic chips are relatively fragile, with poor tolerance to pressure, temperature, and chemical corrosion, making them prone to damage and difficult to maintain.[60] Moreover, microfluidic devices usually require precision pump systems and pressure control equipment. These ancillary devices not only increase the overall cost but also raise the complexity of system operation. Another important limitation is the complexity of technology itself. Microfluidics demands high standards in design and operation, involving multiple fields such as fluid mechanics, materials science, and bioengineering.[58] In practical industrial applications, the technical barrier is high, and the training of specialized personnel and system maintenance further increases the difficulty of application. Finally, standardization issues also pose a challenge to the industrialization of microfluidic technology. Since the design of microfluidic chips and devices is often customized for specific applications, there is a lack of unified standards, making it difficult to achieve cross-industry compatibility and modular production. Although this customization suits laboratory needs, it may lead to high costs and low efficiency in industrial production. As a promising technology for the future, microfluidic fabrication of hydrogel microspheres still faces many challenges in terms of technology transfer.

## Spray Method

The spray method is the simplest technique for preparing hydrogel microspheres in industrial production.[59] Its principle involves dispersing a prepared solution or emulsion into extremely small droplets through a sprayer, separating the powders from the water mist, and then collecting the powders to obtain the microsphere agents.[59] The spray method is a convenient and efficient technique for preparing hydrogel microspheres in industrial production, offering several advantages.[61] First, the method is operationally simple, as the process of dispersing the solution or emulsion into droplets through a sprayer can rapidly generate microspheres, making it suitable for large-scale production and meeting industrial demands.[61] Second, the spray method allows precise control over the size and morphology of hydrogel microspheres.[61] By adjusting spray parameters (such as nozzle diameter, pressure, temperature) and liquid composition (such as material concentration and viscosity), uniform microsphere particles can be produced, thereby enhancing product consistency and quality.[62] Additionally, hydrogel microspheres prepared by the spray method exhibit good drug-loading capacity. By controlling the composition of the emulsion and crosslinking conditions (such as crosslinker concentration and cross-linking time), efficient drug encapsulation can be achieved while avoiding drug degradation or loss of activity.[63] Another significant advantage of the spray method is its environmental friendliness.[64] Compared to traditional preparation methods, the spray method typically does not require large amounts of organic solvents, thereby reducing the risk of environmental pollution.[64] Moreover, due to its rapid and non-contact nature, the spray method can minimize contamination and cross-infection during the preparation process, making it particularly suitable for encapsulating sensitive drugs or bioactive molecules. Because of its relative ease of operation, the spray method is well-suited for industrial processes.

Overall, the spray method, with its high efficiency, flexibility, environmental friendliness, and scalability, has become an important technological choice for the preparation of hydrogel microspheres, especially in the biomedical field, where it shows broad application prospects. In the future, by combining with other advanced technologies such as 3D printing and microfluidics, the spray method is expected to further enhance its preparation efficiency and product performance.

## Electrospray Method

Electrospray is a method for preparing polymer microspheres or fibers by forming charged jets of polymer solutions or melts in a high-voltage electrostatic field.[65] It is widely used not only as an ionization technique for biomolecule mass spectrometry in analytical chemistry but also for fabricating hydrogel microspheres through electrostatic forces and ionic crosslinkers.[66] The principle of preparing hydrogel microspheres involves spraying a pre-mixed solution through the

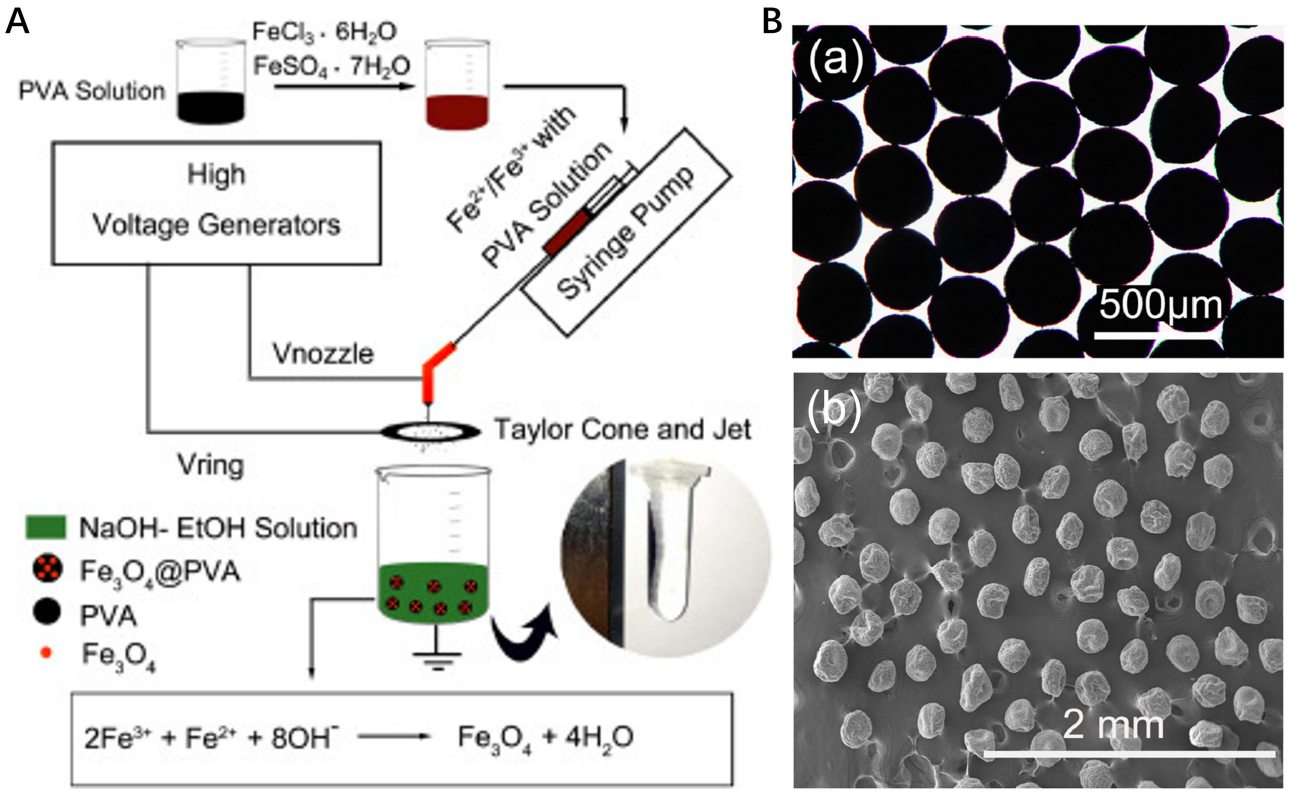



**Figure 2** Schematic illustration of one-step electrospray process to prepare the Fe3O4@PVA microspheres (**A**) and Optical and SEM micrographs of Fe3O4@PVA microspheres (**B**). Reproduced with permission from *Acta Biomater.* Volume 131. Li J, Wang J, Li J, et al. Fabrication of Fe(3)O(4)@PVA microspheres by one-step electrospray for magnetic resonance imaging during transcatheter arterial embolization. 532–543. Copyright 2021, with permission from Elsevier.[69]

nozzle of a syringe pump and applying a high voltage at the nozzle exit. In this method, the nozzle of the syringe pump is connected to the positive electrode, and the collection device is connected to the negative electrode.[67] Under the combined action of gravity, electric field force, and surface tension, droplets flowing out of the nozzle gradually form a Taylor cone and are atomized into fine charged droplets.[68] Subsequently, the droplets fall onto a solid collection device (metal collection plate) or a conductive liquid collection device and immediately react with other substances to form nanoparticles as shown in Figure 2.[23,69] Currently, electrospray has become an effective method for preparing hydrogel microspheres with uniform size and high drug loading, although further optimization of influencing factors is still needed to obtain microspheres with more precise size, structure, and mechanical strength.[70] For example, optimizing the applied electric field strength, electrode spacing and shape, material properties such as surface tension, electrical conductivity, and colloidal viscosity, as well as the flow rate of the syringe pump.[71] To meet the demands of practical applications, response surface methodology (RSM) is commonly used to optimize the above experimental parameters and adjust the mixing ratio.[72] RSM can establish and analyze models of relationships between multiple input variables and response variables, thereby guiding subsequent design and optimization through limited experiments and significantly reducing cost and time investment. For example, increasing the electric field strength and the surface tension of the mixture can reduce the size of hydrogel microspheres, while increasing the flow rate of the syringe pump and the viscosity of the mixture can decrease the diameter of the microspheres.[73] These findings provide theoretical basis and practical guidance for further precise control of material properties, which helps advance the optimized preparation of hydrogel microspheres in complex application scenarios.

