Journal of Nanomaterials

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Lead Guest Editor: Jinjian Huang
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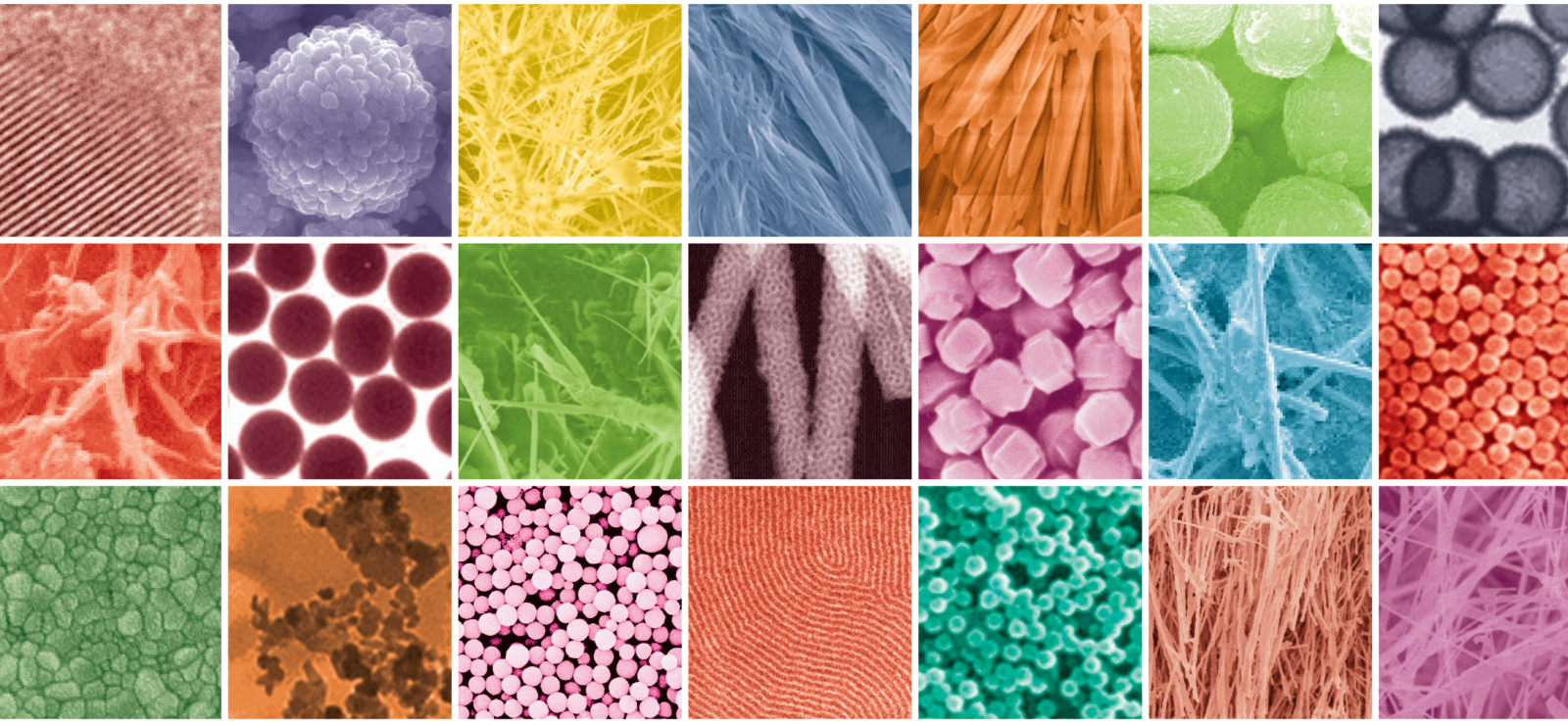

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Review Article

Application of Nanomaterial in Hydrogels Related to Wound Healing

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Traditional dressings used for wound repair, such as gauze, have shortcomings; for example, they cannot provide a suitable microenvironment for wound recovery. Therefore, it is necessary to find a better dressing to overcome shortcomings. Hydrogel provides a suitable wet environment, has good biocompatibility, and has a strong swelling rate to absorb exudate. Nanomaterial in hydrogels has been used to improve their performance and overcome the shortcomings of current hydrogel dressings. Hydrogel dressing can also be loaded with nanodrug particles to exert a better therapeutic effect than conventional drugs and to make the dressing more practical. This article reviews the application of nanotechnology in hydrogels related to wound healing and discusses the application prospects of nanohydrogels. After searching for hydrogel articles related to wound healing, we found that nanomaterial can not only enhance the mechanical strength, antibacterial properties, and adhesion of hydrogels but also achieve sustained drug release. From the perspective of clinical application, these characteristics are significant for wound healing. The combination of nanomaterial and hydrogel is an ideal dressing with broad application prospects for wound healing in the future.

1. Introduction

A skin wound, one of the most common clinical diseases, is defined as damage to the structure or integrity of skin tissue due to various causes [1–4]. In recent years, with the changes in the spectrum of human diseases, the number of patients and the cost of skin wounds have increased significantly. According to statistics, the total annual direct cost of wound treatment in the United States exceeds $25 billion [5]. Repairing wounds quickly and with high quality is challenging. Following the introduction of the theory of moist healing by Dr. George Winter of the University of London in the United Kingdom [6], the US Food and Drug Administration (FDA) pointed out in the industry guidelines in 2000 for wound medical supplies (external drugs and dressings) that maintaining a moist environment on the wound surface is the standard treatment method.

Studies have shown that moist wounds, those with a moist microenvironment, are less susceptible to infection than dry wounds, inhibit wounds more effectively, and promote healing. A moist wound dressing can create and maintain a moist environment around the wound and promote the regeneration and repair of the dermis and epidermal tissue during the wound healing process. The ideal wound dressing should have the following characteristics: good biocompatibility, antibacterial activity, water absorption, water retention, noncytotoxicity, and good biodegradability [7].

New wet wound dressings have the characteristics of moisture permeability and oxygen permeability and are mainly used as a physical barrier to protect the wound surface from microorganisms. As a new type of wet wound dressing that has emerged in recent years, hydrogel dressings have a three-dimensional network structure that can absorb or retain large amounts of water or biological fluids. Compared with traditional dressings, hydrogel dressings can provide a moist healing environment and speed up the wound healing time [8]. They have the advantages of good biocompatibility, strong water absorption, less bacterial growth, and