Electrospray and spray methods have distinct differences in the preparation of hydrogel microspheres. Electrospray uses the electric field force generated by high voltage to atomize charged droplets into finer droplets, ultimately forming microspheres or nanoparticles. This method is suitable for preparing microspheres at the nanoscale and is more commonly used in applications where the activity of biomolecules needs to be maintained.[23] In contrast, the spray

method relies on physical means, such as pressure differences or ultrasonic vibrations, to disperse solutions or emulsions into fine droplets, which are then formed into microspheres through drying or other solidification methods. The spray method is suitable for industrial-scale production and rapid, large-scale preparation.[74] Electrospray can precisely control the size and distribution of microspheres by adjusting parameters such as voltage and flow rate, while the size control in the spray method depends on the design and operating conditions of the sprayer. Microspheres prepared by electrospray may possess specific surface charge characteristics, whereas the surface properties of microspheres prepared by the spray method mainly depend on the raw materials and solidification process. Additionally, electrospray does not produce additional chemical waste during operation, making it more environmentally friendly, while the spray method may involve the use and recovery of solvents, with environmental impact depending on specific operating conditions and post-treatment. Therefore, the choice between electrospray and the spray method mainly depends on specific application requirements, cost-effectiveness, and production scale. However, electrospray microspheres also have some disadvantages, including uncontrollable morphology, rapid drug release from single-polymer drug-loaded microspheres, and complex preparation processes for composite microspheres.[75]

Compared to microfluidic methods, electrospray technology is simpler and more efficient in operation, without the need for surfactants, thereby avoiding complex cleaning steps during preparation. Compared to double emulsion methods, electrospray can efficiently prepare uniform hydrogel microspheres with sizes ranging from a few micrometers to several thousand micrometers by adjusting system parameters. It also offers higher drug encapsulation efficiency and better biocompatibility.[69] This characteristic provides significant advantages in protecting biomacromolecules whose structures are susceptible to dissociation and ionization processes. Additionally, electrospray can generate multiply charged ions under specific voltage conditions, making it a powerful tool for strictly controlling the deposition of each component and inducing the formation of hydrogel microsphere structures. These structures can further be applied in the production of biomedical composite materials, including homogeneous membranes, arrays, three-dimensional microstructures, packaging systems, and nanofibers.[71]

## Emulsion Method

The emulsion method is one of the most used techniques for synthesizing hydrogel microspheres, allowing the formation of stable emulsions containing dispersed hydrogel microspheres. The emulsion system typically consists of four components: monomers, aqueous dispersing medium, water-soluble initiators, and emulsifiers. Specifically, the emulsion method can be broadly categorized into three types: emulsion crosslinking, emulsion polymerization, and inverse emulsion polymerization.[23] The principle of the emulsion crosslinking method is to disperse the hydrogel precursor solution in the continuous phase to form stable droplets using emulsifiers and mechanical stirring. Subsequently, crosslinking agents are added, or external conditions (such as temperature or pH changes) are used to induce crosslinking reactions within the droplets, resulting in the formation of hydrogel microspheres. After crosslinking, the microspheres are separated and purified through centrifugation, filtration, or washing.[76] Emulsion polymerization is a method that uses the emulsion system to induce monomers to prepare hydrogel microspheres. Common systems include oil-in-water (O/W) or water-in-oil (W/O) emulsions, where monomers, initiators, and functional components are dispersed in the dispersed phase. Polymerization reactions are promoted by thermal, photochemical, or chemical initiation, forming solid hydrogel microspheres.[77] Inverse emulsion polymerization is a special type of emulsion polymerization that mainly uses a water-in-oil (W/O) emulsion system. Water-soluble monomers and functional components are dispersed in the aqueous phase, forming a stable inverse emulsion through the oil phase. Subsequently, polymerization reactions are induced by initiators or external energy (such as light or heat) within the emulsion system, solidifying to form hydrogel microspheres.[78] Each of these three methods has its own advantages and disadvantages. The emulsion crosslinking method is simple to operate, with low requirements for monomer materials, and is suitable for the preparation of various hydrogel materials.[79] The emulsification process allows for the control of microsphere size by adjusting the concentration of emulsifiers and stirring conditions, resulting in highly uniform microspheres. However, the crosslinking process is sensitive to reaction conditions (such as temperature or crosslinking agent concentration), which can easily affect the performance of the microspheres.[80] Additionally, the use of emulsifiers may introduce impurities, requiring additional purification steps, especially in biomedical applications, which can increase costs and complexity. Emulsion

polymerization is suitable for large-scale production, yielding microspheres with high stability and functionality.[81] By optimizing emulsifiers and emulsion stability, precise control of microsphere size can be achieved, and complex structures such as core-shell or hollow microspheres can be prepared. However, the emulsification process may result in non-uniform size distribution, especially in high-viscosity systems, making the operation more challenging. The removal of emulsifiers and residual monomers increases purification costs, and certain active substances may degrade or become inactive during the polymerization process. Inverse emulsion polymerization can produce hydrogel microspheres with extremely small particle sizes (typically at the micrometer or nanometer level), with high dispersibility and stability, making it suitable for high-precision applications such as drug delivery or biosensing.[81] Due to the inverse nature of the emulsion, it can more efficiently load water-soluble active substances, and particle size can be controlled by adjusting the ratio of oil to water phases. However, the system is highly dependent on emulsifiers and the oil phase, and the preparation process is more complex. The selection and dosage of emulsifiers needs to be precisely controlled, which may result in residual emulsifier in the product, affecting biocompatibility. Moreover, inverse emulsion polymerization typically requires strict reaction conditions and sophisticated equipment, increasing the complexity and cost of operation.

In summary, all three methods utilize emulsion systems but focus on different aspects: the emulsion crosslinking method focuses on driving crosslinking reactions, emulsion polymerization centers on monomer polymerization, and inverse emulsion polymerization emphasizes the control of particle size and functionality through the inverse system. The emulsion crosslinking method is relatively simple to operate, emulsion polymerization is suitable for large-scale production, and inverse emulsion polymerization is more suitable for preparing high-precision microspheres. The choice of a specific method depends on the target application requirements, microsphere size, and functional characteristics. Compared to other methods, the emulsion method can easily control microsphere size by adjusting emulsification conditions (such as stirring speed, emulsifier concentration, and the ratio of dispersed phase to continuous phase). Compared to high-precision methods such as microfluidics, the emulsion method has relatively lower equipment and operational costs, making it particularly suitable for large-scale production and more cost-effective. However, its disadvantages are also relatively apparent. Compared to microfluidic or spray drying methods, the uniformity of particle size distribution is poorer, and it is prone to polydisperse systems, limiting its use in high-precision applications. Emulsifiers play a key role in the emulsification process, but their residues may introduce impurities, especially in biomedical applications, which can affect the biocompatibility and safety of the product. Additionally, the removal of emulsifiers increases the complexity and cost of subsequent purification.

## Phase Separation

Phase separation refers to the process of creating a new condensed phase by adding another material or using other means to reduce the solubility of complementary substances in a mixture of drugs and excipients. Simply put, its basic principle is to place the hydrogel precursor solution under specific environmental conditions (such as temperature, solvent composition, or addition of non-solvents) to induce phase separation of the solutes in the solution, thereby forming microsphere-like gel structures in the dispersed phase. Subsequently, these microspheres are stabilized by crosslinking to obtain stable hydrogel particles. The key to this method lies in using external stimuli (such as temperature, solvent, non-solvent, chemical reaction) to trigger phase separation. The main methods currently used to achieve phase separation include single coacervation, complex coacervation, solvent-nonsolvent method, and temperature variation method. The core mechanism of these methods is consistent. Whether through chemical reactions (single coacervation, complex coacervation) or physical changes (solvent-nonsolvent, temperature variation), the essence is to induce materials to separate from a homogeneous system into a dispersed phase. They all rely on the differences in interactions between materials and solvents, non-solvents, or other materials to trigger phase separation. Moreover, subsequent steps such as crosslinking, cooling, or curing are required to stabilize the particles generated by phase separation into hydrogel microspheres.[82,83]

However, these four methods also have certain differences. Single coacervation is one of the classic forms of phase separation, which triggers the aggregation and separation of materials by changing the environmental parameters of the system (such as pH, ionic strength, or solvent composition). For example, charged polymers precipitate in salt solutions to form particles, which is a typical manifestation of the phase separation process. Complex coacervation also utilizes the principle of phase separation but introduces interactions among multiple materials (such as electrostatic, hydrogen

bonding, or hydrophobic interactions), allowing two or more materials to participate in phase separation and particle formation. It expands the material applicability and functional design space of phase separation methods. The solvent-nonsolvent method directly relies on the solubility differences in the solution system for phase separation. Materials dissolved in a good solvent precipitate upon the introduction of a nonsolvent, forming a dispersed phase. This process essentially induces phase separation by regulating the solubility of the solution. The temperature variation method triggers changes in the solubility of materials by adjusting the temperature, directly using temperature changes as an external driver for phase separation. For example, some thermosensitive polymers precipitate to form particles when their solubility sharply decreases at a specific temperature.[84,85]

## Photomask Method

The photomask method is a technique for preparing microspheres using photolithography and photochemical reactions. It involves the use of photomasks and photo initiators to precisely direct light energy onto the material, thereby enabling the formation and curing of microspheres.[74] Specifically, a photomask with a specific pattern is used to control the projection area of light. The transparent regions of the mask allow light to pass through, while the opaque regions block the light. The precursor material is exposed to a light source (such as ultraviolet light or laser), and the photomask enables spatially selective irradiation. In the irradiated areas, the photo initiator absorbs light energy to produce free radicals or ions, which initiate the polymerization or crosslinking reactions of monomers, forming microspheres. This method combines the high resolution of photolithography with the rapid nature of photochemical reactions, allowing for the preparation of microspheres with uniform particle size and controllable morphology. It is particularly suitable for applications that require high precision and complex structural design. However, the photomask method requires specialized equipment, such as photolithography machines, laser devices, and high-precision photomasks, which increases the technical threshold and cost. Moreover, this method relies on the photo reactivity of the material and is only applicable to material systems containing photo initiators. Non-photoreactive materials may require modification for this process.

## 3D Printing

In recent years, 3D printing technology has been developed for the preparation of various hydrogel microspheres. By precisely controlling the deposition and curing of materials, it can directly manufacture microspheres with uniform particle size and controllable morphology, especially suitable for the development of complex structures and functional microspheres.[86] Specifically, hydrogel precursor materials are prepared into flowable liquid or semi-liquid substances suitable for 3D printing. These materials typically contain photo initiators, crosslinkers, and other auxiliary components, which form stable structures during the subsequent printing and curing processes. Common methods include Direct Ink Writing (DIW), Digital Light Processing (DLP), and Photolithography. DIW involves the ejection of hydrogel precursor materials drop by drop through a micro-nozzle to form droplets, which are then cured by UV light or thermal initiation to form microspheres. The method relies on the photopolymerization of bio-ink also can be called DLP. Photopolymerization printing mainly relies on liquid photoreactive hydrogel materials, using laser or digital projection to precisely irradiate droplets for photopolymerization, forming microspheres.[87]

Compared to other technologies, 3D printing can precisely control the deposition location and number of materials at the micrometer level, thereby achieving high consistency in microsphere size and morphology. It is suitable for the preparation of microspheres with high uniformity requirements. Moreover, 3D printing is compatible with a variety of hydrogel materials, including natural polymers (such as gelatin, alginate) and synthetic polymers (such as hydrophilic polymers). It can also achieve composite functionality through the printing of multiple materials. Microfluidics combined with 3D printing is also utilized for the preparation of hydrogel microspheres. Microfluidic devices, which are microchannels capable of handling and transporting small fluid volumes, offer precise control over the formation of microspheres. In the context of 3D printing, microfluidic chips can be designed and printed to facilitate the generation of hydrogel microspheres with uniform morphology. Additionally, 3D printing eliminates the need for complex molds or equipment, allowing rapid validation and optimization of new designs, significantly reducing the development cycle. Moreover, material waste is minimal during the 3D printing process, and there is no need for emulsifiers or large amounts of organic solvents, making it more environmentally friendly and suitable for applications in the biomedical field.[88]

**Table 1** Various Preparation Methods of Microspheres and Their Advantages and Disadvantages

| Method | Advantages | Disadvanges |
|---|---|---|
| **Microfluidics** | - High precision in controlling microsphere size, shape, and structure.<br>- Capable of producing complex structures (eg, core-shell, multilayer microspheres).<br>- Low material waste, suitable for sensitive active components.<br>- High-throughput production with good batch-to-batch reproducibility. | - High equipment cost and complex operation.- Low production efficiency, difficult to scale up for industrial use.<br>- Microchannels prone to clogging, limited device lifespan.<br>- High requirements for operating environment, fragile equipment. |
| **Spray Method** | - Simple operation, suitable for large-scale production<br>- Precise control over microsphere size and morphology.<br>- Environmentally friendly, reduces organic solvent use.<br>- Suitable for encapsulating sensitive drugs or bioactive molecules. | - Broad particle size distribution, less uniform than microfluidics.<br>- May require solvents, environmental impact depends on operating conditions.<br>- Surface properties depend on raw materials and solidification process. |
| **Electrospray Method** | - Capable of producing nanoscale microspheres, suitable for biomolecule encapsulation.<br>- Precise control over microsphere size and distribution.<br>- No need for surfactants, avoids complex cleaning steps.<br>- Environmentally friendly, no chemical waste produced. | - Low efficiency with single-nozzle operation, difficult for large-scale production.<br>- Uncontrollable morphology, rapid drug release from single-polymer microspheres.<br>- Complex preparation process for composite microspheres. |
| **Emulsion Method** | - Simple operation, suitable for large-scale production.<br>- Can control microsphere size, suitable for various hydrogel materials.<br>- Low equipment cost, suitable for industrial production.<br>- Can produce core-shell or hollow microspheres. | - Non-uniform particle size distribution, prone to polydisperse systems.<br>- Emulsifier residues may affect biocompatibility.<br>- Active substances may degrade or lose activity during polymerization.<br>- Complex purification steps increase costs. |
| **Phase Separation** | - Can be triggered by external stimuli (eg, temperature, solvent).<br>- Suitable for high-precision microspheres.<br>- Can control microsphere functionality and structure. | - Sensitive to reaction conditions, may affect microsphere performance.<br>- Requires precise control of emulsifiers and oil phase, complex operation.<br>- Emulsifier residues may affect biocompatibility. |
| **Photomask Method** | - High resolution, suitable for high-precision and complex structures.<br>- Rapid photochemical reactions, suitable for high-precision applications. | - Requires specialized equipment (eg, photolithography machines, laser devices).<br>- High technical threshold and cost.<br>- Only applicable to materials containing photoinitiators. |
| **3D Printing** | - High precision in controlling microsphere size and morphology.<br>- Suitable for complex structures and functionalized microspheres.<br>- No need for complex molds, short development cycle.<br>- Low material waste, environmentally friendly. | - Low printing efficiency, difficult to scale up for industrial use.<br>- High requirements for material rheology and curing properties.<br>- High equipment cost and technical complexity. |

**Notes**: Microfluidics is suitable for high-precision, small-scale production but has high equipment costs and is difficult to scale up. Spray Method and Emulsion Method are suitable for large-scale production but may have issues with uniformity and biocompatibility compared to microfluidics. Electrospray Method is suitable for nanoscale microsphere preparation but has low efficiency and complex morphology control. Phase Separation and Photomask Method are suitable for high-precision applications but involve complex operations and high costs. 3D Printing is suitable for complex microsphere structures but has low efficiency and high equipment costs.