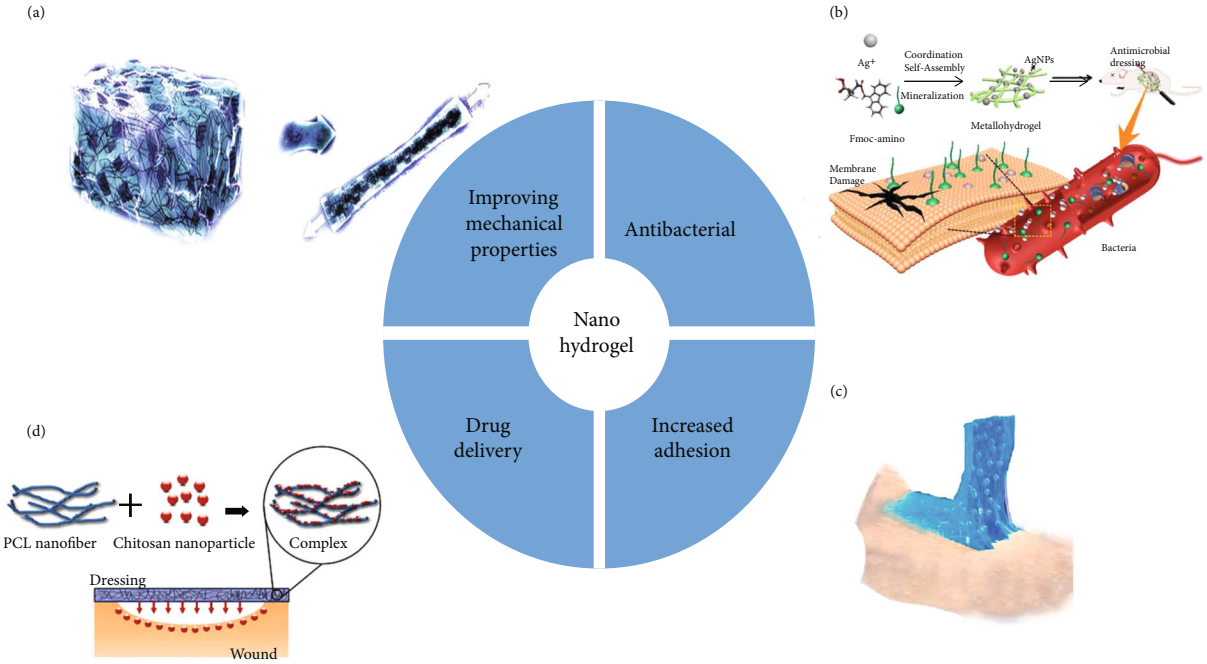

Figure 1: The role of nanomaterial in wound hydrogels. (a) Reproduced with permission from ref. [21]. Copyright ©2019 WILEY-VCH. (b) Reproduced with permission from ref. [22]. Copyright © 2020 WILEY-VCH. (c) Reproduced with permission from ref. [23]. Copyright © 2021 Elsevier. (d) Reproduced with permission from ref. [24]. Copyright © 2021 Taylor & Francis.

less discomfort to patients [9, 10]. Moreover, the internal porous structure of hydrogel mimics the natural extracellular matrix (ECM), which is an ideal scaffold for tissue engineering [11]. However, hydrogels also have disadvantages, such as low mechanical strength, high brittleness, and poor antibacterial ability, so their application is limited [12–14].

Nanomaterials have unique size effects and interface effects and have shown great application prospects in chip preparation, construction chemicals, and biomedicine [15–18]. Through template molding, self-assembly, microfluidics, and 3D printing technologies, building ultrastructures or dispersing nanoparticles in hydrogels to form composite materials can significantly improve the mechanical properties and stability of hydrogels while endowing hydrogel dressing with more functions. At the same time, nanohydrogel can achieve sustained drug release by wrapping or loading drugs, thereby promoting wound healing (Figure 1). At present, few studies have summarized the prospects and challenges to nanohydrogel applications in wound healing [19, 20]. In this article, we summarize the latest developments of nanohydrogel dressings and their application prospects in wound healing, and we further analyze the current opportunities and challenges in wound healing.

2. The Role of Nanomaterial in Wound Hydrogel

2.1. Improving Mechanical Performance. Hydrogel is a three-dimensional hydrophilic network, insoluble in water or aqueous solutions, and capable of absorbing water or other biological fluids [25, 26]. It can promote the healing process, rehydrate necrotic tissue and increase the healing of debridement, and cool the wound surface, and it is suitable for cleaning dry, loose, or necrotic wounds [21, 27]. Furthermore, it does not react with organisms, is nonirritating and nonadhesive, and has permeable metabolites [28]. As an important wet wound dressing, hydrogels meet the requirements for ideal wound dressings [29]. Natural polymers are superior to synthetic polymers owing to their excellent biodegradability and biocompatibility [30]. However, the high water contents of natural polymer hydrogels often result in poor mechanical properties, low mechanical strength, and high brittleness, which greatly limit the speed of wound repair [31, 32].

There are often multiple interactions in the hydrogel network to maintain systematic stability. Nanomaterials in hydrogel have greatly improved the mechanical strength of hydrogels through strong physical crosslinking, such as the formation of hydrogen bonds, electrostatic interactions, covalent bonds, hydrophobic interactions, and other physical crosslinks [33, 34] (Table 1). Although hydrogen bonding groups (-OH, -NH, and -C-O) are ubiquitous in a variety of natural and synthetic polymers, the water molecules in hydrogel usually screen the hydrogen bonding interactions in it [35]. Most polymers can only form hydrogels with weaker mechanical strength through hydrogen bonding, such as gelatin, agarose, and carrageenan [36]. PVA, chitosan, and cellulose can form crystalline domains ranging in size from nanometers to micrometers through hydrogen bonding under certain conditions. This strong physical crosslinking can endow hydrogel with excellent mechanical strength and maintain the stability of the system through a variety of interactions.

Electrostatic interaction usually occurs between the fixed charged polymer and the corresponding ion, such as the physical crosslinking of alginate with divalent cations and

Table 1: The role of nanotechnology in wound hydrogel.

Effective	Mechanism
Improving mechanical properties	In situ polymerization [59], electrospinning technology [60], casting and coordination interaction [61–64]
Drug release	Three-tier structure [65] and embedding [66]
	Cross-linked and direct interaction [26, 49, 58, 67–70]
Antibacterial effect [39]	Interfering with DNA replication and RNA production
	Destroy cell membranes
	Interference with cellular respiration
	Change enzyme conformation and inactivate enzyme activity
Increased adhesion	Covalent coupling [71] and noncovalent complex [60, 72]

the crosslinking of chitosan with multivalent anions [37, 38]. Other biopolymers that can form ionically crosslinked hydrogels include chiral polysaccharides, pectins, cellulose, and sodium polygalacturonate. A series of tough and self-healing hydrogels are produced through the formation of polyionic complexes and the gradual polymerization of oppositely charged monomers. Moreover, electrostatic interactions do not work in isolation. These bonds are caused by other noncovalent interactions, such as van der Waals interactions and hydrogen bonding, to further stabilize the hydrogel network.

Covalent bonds are important structures constituting the hydrogel network, and covalent crosslinking is also a common way for nanotechnology to enhance the strength of hydrogels. Chemical crosslinking occurs between the polymer matrix and the crosslinking agent to form a covalent bond, thereby forming a fairly stable, strong, and heat-resistant hydrogel [39]. For example, cobalt oxide magnetic nanoparticles can be used as a covalent crosslinking agent to form acrylamide-based, magnetically responsive hydrogels.