Despite its great potential in the preparation of hydrogel microspheres, 3D printing currently faces some challenges, such as low printing efficiency, limited industrial scale, and high requirements for the rheological and curing characteristics of printing materials. In the future, by integrating with automation, high-throughput technologies, and smart manufacturing, 3D printing is expected to overcome these bottlenecks and achieve large-scale, functionalized preparation of hydrogel microspheres, further expanding its potential.[89] Finally, we have summarized and concluded all the methods mentioned above for preparing microspheres, outlining their advantages and disadvantages, as shown in Table 1.

## Hydrogel Microspheres for the Treatment of Diabetic Wounds

The formation and progression of chronic diabetic wounds is a complex pathological process closely related to the systemic metabolic abnormalities caused by diabetes. The long-term hyperglycemic state leads to vascular lesions, nerve damage, and immune dysfunction, which collectively contributes to the propensity for chronic, non-healing wounds in diabetic patients following skin injury. First, the micro- and macro-vasculatures of diabetic patients are chronically affected by hyperglycemia, resulting in endothelial dysfunction and reduced blood flow. This leads to insufficient oxygen and nutrient supply to the wound site, thereby inhibiting tissue repair capacity. Additionally, neuropathy reduces the patients' ability to sense pain from wounds, often leading to minor injuries going unnoticed and further exacerbating wound damage. Secondly, immune dysfunction in diabetic patients is characterized by persistent abnormal inflammatory responses. Macrophages and neutrophils within the wound are functionally impaired, reducing their ability to clear bacteria and making the wound more susceptible to infection. Infection, in turn, exacerbates local inflammation, creating a vicious cycle that further delays the healing process. Moreover, the diabetic state affects the migration and proliferation of fibroblasts and keratinocytes, impairs collagen synthesis, and reduces angiogenesis, leading to insufficient granulation tissue formation and difficulty in re-epithelialization. The lack of an effective repair mechanism causes the wound to remain in the inflammatory stage for extended periods, failing to progress to the proliferative and remodeling stages and thus becoming a chronic, non-healing wound. In summary, the formation of chronic diabetic wounds is the result of the combined effects of vascular lesions, nerve damage, infection, and tissue repair impairment. The complexity of its pathology determines the difficulty of treatment, which requires multidisciplinary interventions to achieve better outcomes. To address this, numerous hydrogel microsphere designs have been reported in laboratories.

### Treatment of Full-Thickness Defects in Diabetic Wounds

Basic fibroblast growth factor (bFGF) is a multifunctional cytokine with a molecular weight of approximately 17–18 kDa, belonging to the fibroblast growth factor family. It is widely present in mammalian tissues and plays roles in promoting cell proliferation, differentiation, migration, as well as regulating angiogenesis and tissue repair.[90] It primarily activates downstream signaling pathways such as MAPK and PI3K/AKT by binding to its specific receptor, thereby regulating cell behavior and participating in various physiological and pathological processes. For example, it enhances the differentiation capacity of vascular endothelial cells to promote neovascularization and stimulates the proliferation and migration of keratinocytes. As a commonly used topical wound medication in clinical practice, bFGF-based hydrogel microspheres have been widely reported. In non-diabetic wounds, bFGF has been encapsulated in polycaprolactone for treatment.[91] However, to address the more complex pathological state of diabetic wounds, a novel charge-driven self-assembled microsphere hydrogel scaffold based on electrostatic interactions has been reported. This scaffold combines positively charged microspheres containing black phosphorus and chitosan with negatively charged microspheres containing bFGF and hyaluronic acid methacrylate (HAMA). The weak electrostatic attraction between the microspheres endows the scaffold with excellent injectability. The incorporation of black phosphorus allows near infrared photothermal effects, which significantly influence the degradation behavior and drug release characteristics. The combination of short-term physical (photothermal) intervention and long-term chemical (drug release) intervention shows great potential in spatiotemporal control of the regenerative microenvironment, significantly promoting cell proliferation, migration, angiogenesis, and macrophage polarization, thereby enhancing its potential in tissue regeneration.[92] In addition to this simple encapsulation and assembly, studies have also utilized magnetic biohybrid microspheres for the efficient purification of bFGF. Specifically, glutathione (GSH) was used to prepare microspheres. GSH is a naturally occurring tripeptide (L-γ-glutamyl -L-cysteinyl-glycine) that plays a key role in cellular redox reactions. GSH can bind with high affinity to glutathione