2.2. Antibacterial Effect. The danger of antibiotic resistance is the human and economic losses it causes. Owing to the improper use of antibiotics, bacteria with resistance to traditional therapies have developed. Approximately 700,000 people die each year in the world from infections caused by antibiotic-resistant bacteria [40]. For example, in 2017, methicillin-resistant Staphylococcus aureus (MRSA) caused nearly 120,000 blood-borne infections and 20,000 related deaths in the US [41]. Excessive and inappropriate use of antibacterial drugs has led to the emergence of stronger strains, which are less vulnerable to treatment [42]. In addition, traditional antibacterial drugs also have many problems, such as low water solubility, reduced stability, minimal oral bioavailability, complexity of drug targeting, and reduced patient compliance due to frequent medication and different toxicities [43]. Nanomaterial plays an important role in improving the effectiveness of existing treatment methods by improving the physical and chemical properties and stability of antibiotics, increasing the opportunities for internalization of biofilms, extending the release time of antibiotics, and improving the effectiveness of drugs [44]. Nanosystems mainly include inorganic nanosystems, such as metal-based nanoparticles (e.g., silver (Ag NP), copper (Cu NP), metal oxide nanoparticles titanium oxide (TiO_2 NP), zinc oxide (ZnO), cerium oxide (CeO_2), and yttrium oxide (Y_2O_3)) and other substances with antibacterial activity, and they are used for wound healing [22, 24, 45]. The mechanism of action is generally as follows: (1) inhibiting bacterial replication by interfering with bacterial DNA replication and RNA production, (2) destroying cell membranes, (3) interfering with cell respiration, and (4) changing enzyme conformation and inactivating enzyme activity [46] (Table 1).

There are two types of mechanisms of antimicrobial action of AgNPs: inhibitory action and bactericidal action [47]. The mechanism involves the formation of reactive oxygen species (ROS) resulting from the inhibition of a respiratory enzyme by the $Ag+$ ions, which kills the cell. The bacterial cell contains sulfur and phosphorous, which are the soft bases that interact with the AgNP as soft acid, leading to apoptosis [48].

Biocompatibility, high surface reactivity, antibacterial, antioxidation, antiplasmon resonance, and other necessary properties make AuNPs an integral part of the field of therapeutics and diagnosis [49]. AuNPs could inhibit the lipid from peroxidation and prevent the formation of ROS to restore antioxidant discrepancies.

ZnO NPs have been used in nanocomposites for wound healing applications as well as for skin infections. The mechanism involved is (a) the inhibitory action and (b) the bactericidal action [48] (Figure 2).

The protection against oxidative stress damage for wound treatment by CeO_2 NPs and Y_2O_3 NPs can be explained by the three possible mechanisms that follow [50]: (a) these nanoparticles act as direct antioxidants and restrict the generation of ROS, which inhibits the programmed cell death pathway; (b) these nanoparticles directly cause a low level of ROS production, which rapidly induces the ROS defense system before the glutamate-induced cell death program is complete; and (c) the latter is a form of preconditioning that may be caused by the exposure of cells to particulate material known to induce low levels of ROS.

2.3. Achieve Sustained Drug Release. A controlled-release drug delivery system minimizes the side effects of drugs by delivering active substances to the site of action, and this

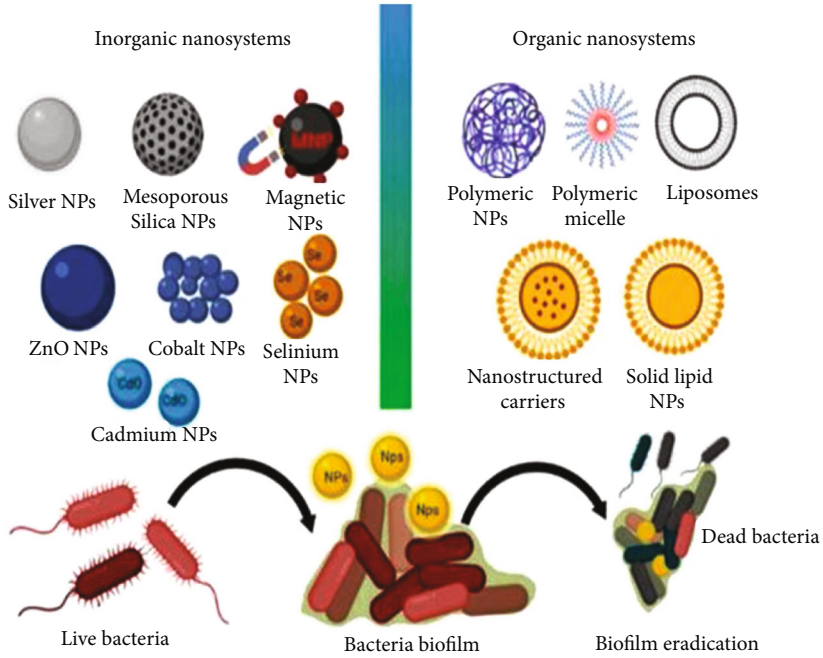

Figure 2: The role of nanomaterial in wound hydrogels. Panel is reproduced with permission from ref. [42]. Copyright © 2020MDPI.

type of system has attracted widespread attention [51]. The goal of a controlled-release drug delivery system is to achieve the temporal and spatial distribution of drugs. Nanomaterials are biocompatible, biodegradable, and nontoxic [23]. They combine the characteristics of hydrogels (high water content and flexible mechanical properties) in drug delivery (Figure 3). Fortunately, advances in nanomaterial have promoted the development of smarter nanocarriers, such that various drugs can be packaged, and the wound can be treated in a better way, thereby improving patient compliance. In addition, the nanohydrogel surface can be combined with different types of ligands to improve site-specific delivery, thereby reducing toxicity [52]. Various forms of nanocontrolled release systems are realized by encapsulating or loading drugs (Table 1), including nanospheres, nanogels, solid lipid nanoparticles (NPs), polymer nanoparticles, nanoemulsions, nanofiber mats, graphene-based nanocomposites material, and other forms [53]. In recent years, the incidence of various infectious diseases has increased significantly, which has placed a huge burden on the global economy and public health. Although antibiotics have played a critical role in wound treatment, the abuse of antibiotics has led to the emergence of drug-resistant pathogens, such as Pseudomonas aeruginosa, methicillin-resistant Staphylococcus, and vancomycin-resistant enterococcus [54, 55]. In the process of wound healing, nanomaterials can directly deliver antibacterial drugs to the wound site to make them continue to work [56], reduce the production of multidrug resistant bacteria, and promote wound healing. In addition, they eliminate the main problem of conventional dosage forms—frequent administration—which is beneficial for the treatment of chronic wounds.

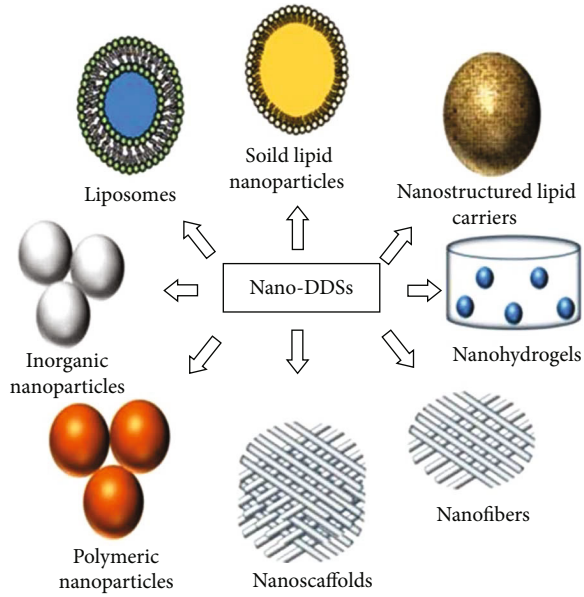

Figure 3: The role of nanotechnology in wound hydrogels. Panel is reproduced with permission from ref. [47]. Copyright © 2019 Wei Wang et al.

2.4. Increased Adhesion. For drug delivery, bioadhesion refers to the attachment of a drug carrier system to a designated biological location. The biological surface can be epithelial tissue or a mucous coating on the surface of the tissue. If it adheres to the mucus coating, this phenomenon is called adhesion [57]. Mucosal adhesions should not be confused with biological adhesions or bioadhesions. In bioadhesion, the polymer attaches to the biofilm. If the

Table 2: The common materials used to design nanohydrogel for wound repair.

Type	Advantage	Application
Collagen-based hydrogel	Forms microenvironment similar to extracellular mechanisms	Spontaneous migration of fibroblasts [73], antibacterial [26, 58, 67], and improve mechanical properties [61]
Gelatin-based hydrogel	Good biocompatibility, solubility	Improved mechanical properties [59], adhesion [71], antibacterial [68], drug release [65], and regeneration [71]
Fibrin-based hydrogel	Good adhesion, biocompatibility	Increase mechanical strength [62, 63] and regeneration [62]
Chitosan-based hydrogel	Good hemostatic properties	Increase mechanical strength [72], adhesion [60], antibacterial [74], and drug release [66]
Cellulose-based hydrogel	Most distributed, content	Increase the scope of application [75], antibacterial [69, 75], drug release [69], and regeneration [49]
Hyaluronic acid-based hydrogel	Good hydrophilicity, biocompatibility	Increase mechanical strength [64] and regeneration [76]
Polyethylene glycol-based hydrogel	High molecular polymer	Promote cell proliferation [77], antibacterial [77], and drug release [70]

substrate is a mucous membrane, the polymer adheres to the mucous membrane. Combining nanotechnology with hydrogels can improve the adhesion of hydrogels and the efficiency of drug delivery by covalent coupling and noncovalent complexes, for example. Furthermore, hydrogels can be used directly on the wound site and can fill the wound area to promote wound healing and the growth of hair follicles and capillaries (Table 1).

2.5. Nanoenzyme. A nanoenzyme is a kind of mimetic enzyme that has the characteristics of nanomaterials and the catalytic performance of natural enzymes. Compared with natural enzymes, nanoenzymes have the advantages of high stability, strong catalytic activity, and low cost, so they are widely used in disease diagnosis, treatment, and biosensing. The oxidoreductase activity of nanoenzymes includes peroxidase, catalase, and superoxide dismutase, for example. The catalytic activity of nanoenzymes is determined by the electron transfer process on the surface. At present, the design of nanoenzymes focuses on improving the catalytic activity of nanoenzymes. Although the activity of nanoenzymes has been greatly improved, it is a challenging task to use nanoenzymes to construct multifunctional biological materials so as to meet the needs of different environments [58].

3. The Common Materials Used to Design Nanohydrogel for Wound Repair

Wound healing is a complex pathophysiological process involving a variety of cytokines, growth factors, blood, and the ECM [61, 73]. It is a dynamic and complex phenomenon consisting of three main continuous events: inflammation, cell proliferation, and remodeling [67, 78, 79]. Wound healing is divided into four stages [71]: hemostasis, inflammation, new granulation, and tissue remodeling [65]. Treatment at each phase is conducive to wound healing. The natural polymer hydrogel dressings reported in recent years can improve the microenvironment of the wound and promote healing at different stages. It is expected to play a pivotal role in wound healing (Table 2).

3.1. Collagen. Collagen is a common protein in the human body and an important component of the ECM. In addition to being an indispensable part of the body, collagen also promotes cell migration and protein secretion. As a collagen hydrogel can form a microenvironment similar to that of the ECM on the wound surface, it has obvious advantages in wound healing. However, the mechanical properties of collagen hydrogels are poor, and the degradation rate is relatively fast, which limits further clinical application [59]. Therefore, adding nanoparticles or chemical cross-linking can improve mechanical properties and stability. In addition, this addition can also provide anti-inflammatory, antioxidant, and antibacterial effects [68, 80]. Moreover, the combination of fiber membranes prepared by nanotechnology and collagen hydrogels is also a useful method, which not only enables the spontaneous migration of fibroblasts but also promotes local cell proliferation activity [81]. Sun et al. [62] adopted electrospinning technology to load zinc oxide on collagen/chitosan nanofibers. Curcumin- (CUR-) chitosan nanoparticles (CSNPs) can also be impregnated into collagen scaffolds. Moreover, CSNPs can improve the stability and solubility of CUR, play an anti-inflammatory and antioxidant role, protect the wound surface, and promote healing [32].

3.2. Gelatin. Gelatin is a hydrolysate of collagen and is widely used in food processing and biomedicine. Because its structure is similar to collagen, it has good biocompatibility, poor mechanical properties, and faster biodegradation than synthetic polymers in wound treatment. However, the difference is that the solubility of gelatin is significantly better than that of collagen, which greatly promotes its application in 3D printing [63]. Modified by electrospinning technology, the nanohydrogel fiber membrane formed by modified gelatin can be used as a cell presentation system and promote the healing of rabbit full-thickness skin wounds by transplanting human umbilical vein endothelial cells [82]. A recent study showed that the mechanical properties and adhesion of gelatin methacryloyl (GelMA) hydrogels modified with silicate nanosheets (Laponite) were significantly improved, and they exhibited the sustained

release of epidermal growth factor (EGF) and the ability to stop bleeding to stimulate complete skin regeneration [66]. Lin produced a multifunctional three-layer wound dressing (sandwich dressing). The inner layer consists of activated carbon fiber and gentamicin, and the outer layer consists of gelatin/chitosan/EGCG nanoparticles and a c-PGA gelatin hydrogel. While preventing bacterial infection and controlling inflammation, this dressing was easy to remove from the wound, promoted the reepithelialization of wound tissue, and accelerated the wound healing process [60]. Xu discovered a composite hydrogel. The hydrogel template is stabilized by a colloidal hybrid of carbon nanotubes (CNTs) and gelatin methenoyl (GelMA) and then undergoes in situ polymerization and antimicrobial peptide incorporation, which can significantly improve the electrical conductivity and mechanical properties of the hydrogel. The GelMA inside of it was found to support cell adhesion and proliferation [72]. The chitosan nanoparticles loaded with curcumin were mixed into the fiber network scaffold of electrospun polycaprolactone and gelatin. The nanoparticles improved its hydrophilicity, wettability, and degradability, enhanced the effect of wound healing, and also played a role as an anti-inflammatory agent, promoting cell adhesion and proliferation [74].