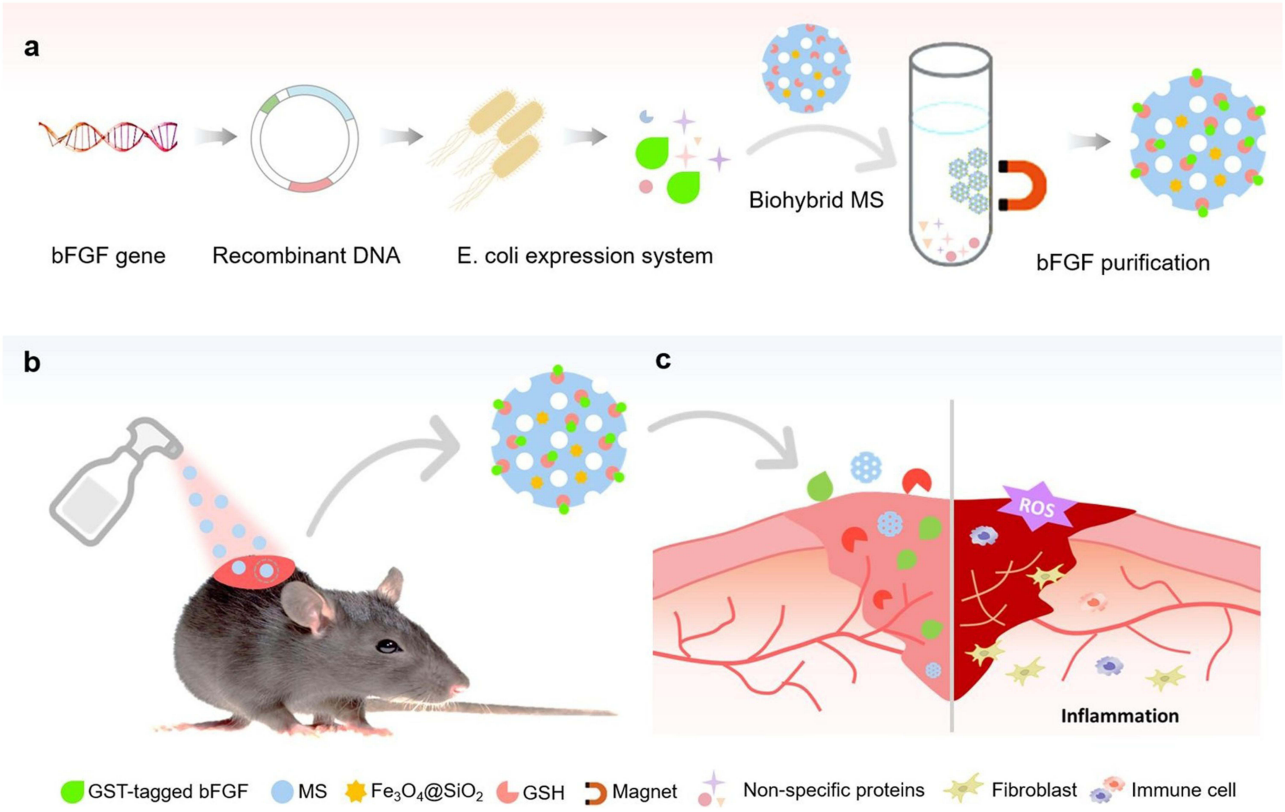



**Figure 3** Schematic illustration of the fabrication of multifunctional biohybrid microsphere system and its application in chronic wound healing. (**a**) Microspheres were designed to isolate bFGF protein which was produced using E. coli expression system. (**b** and **c**) Conjugated with bFGF, the biohybrid microspheres were applied for enhanced wound healing in diabetic mice. Reproduced with permission from Lei L, Zhu Y, Qin X, et al. Magnetic biohybrid microspheres for protein purification and chronic wound healing in diabetic mice. *Chem Enginee J*. 2021;425:130671.[93]

S-transferase (GST), which is widely used in fusion protein purification, such as in the recombinant human synthesis of bFGF. By capturing GST-tagged bFGF, recombinant bFGF was expressed in E. coli using the GST fusion system. Subsequently, bFGF protein was isolated from cell lysates using GSH microspheres to form bFGF-GSH complex microspheres, which efficiently separated the target protein while effectively removing non-specific proteins. In addition to the therapeutic effects of bFGF itself, GSH used to capture GST-fusion target proteins also exhibited synergistic antioxidant and anti-inflammatory activities during wound healing, achieving excellent therapeutic effects in diabetic mouse wounds (Figure 3).[93] This method can be extended to various recombinant human products that can be fused with GST, such as vascular endothelial growth factor (VEGF), which has already been reported.[94] Although this study was not focused on diabetic wound treatment, it is believed to have potential for expanded applications. In fact, VEGF-encapsulated hydrogel microspheres have been reported and combined with black phosphorus. In this study, to develop a hydrogel system that can adapt to the shape of the wound, the photothermal effect of black phosphorus quantum dots and the thermos-reversible stiffness changes of dynamic hydrogels were utilized to achieve adhesion between microspheres under near-infrared irradiation. Moreover, photothermal effects can regulate the release of VEGF encapsulated in the microspheres. More importantly, the drug release process can be monitored in real-time through visual color changes.[95]

In addition to the local application of growth factors, classic drugs such as metformin and insulin have also shown potential in promoting wound healing in diabetic wounds. These drugs support tissue repair by regulating glucose metabolism and improving systemic and local microenvironments. Metformin, a widely used antidiabetic drug, not only improves systemic metabolic status by lowering blood glucose levels but also exhibits anti-inflammatory and angiogenic effects. Metformin regulates the function of fibroblasts and keratinocytes by activating the AMPK signaling pathway, thereby enhancing wound healing capacity. Insulin, while controlling blood glucose levels, can also directly act on local tissues to promote cell proliferation and migration.[96] Insulin promotes the function of endothelial cells and fibroblasts through the PI3K/AKT

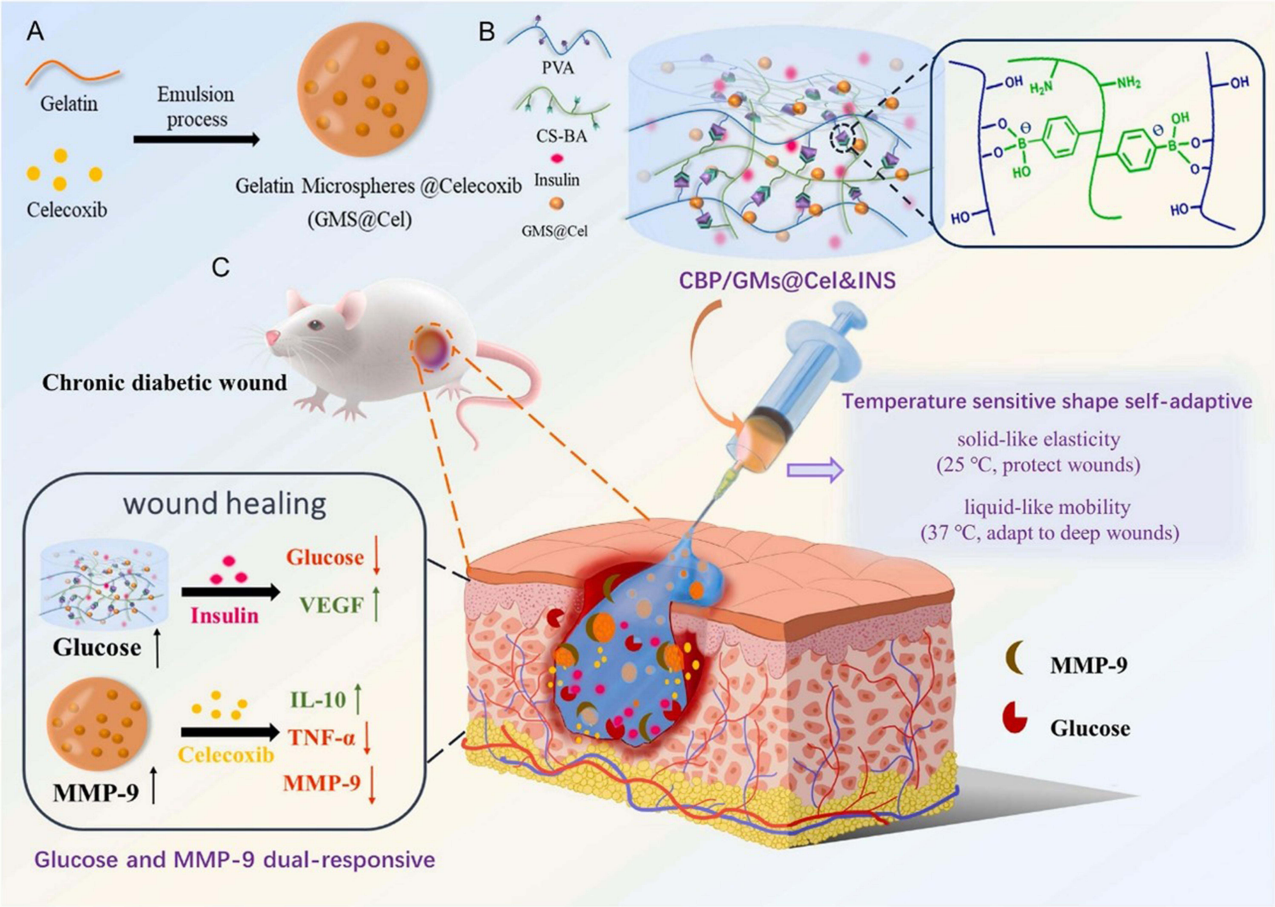



**Figure 4** The design strategy of glucose and MMP-9 dual-responsive shape self-adaptive hydrogels for treating chronic diabetic wound. (**A**) Preparation of gelatin microspheres containing celecoxib (GMs@Cel). (**B**) Preparation of the CBP/GMs@Cel&INS hydrogel and the characteristics of temperature sensitive shape self-adaptive. (**C**) The process of treating chronic diabetic wounds with the CBP/GMs@Cel&INS hydrogel through the glucose and MMP-9 dual-response system. Reproduced from Zhou W, Duan Z, Zhao J, et al. Glucose and MMP-9 dual-responsive hydrogel with temperature sensitive self-adaptive shape and controlled drug release accelerates diabetic wound healing. Bioact Mater. 2022;17:1–17. Creative Commons.[98]

pathway, enhancing angiogenesis and collagen synthesis, thus accelerating wound healing.[97] Combining these classic therapies with microspheres will be a future direction for breakthroughs. Metformin was encapsulated in gelatin to prepare microspheres and loaded into a pH-responsive "double hydrogen bond" (hydrogen bond and hydrazone bond) hyaluronic acid-collagen hydrogel matrix. This hydrogel rapidly self-cured in neutral and alkaline environments, but the weak acidic inflammatory microenvironment of diabetic wounds accelerated its degradation and promoted the release of metformin. In vitro experiments showed that hydrogel significantly enhanced fibroblast adhesion and infiltration while inhibiting excessive macrophage growth. The released metformin induced macrophage polarization from the M1 phenotype to the M2 phenotype, thereby accelerating fibroblast migration and collagen production in a high-glucose environment. In vivo experiments further confirmed that the hydrogel effectively remodeled the extracellular matrix in diabetic mouse wounds.[27] Such a responsive release system has also been reported in insulin-based treatments. Diabetic wounds have unique microenvironmental characteristics, including high glucose levels, elevated matrix metalloproteinase-9 (MMP-9), and chronic inflammatory states. To address this complex environment, a glucose- and MMP-9-responsive temperature-sensitive shape-adaptive hydrogel was designed and constructed using polyvinyl alcohol and chitosan grafted with phenylboronic acid. This hydrogel was used to deliver insulin and celecoxib-loaded gelatin microspheres. The hydrogel exhibits fluid-like flowability at 37°C, allowing it to quickly adapt to deep wounds, while at 25°C, it has solid-like elasticity, effectively protecting the wound from external damage as shown in Figure 4.[98] Of course, to adapt the gel to the wound shape, there is more than one way. Directly using a photocurable hydrogel matrix can achieve this effect. Rhodiola crenulate saponin-loaded sodium alginate hydrogel

microspheres were further loaded into an HAMA hydrogel system to achieve in situ photo crosslinking to adapt to the wound shape.[99] In addition, endowing the gel system with excellent adhesive properties is another form. Marine mussel-derived mussel proteins or dopamine have excellent adhesive properties, mainly due to their catechol functional groups. Oyster peptides also have such groups. By combining oyster peptides with chitosan to endow chitosan with adhesive ability, catechol-functionalized chitosan microspheres were prepared for the treatment of diabetes, achieving excellent therapeutic effects.[100]

Inflammation plays a key role in the formation and non-healing of diabetic wounds and is one of the main reasons for abnormal healing processes. Under normal circumstances, wound healing is divided into the inflammatory, proliferative, and remodeling phases, with inflammation being the first step in initiating the healing process. However, the chronic hyperglycemic state in diabetic patients leads to abnormal inflammatory responses, not only prolonging the inflammatory phase but also keeping the wound in a chronic inflammatory state, which hinders subsequent tissue repair. Under hyperglycemic conditions, the function of macrophages and neutrophils is impaired. The levels of pro-inflammatory cytokines released by these immune cells (such as TNF-α, IL-6, and IL-1β) are significantly elevated, causing excessive local inflammation. Moreover, macrophages have difficulty transitioning from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, further prolonging the inflammatory phase. Additionally, oxidative stress caused by hyperglycemia leads to excessive accumulation of reactive oxygen species (ROS) in the wound, which not only damages tissues but also further activates the inflammatory response, creating a vicious cycle. In one study, excessive cell-free DNA (cfDNA) was found to activate chronic inflammation by engaging intracellular nucleic acid sensors. Intervening in cfDNA-mediated pro-inflammatory signaling may be a potential strategy to alleviate chronic inflammation. However, how to effectively and specifically reduce the concentration of cfDNA in the pathological microenvironment remains an urgent challenge. By guiding the assembly with cfDNA, independent polydopamine nanosheets were successfully prepared and embedded into Gelatin Methacrylate (GelMA) microfluidic hydrogel microspheres. The polydopamine nanosheets interact with cfDNA bases through π-π stacking and hydrogen bonds, achieving efficient capture, while the caged space provided by the hydrogel polymer network further ensures stable storage of cfDNA. Moreover, the catechol groups in polydopamine nanosheets can reduce ROS levels. This system can efficiently bind cfDNA without relying on serum proteins, specifically blocking cfDNA-mediated abnormal activation of Toll-like receptor 9 (TLR9) and significantly downregulating inflammation-related cytokines and ROS levels (Figure 5).[101] However, in wound healing, excessive removal of ROS may sometimes damage the oxygen supply system, and how to balance this effect is a current therapeutic challenge.[13] To address such issues, core-shell microspheres were prepared, with the shell combined with catalase to efficiently convert $H_2O_2$ in the released PVP/$H_2O_2$ into $O_2$. This design effectively prevents $H_2O_2$ from escaping from the microspheres, thus avoiding potential issues. In terms of results, the microspheres can rapidly release sufficient oxygen to maintain cell survival under hypoxic conditions and continuously release oxygen for at least 2 weeks. Unlike most current oxygen-generating systems, which first release toxic $H_2O_2$ into the tissue environment and then produce oxygen through its decomposition, the designed oxygen-releasing microspheres directly release oxygen, avoiding the potential risk of excessive ROS.[102] However, wound healing is a continuous and phased process, and when using hydrogel microspheres, combining more complex sequential designs can achieve phased treatment. A multilayer injectable active substance sequential delivery system was designed to promote wound regeneration in stages. The outer layer of this system consists of an injectable alginate/bioactive glass hydrogel that can release bioactive glass ion products in the early stages of wound healing to modulate the host's inflammatory response. The middle layer is alginate-based hydrogel microspheres dispersed in the hydrogel, which release RAW264.7 cell conditioned medium activated by bioactive glass ions to promote cell proliferation and granulation tissue formation. Finally, pirfenidone encapsulated in the hydrogel microspheres is used to regulate extracellular matrix synthesis, inhibit angiogenesis, prevent excessive fibrosis and scar formation, thereby promoting full-thickness skin regeneration.[26]

In addition to drug or gene delivery, there are also reports of hydrogel microspheres being used for cell delivery to treat diabetic wounds. Gelatin microspheres were used to provide a suitable microenvironment for the survival of rat adipose tissue-derived stem cells (ADSCs). Using microfluidic technology, uniform and well-dispersed microspheres were manufactured. Due to their geometric shape, the protease degradation rate of the microspheres was four times that of bulk hydrogels. The microspheres were well-integrated into the regenerating tissue and exhibited good biocompatibility and adaptive biodegradation rates. Histological examination showed that ADSC-loaded gelatin microspheres