3.3. Fibrin. Fibrin is a type of protein that is mostly found in blood and is insoluble in water. It has good biocompatibility and adhesion. Currently, fibrin glue is often used clinically as a hemostatic agent, wound healing agent, and plugging agent. In the treatment of trauma, protein-based polymers also play an important role. In recent years, autologous fibrin glue has been widely used in clinical practice because it is rich in cell growth factors and reduces the risk of viral infection and allergic reactions [83]. However, fibrin glue has relatively poor stability and is easily hydrolyzed [69]. Wang et al. prepared fibrin-silica hydrogel. The nanofiber hydrogel exhibited higher mechanical properties than pure fibrin while retaining its ability to support the proliferation of myoblasts, which had a great effect on the formation and regeneration of tissues [75]. According to a study by Scionti et al. [84], adding nanoparticles to the fibrin-agarose hydrogel significantly increased the density of chemical bonds and improved the mechanical properties of the hydrogel.

3.4. Chitosan. Chitosan (CS) is a product of chitin N-deacetylation and a natural polymer with huge reserves. CS is the only alkaline polysaccharide in nature because of its positively charged amino group [64, 76, 85]. This endows CS with many important characteristics of biomedical value, including among others excellent biocompatibility, biodegradation, nontoxicity, adhesion, antimicrobial, antioxidative, and hemostasis [70, 77, 86]. Because of its hemostatic activity, excellent biocompatibility, and antibacterial effect, it is widely used in biomedical fields such as wound dressings, slow drug release, gene transduction, and tissue engineering [87]. At present, we often increase the mechanical strength of chitosan hydrogels by modifying, cross-linking other polymer materials, and electrospinning, thereby increasing its application scenarios. According to Kumbar's research, chitosan microspheres can be cross-linked by several methods, including the chemical substances glutaraldehyde and sulfuric acid, and heat treatment for drug encapsulation and delivery [88]. Genipin was added to the nanocomposite as a cross-linking agent, which could effectively control the release of the drug from the hydrogel by forming a physical bond. To overcome the disadvantage of poor mechanical properties, Xie et al. synthesized a chitosan hydrogel based on an alkaline urea aqueous solution, using Ag nanoparticles as a filler and secondary reinforcing agent, and using the amino group of chitosan as a chelating agent. Xie et al. improved the mechanical strength of the chitosan hydrogel through coordination interaction [89].

3.5. Cellulose. Cellulose is the most widely distributed and polysaccharide in nature that is used in many fields, such as food processing, construction, and biomedicine. Cellulose has good mechanical strength and thermal stability, but it does not have antibacterial activity. In addition, its poor hydrolysis greatly limits its application. Nanocellulose prepared by nanotechnology opens up new application scenarios. Nanocellulose is divided into three main categories: bacterial nanocellulose (also known as microbial cellulose or biocellulose), cellulose nanofibers (also known as nano/microfiber cellulose), and cellulose nanocrystals (also known as nanocrystalline cellulose). Among them, bacterial nanocellulose is applied in antibacterial wound healing and biosensing. By carrying antibacterial and healing factors, a bacterial nanocellulose wound dressing can safely and effectively promote wound healing [90]. Koneru used citric acid (CA) croscarmellose sodium (NaCMC)/hydroxypropyl methylcellulose (HPMC) to prepare a hydrogel film and loaded it with grape seed extract (GFSE). This hydrogel showed excellent antibacterial properties. So it could be used as an antibacterial dressing to meet the needs of wound healing [91]. Loh et al. studied a nonbiodegradable bacterial nanocellulose/acrylic acid (BNC/AA) hydrogel to explore the potential of transferring human dermal fibroblasts (HDF) to the wound surface and the healing effect of HDF in breast-free mice after transfer. The results showed that hydrogel had good properties. Thus, it is beneficial to wound healing and can be used as a wound dressing and cell carrier [92]. To solve the problems of bacterial infection and uncontrollable bleeding during wound healing, Liu et al. designed a green nanocomposite hydrogel, which is a noncovalent (dynamic ionic bridge) cross-linked hydrogel, by introducing aminated silver nanoparticles (Ag-NH_2NPs) and gelatin (G) into carboxylated cellulose nanofibers (CNF). The hydrogel had strong mechanical properties, self-healing properties, antibacterial properties, good hemostatic properties, and a suitable liquid balance on the wound surface. More importantly, it showed excellent biocompatibility and wound-healing effect, so it can be used as an improved wound dressing [93].

3.6. Hyaluronic Acid. Hyaluronic acid (HA) is a natural acid mucopolysaccharide. HA has excellent hydrophilicity and biocompatibility and is often used as a filler in cosmetology,

ophthalmology, and joint surgery. Further, as an ECM, it also plays an important role in wound healing and tissue regeneration. In recent years, with the promotion of the theory of wet wound healing, the potential application value of HA as a dressing matrix has been fully developed. Electrospinning technology, embedded metal nanoparticles, and other methods can strengthen the mechanical strength of HA dressing, allowing it to play a role as an antibacterial agent, promote cell proliferation and adhesion, and effectively promote wound healing [94, 95]. Karimi Dehkordi et al. developed a composite material, that is, nanocrystalline cellulose- (CNC-) reinforced HA chitosan nanoparticles, which improved the mechanical properties of HA-based composite materials. As an effective wound dressing, composite materials have good mechanical properties, release GM-CSF slowly, enhance reepithelialization, and provide an improved healing environment [96]. Moreover, Uppal et al. studied a wound dressing formulation based on HA nanofibers; compared with natural solid HA biomaterials, these HA nanofibers helped cell migration and proliferation, promoted tissue growth, and accelerated wound healing [97].

3.7. Polyethylene Glycol. Polyethylene glycol (PEG) is a high-molecular polymer, and different groups can be grafted to the end of PEG according to requirements, which gives it application potential. The adjustable group at the end of PEG can undergo a variety of cross-linking reactions with natural polymer materials, strengthen the internal force of the hydrogel, and improve the mechanical properties of the hydrogel. This can promote the proliferation of wound cells to the greatest extent, and at the same time, improve the sustained release ability of hydrogel drugs. Chu et al. prepared a new type of collagen-nanomaterial-drug hybrid scaffold mediated by PEG that promotes the attachment, proliferation, differentiation of mesenchymal stem cells (MSC), and collagen deposition in diabetic wound repair and angiogenesis [98]. Guo et al. found that polyethylene glycol diacrylate (PEGDA) core/alginate shell-structured hydrogel particles were formed by one-step microfluidic droplets. The granular hydrogel did not contain toxic organic solvents and had good wetting properties, could control the release of corticosteroids, and accelerated wound healing and scar treatment [99]. Li et al. researched and prepared a nanocomposite scaffold composed of PEGDA, forming the main network, and a secondary dynamic network (PABC scaffold) formed between copper-containing bioactive glass nanoparticles (BGNC) and sodium alginate (ALG). It showed strong antibacterial activity and the ability of self-healing and could significantly enhance wound healing and skin tissue formation by promoting early angiogenesis [100].

4. Conclusion

Based on current research, we know that hydrogels have shortcomings, such as low mechanical strength, high brittleness, and poor antibacterial ability. Thus, their application to wounds is greatly restricted. Nanomaterial can play a critical role in hydrogels, which improves the application potential of nanohydrogels as wound dressings. Nanotechnology not only improves the mechanical properties, water-solubility, and cell adhesion of the hydrogel but also packages or loads various particles with antibacterial or biological activity in the nanohydrogel to achieve long-term slow drug delivery and play a better curative effect than conventional topical drugs. Moreover, owing to the large surface area of the nanoparticles, a small number of drugs can play a better role, which also reduces the toxicity of the drugs to a certain extent. Therefore, the combination of nanomaterial and hydrogels has unlimited possibilities and will be further explored in future research.

Conflicts of Interest

There is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Peige Wang and Xiaoyan Zhu designed the review. Yangyang Liu and Shurui Song both reviewed the paper. Shuangyong Liu participated in the design and drafting of this paper. Peige Wang and Xiaoyan Zhu criticized the original paper. All authors read and approved the final manuscript.

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Research Article

Liquid-Assisted Electrospinning Three-Dimensional Polyacrylonitrile Nanofiber Crosslinked with Chitosan

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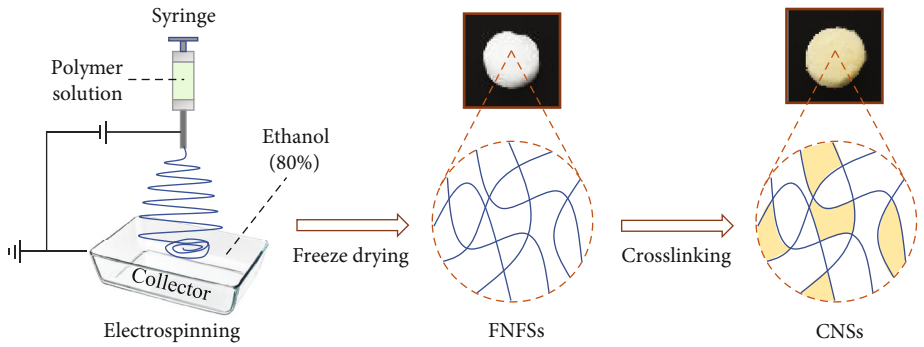
Electrospinning has become a popular nanotechnology for the fabrication of tissue engineering scaffolds, which can precisely regulate fiber diameter and microstructure. Herein, we have prepared a three-dimensional polyacrylonitrile (PAN) nanofiber by liquid-assisted electrospinning. The spacing between PAN nanofibers can reach to 15-20 μm, as the uniform internally connected pore structure can be formed, through the regulation of parameters. Furthermore, the chitosan attached to the as-prepared nanofibers gives the material antibacterial effect and increases its biocompatibility. Meanwhile, the special structure of chitosan also provides the possibility for further loading drugs in dressings in the future. This newly developed nanocomposite seems to be highly suitable for wound healing due to its unique properties of biodegradability, biocompatibility, and antimicrobial effectiveness.

1. Introduction

As the largest organ of the human body, the skin is the first barrier for the human body to protect itself. However, due to the physical characteristics of the skin and its associated soft tissue, it is vulnerable to external stimulation and is damaged easily. In China, the number of trauma patients seeking medical treatment is as high as 62 million times per year, among which the number of deaths is about 600-700 thousand. Therefore, there is a great clinical demand for trauma repair.

Skin dressing materials can replace the damaged skin and resist the secondary injury caused by external mechanical factors, such as the invasion and touch of foreign bodies, as well as resist the secondary pollution and injury caused by chemical factors, and prevent the wound from drying and the loss of body fluid. Traditional medical dressings mainly include cotton ball, cotton gauze and other natural fiber materials, which play the role of physical isolation. However, there are some defects, such as retearing at the scab of the wound during cleaning, easy infection of the wound, and poor hemostatic performance. Biomedical dressings can overcome those shortcomings and become the focus of researchers.

Electrospinning is an effective, versatile, and scalable technique of preparing the skin dressing materials from a variety of synthetic or natural polymers with diameters down to the nanoscale [1, 2]. Moreover, there are many controllable parameters to control the fiber diameter, morphology, and secondary structure more accurately. In particular, the use of liquid as a receiving device for electrospinning nanofibers could obtain the interconnected 3D (three-dimensional) structure [3, 4]. Compared with most multilayer electrospinning techniques [5, 6] and template techniques [7], the 3D electrospinning technique of liquid reception is more concerned with increasing fiber spacing than with the construction of macroscopic 3D structure [8–10]. And compared



Scheme 1: Schematic illustration of the setup for liquid-assisted electrospinning.