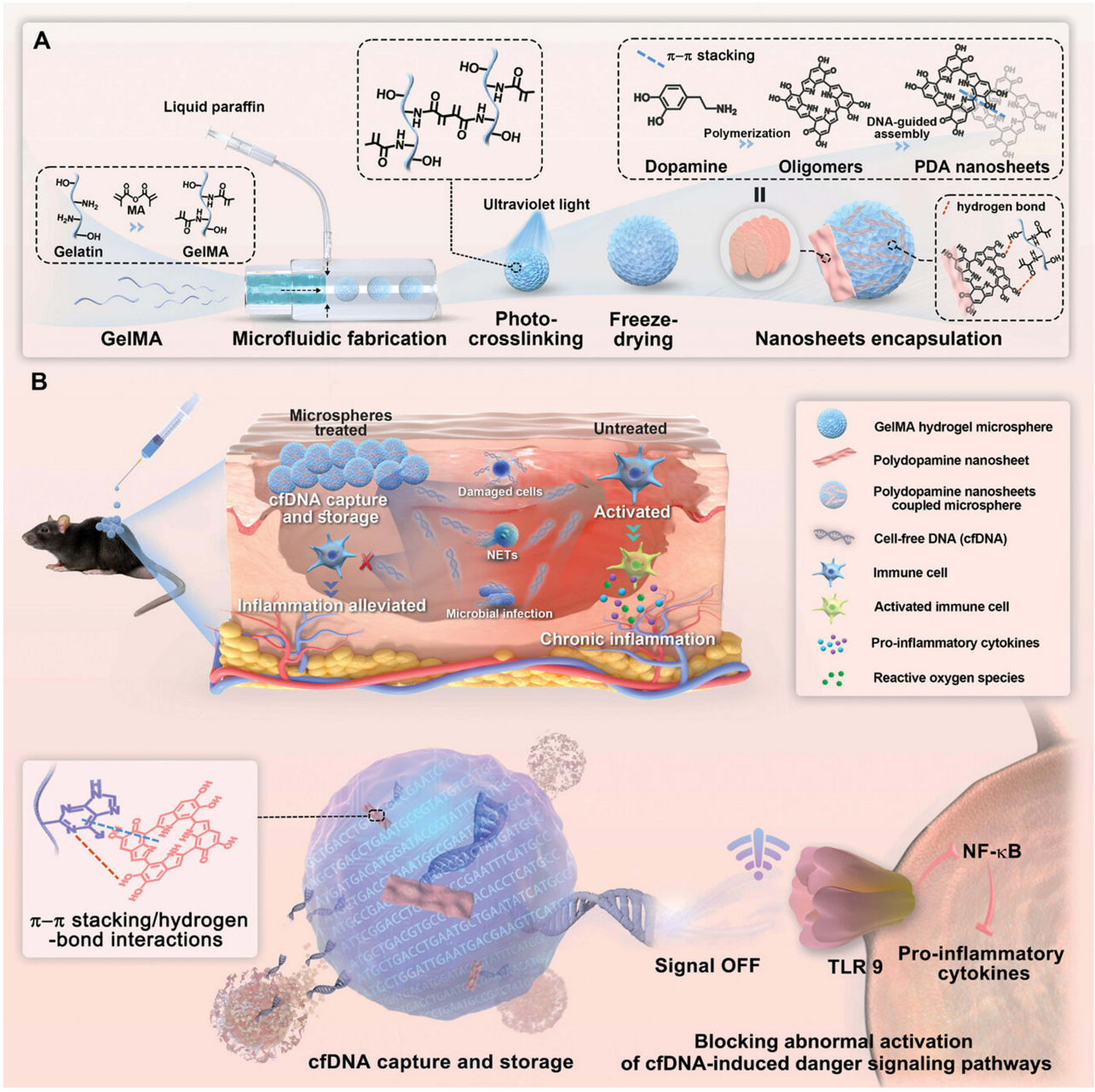



**Figure 5** Schematic of the microfluidic fabrication of PDA@GM MSs (**A**) and the mechanism behind the capture and storage of cfDNA and the intervention of pro-inflammatory signaling in a diabetic wound model (**B**). Reproduced from Ding T, Xiao Y, Saiding Q, et al. Capture and storage of cell-free DNA via bio-informational hydrogel microspheres. *Adv Mater.* 2024;36(33):e2403557. © 2024 Wiley-VCH GmbH.[101]
**Abbreviations**: cfDNA, cell-free DNA; GM MS, GelMA hydrogel microsphere; PDA, polydopamine.

significantly accelerated wound healing by promoting M2 macrophage polarization, collagen deposition, angiogenesis associated with peripheral nerve recovery, and hair follicle formation. Notably, the relative fluorescence intensity around the hair follicles was 17 times higher than that of the control group, indicating that ADSCs participated in the healing process through exosomes.[103] In addition to direct ADSC application, studies have also developed a system loaded with ADSC-derived apoptotic bodies (ABs) to achieve sustained activity of ABs and explore their role in macrophage polarization regulation. The study first demonstrated that ABs could balance the polarization state of macrophages by regulating the JAK-STAT1 signaling pathway and reducing the upregulation of arginosuccinate synthase induced by lipopolysaccharide, thereby inhibiting inflammatory responses and promoting diabetic wound tissue regeneration

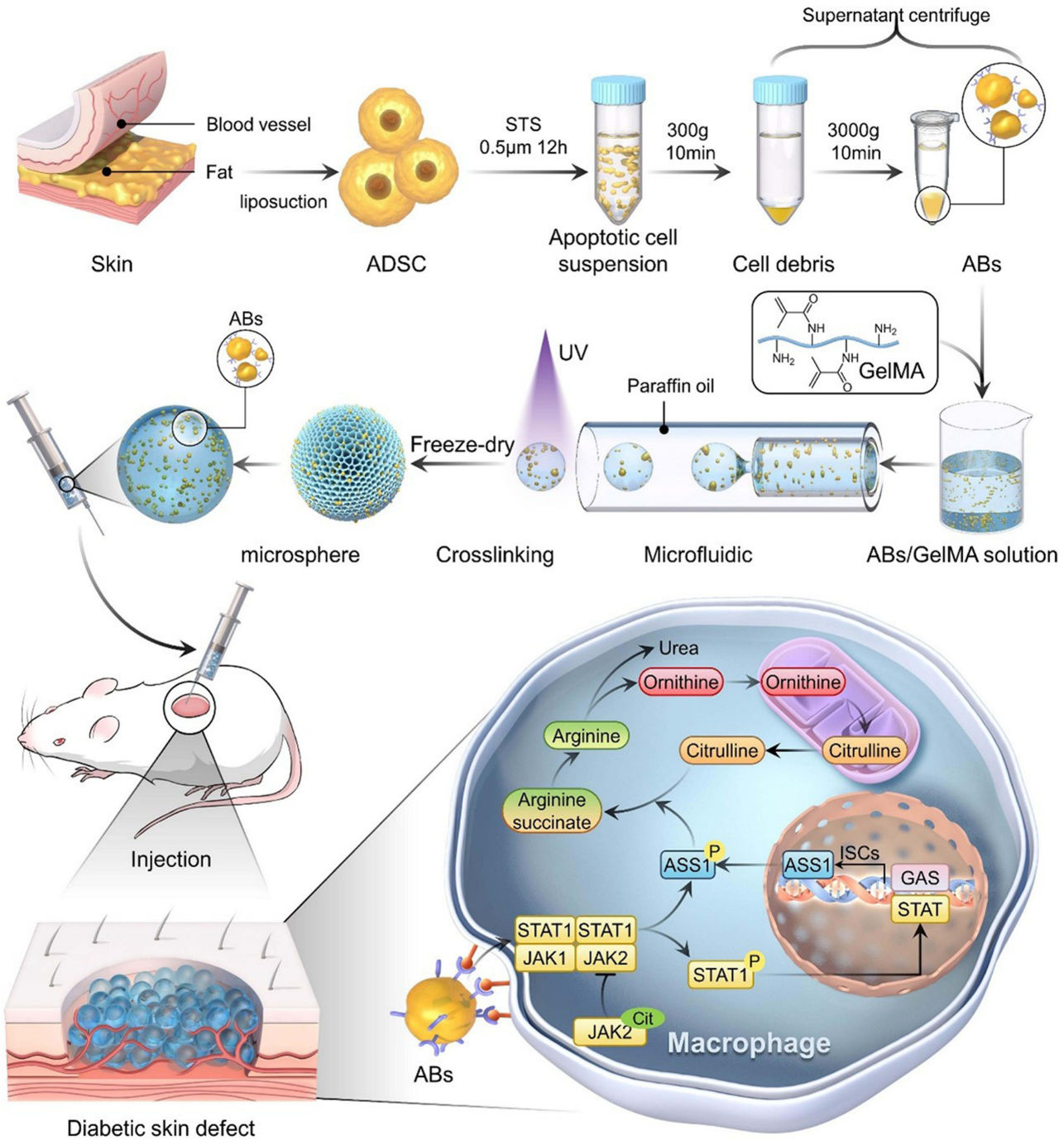



**Figure 6** Schematic illustrating the preparation of AB@GMSs and their modulation of local macrophages in wounds. ABs, isolated from ADSCs by STS induction and differential centrifugation, were encapsulated in GMSs. With slowly released at the wound site, they promoted M2 macrophage polarization via the JAK-STAT pathway, influencing ARG expression and enhancing wound healing. Reproduced with permission from Mao J, Qian S, Zhao Q, et al. Balancing macrophage polarization via stem cell-derived apoptotic bodies for diabetic wound healing. *Med.* 2024;5(2):148–168.e8. Creative Commons.[104]
**Abbreviations**: ADSC, Adipose tissue-derived stem cell, Abs, Apoptotic bodies.