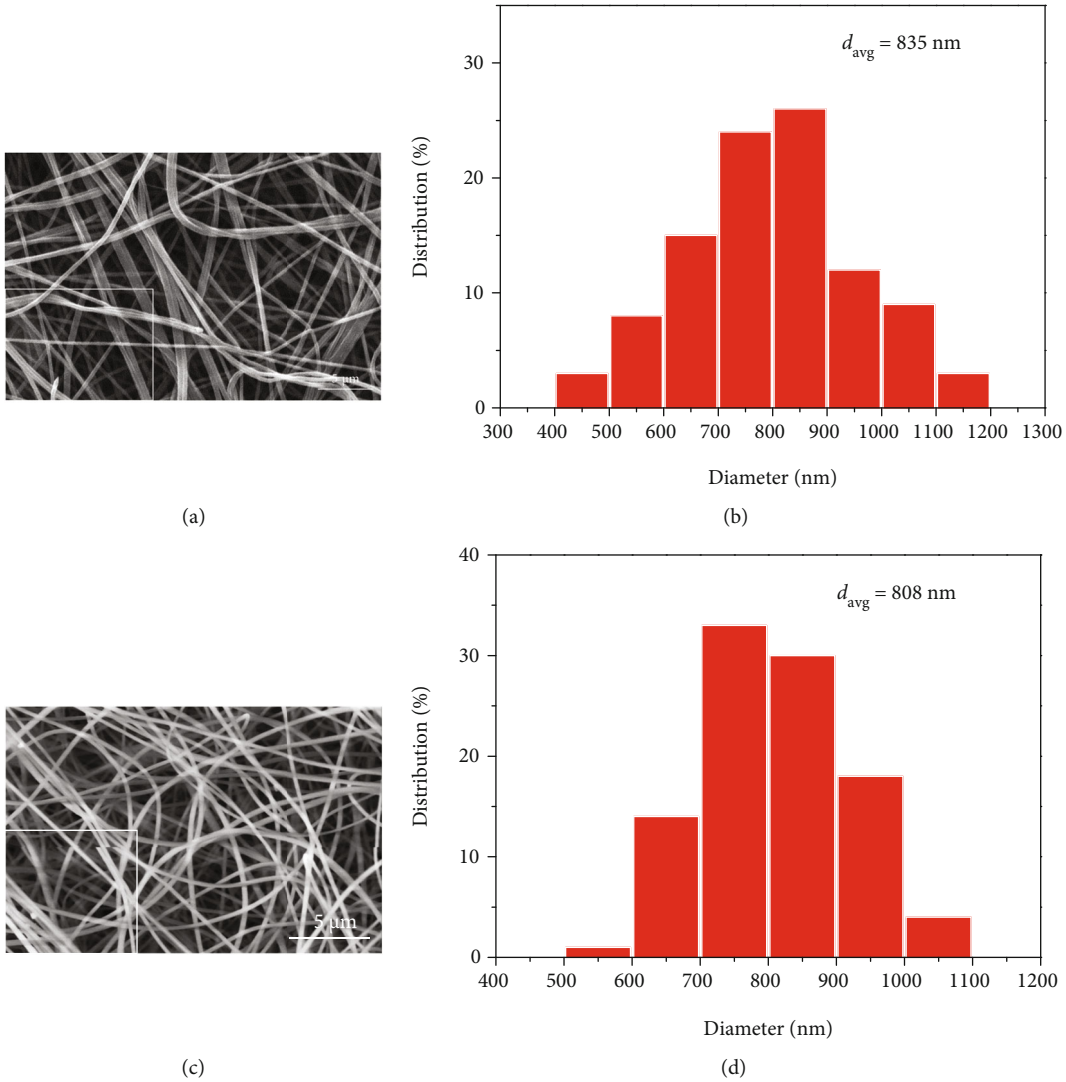


Figure 1: (a) SEM images and (b) fiber diameter distribution of electrospinning 2D-PAN fiber mats; (c) SEM images and (d) fiber diameter distribution of the electrospinning 3D-FNFS nanofiber.

with the pore-forming agent technique [11], the aperture is not too large and the pore distribution is more uniform [3]. In addition, this technique is convenient to operate and does not need other complex postprocessing technology such as salt leaching or template removal [12].

Polyacrylonitrile (PAN) is a kind of semicrystalline synthetic organic polymer, which is an important raw material of the ultrafiltration membrane, reverse osmosis hollow fiber, fabric fiber, and carbon fiber [13]. Meanwhile, the cost of industrial PAN staple fibers is low while the mechanical

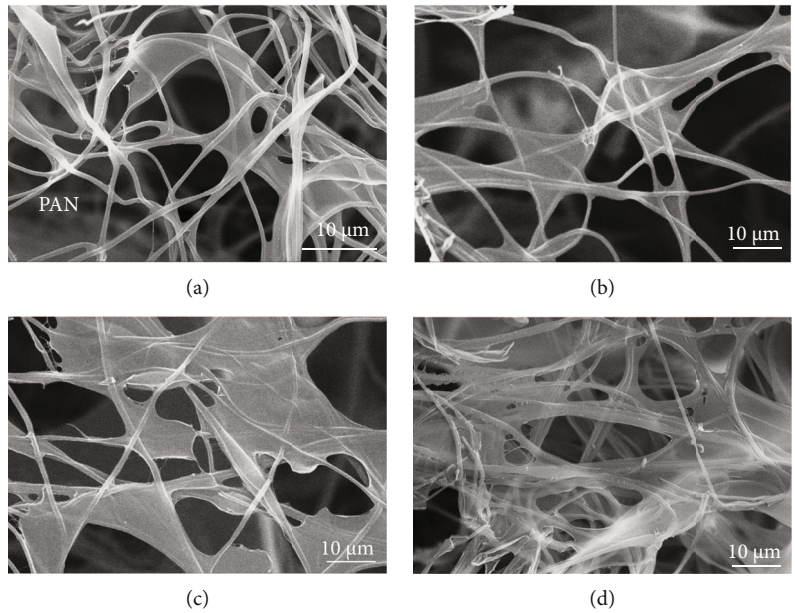


Figure 2: SEM images of (a) $CNS_{0.050}$, (b) $CNS_{0.075}$, (c) $CNS_{0.100}$, and (d) $CNS_{0.125}$ (scale bar, 10 μm) composites.

properties are stable. In addition, PAN solution in electrostatic field is easy to be spun into silk, which could provide the cells with an environment that closely resembles their native extracellular matrix (ECM). It is expected that compared with general materials, it has better cytocompatibility, which is conducive to promoting the reproductive growth of wound cells and accelerating wound healing. In addition, 3D structure provides more crosslinking sites, which is conducive to the adhesion of materials and the adsorption of tissue fluid [14–16]. Consequently, electrospinning nanofibers are considered to be an effective materials in tissue engineering application, which is of great significance in real life [17–20]. As a biomaterial with rich pore structure, electrospinning nanofibers can be used as scaffold for three-dimensional cell culture in tissue engineering [21]. Moreover, due to their porous structure which provides sufficient space for cells' uniform distribution and facile delivery of oxygen and nutrients, the 3D nanofiber can mimic the topology and biological functions of the extracellular matrix (ECM) for guided cell growth.

In this study, we report the preparation of 3D PAN nanofiber scaffolds by electrospinning and crosslinking with different concentrations of chitosan. The 3D network structure was formed by the stacking of one-dimensional nanowires, and the amino group of chitosan was used to complete the crosslinking to enhance the antibacterial property of the material. These scaffolds were then characterized by analyzing the morphological structure and chemical structure, as well as examining the cytotoxicity and antibacterial property.

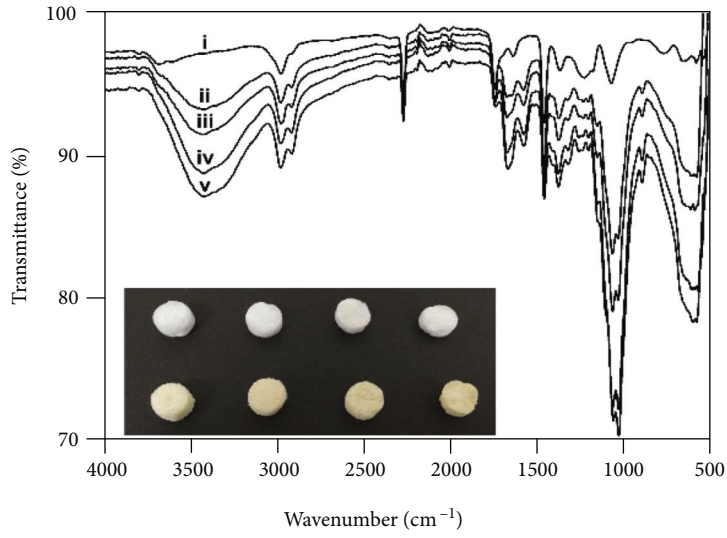
2. Materials and Methods

2.1. Materials. N,N-Dimethylformamide (DMF, ACS reagent), chitosan, glutaraldehyde, acetic acid, glycine, and other reagents used for the preparation of PBS buffer were purchased from Sigma-Aldrich. To prepare the PBS buffer, 8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, and 0.24 g KH_2PO_4 were dissolved in 800 mL deionized water. HCl solution and NaOH solution were used to adjust the pH to 7.4, and finally, the solution was diluted with deionized water to 1000 mL. The PAN staple fibers (Macklin) were dissolved in N,N-dimethylformamide (DMF) at a concentration of 10% (*w*/*v*) and magnetic stirred at 50°C overnight to obtain the electrospinning precursor solution. Other cell culture media and supplements were purchased from Sigma.