(Figure 6).[104] In addition, a study reported the direct assembly of macrophage membranes with synthetic lipid membranes to construct a novel type of nanovesicle that could inherit the cytokine neutralization capacity of its source macrophages. To simulate the layered structure of cells, this study used microfluidic technology to encapsulate the synthetic nanovesicles into GelMA hydrogel microspheres, forming a composite structure. Thus, these nanovesicles were

effectively protected in the external environment and could achieve sustained or controlled release of cytokines, ultimately achieving excellent therapeutic effects for diabetic wounds.[105]

In summary, in the application of hydrogel microspheres for the treatment of diabetic wounds, it is not difficult to see that current research mainly focuses on delivering classic therapeutic drugs, genes, and various cell-derived therapeutic substances. The results show that the combination of various materials and delivery systems has achieved comprehensive wound management capabilities, involving promotion of healing, angiogenesis, anti-inflammatory effects, and anti-ROS. However, diabetic wounds are often complicated by infections, and the application progress of anti-infective hydrogel microspheres will be discussed in the following section.

## Treatment of Diabetic Wounds with Infection

The relationship between wound infection and diabetes is complex and mutually influential. Due to the effects of hyperglycemia, the wound healing process in diabetic patients is often more difficult and slower than in non-diabetic patients. Hyperglycemia favors the growth and proliferation of pathogens such as bacteria and fungi. At the same time, hyperglycemia and metabolic disorders have adverse effects on the body's immune system and immune function, leading to decreased immune capacity and difficulty in resisting pathogen attacks. The biodiversity of healthy skin homeostasis is beneficial for stabilizing the wound bed microenvironment and resisting biological and environmental fluctuations. However, the high levels of oxidative stress in diabetic wounds significantly alter the bacterial wound microbiome. Therefore, diabetic patients have an increased risk of wound infection, and wound healing after infection is even more difficult. This is closely related to the inflammatory response, oxidative stress, immune cell dysfunction, impaired function of stem cells and fibroblasts, and reduced wound microbiome diversity caused by hyperglycemia. Therefore, hydrogel microsphere treatments are also committed to developing antibacterial-related therapeutic models.

As previously mentioned, bFGF is a commonly used means for the treatment of diabetic wounds and has been widely applied in hydrogel microspheres for simple full-thickness defect treatment. On this basis, graphene oxide can be combined as a nanocarrier for antisense nucleotides to experiment with antibacterial effects.[106] At the same time, there are also reports of complex cross-linking of multiple materials to achieve multifunctional encapsulation in microspheres. When dopamine, chitosan, hyaluronic acid, and GelMA are mixed for bFGF encapsulation, dopamine, chitosan, and hyaluronic acid are first cross-linked, and then GelMA is sprayed to form microspheres, achieving anti-inflammatory, antioxidant, antibacterial, and angiogenic biological activities. Specifically, hydrogel microspheres inhibit bacterial proliferation, reduce the release of inflammatory cytokines, alleviate oxidative stress responses, accelerate collagen deposition, promote hair follicle and vessel regeneration, and enhance the repair capacity of infected wounds, thus showing potential for application in diabetic wounds with infection.[107]

In addition, the combination of other forms of treatment has also been widely reported, such as using poly (N-isopropylacrylamide) to prepare hydrogel microspheres for antibacterial effects. Moreover, in this study, smart on-demand treatment methods were also integrated. The integration of a wound exudate management module with a wound infection monitoring and on-demand drug delivery module forms a smart wound dressing, with the wound patch and a flexible circuit equipped with a near-field communication antenna as the two core components. The exudate management module in the wound patch can achieve automatic pumping and storage of liquids, while the sensing module can synchronously monitor the temperature and humidity of the wound, providing key parameters for the diagnosis of infected wounds and drug release. At the same time, the liquid metal heating circuit, controlled by voltage, can trigger the on-demand drug release of thermosensitive hydrogel microspheres. The use of liquid metal as the heating circuit endows the wound patch with flexibility, stretchability, and higher comfort. The modular design of the wound patch not only reduces the cost of component replacement but also adapts to wounds of different sizes. By integrating a near-field communication antenna, the flexible circuit can achieve inductive coupling transmission of data. In addition, a mobile application with a graphical user interface has been developed for recording, analyzing, and visualizing data, as well as controlling the drug release process. Finally, the closed-loop smart wound dressing verified by a mouse wound infection model can continuously monitor temperature and humidity information and provide on-demand drug delivery feedback treatment according to the wound condition. This is important for accelerating wound healing, promoting tissue regeneration, and collagen deposition.[108]

Of course, in addition to infection, there are still many challenges in diabetic wound treatment that need to be addressed, such as biofilm formation, stagnation in the inflammatory phase, pathological scarring caused by abnormal proliferation, and difficulties in skin regeneration. These are also aspects that hydrogel microspheres need to focus on in the future. However, overall, hydrogel microspheres have shown great potential in managing diabetic wounds, and future innovations are highly anticipated.

## Perspectives

In the future, the performance and application scope of hydrogel microspheres are expected to be further enhanced through interdisciplinary integration and the development of new materials. Specifically, the incorporation of smart responsive technologies, such as hydrogels that react to the unique pH, temperature, or enzyme activity of the wound microenvironment, will enable more precise and targeted drug delivery. For instance, pH-responsive hydrogel microspheres could release therapeutic agents specifically in the acidic milieu of diabetic wounds, thereby minimizing systemic side effects. The development of new materials will also play a crucial role in advancing hydrogel microspheres. This includes exploring composite materials that combine natural polysaccharides with synthetic polymers to improve biocompatibility and mechanical properties. Embedding multifunctional nanomaterials, such as gold nanoparticles or magnetic nanoparticles, could endow hydrogel microspheres with additional functionalities like magnetic targeting, imaging capabilities, and enhanced drug delivery efficiency. Meanwhile, in-depth exploration of multi-level drug loading designs, combination with cell engineering, and the development of microenvironment-responsive microspheres will provide more comprehensive and efficient solutions for the treatment of diabetic wounds. Ultimately, those will promote the transition of this technology from the laboratory to clinical applications, benefiting more patients.

## Conclusion

Hydrogel microspheres show unique advantages in treating diabetic wounds, such as efficient drug loading and release, adaptability to complex wound shapes, and multifunctionality in accelerating wound healing. However, challenges remain in practical application. The preparation of hydrogel microspheres is complex, requiring precision equipment and strict condition control, which limits large-scale industrial production. Common preparation methods like microfluidic chips, electro spraying, and emulsion techniques each have limitations, such as low production efficiency, high equipment costs, and broad particle size distribution. Additionally, the design of existing hydrogel microspheres needs further optimization to achieve more precise drug release in response to the unique microenvironment of diabetic wounds and safer in materials. Despite these challenges, hydrogel microspheres hold great potential in the treatment of diabetic wounds.

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## Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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