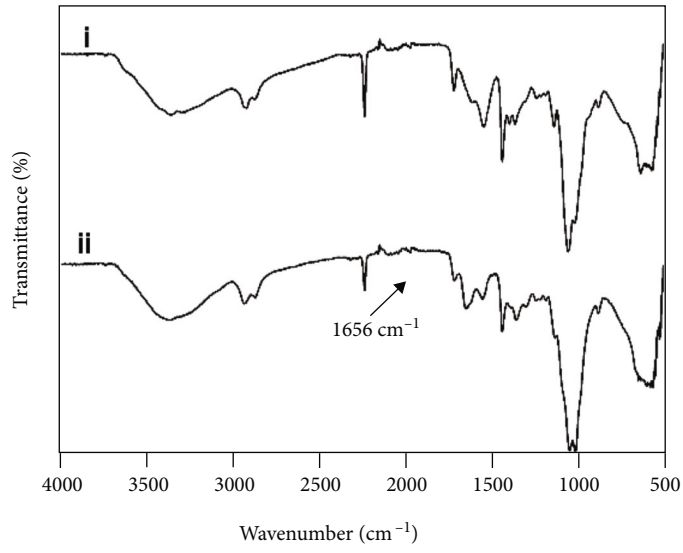
2.2. Electrospinning. The solution for electrospinning was placed in a 1 mL syringe equipped with a 23-gauge needle. The solution was dispersed by a syringe pump at a feeding rate of 1.0 mL/h, with a humidity of 40-53%. The collector was a conductive metal square shell container covered with aluminum foil, wherein the liquid was a mixed solution of ethanol and water (4 : 1, *V*/*V*). The typical distance between the syringe tip and the collector was 10 cm. An electrospinning voltage of 11-12 kV was applied between the syringe tip and the aluminum foil.

The resultant electrospinning nanofibers were collected into a beaker which contained a mixed solution of ethanol and water by a glass rod on the collector. The as-prepared nanofibers were then washed with deionized water for three times. Finally, the appropriate amount of electrospinning nanofibers was allocated to the 12-hole plate or 48-hole plate and freeze dried overnight to obtain the fluffy nanofiber sponges (FNFSs).

2.3. Crosslinking. The chitosan was dissolved in 1.0% (*v*/*v*) acetic acid at a concentration of 0.050%, 0.075%, 0.100%, and 0.125% (*w*/*v*). The as-prepared FNFSs were immersed into the chitosan solutions and then shocked slightly, followed by freeze-drying overnight to remove solvents.



(a)



(b)

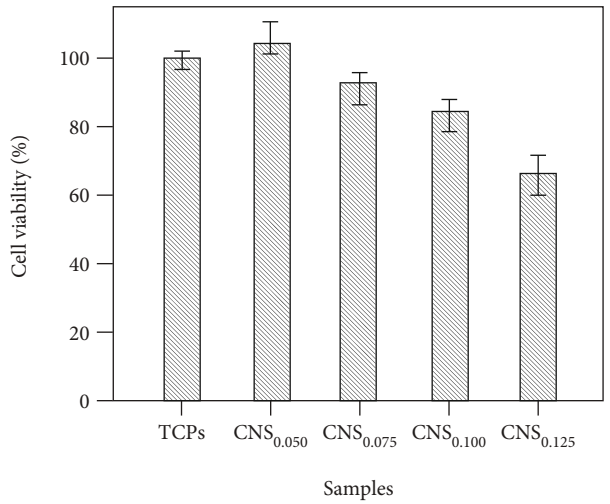
Figure 3: (a) FTIR spectra of PAN (i), $CNS_{0.050}$ (ii), $CNS_{0.075}$ (iii), $CNS_{0.100}$ (iv), and $CNS_{0.125}$ (v), with the inset showing the photographs of $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ from left to right (lower row) and their corresponding constructs before crosslinking (upper row). (b) FTIR spectra of $CNS_{0.100}$ before (i) and after (ii) crosslinking.

A culture dish contained 25% (*v*/*v*) glutaraldehyde solution was placed at the bottom of a dryer. The samples treated with chitosan were placed on the bracket above the culture dish at 40°C for 24 h. The samples were then immersed in glycine solution (0.1 M) to block the unreacted aldehyde group. Then, the samples were rinsed with PBS three times and dried to obtain the chitosan-coated nanofiber scaffolds (CNSs). The CNSs containing chitosan were named $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ according to their respective chitosan concentration.

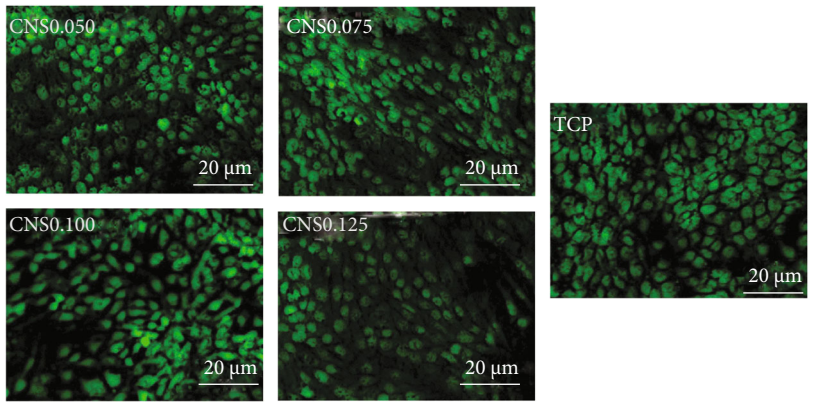
2.4. Characterization. Infrared spectra of the PAN and CNS were obtained using a Nicolet iS10 FTIR instrument (Thermo Fisher Scientific, Waltham, MA) with wavenumber ranges from 400 to 4000 cm^{-1} by accumulating 16 scans at a resolution of 5 cm^{-1}. The morphologies of three-dimensional FNFS, two-dimensional fibrous membrane obtained from the aluminum foil, and CNS with different concentrations of chitosan solution were observed by scanning electron microscopy (SEM). Prior to imaging, the specimens were sputtered with gold.

2.5. Cell Cytotoxicity Assays. The biocompatibility of the as-prepared composites was evaluated by 3T3 fibroblast cells. The 3T3 fibroblast cells were cultured on CNSs with different concentrations of chitosan solution, as the cells cultured on TCPs in the microplate were tested as the blank control.

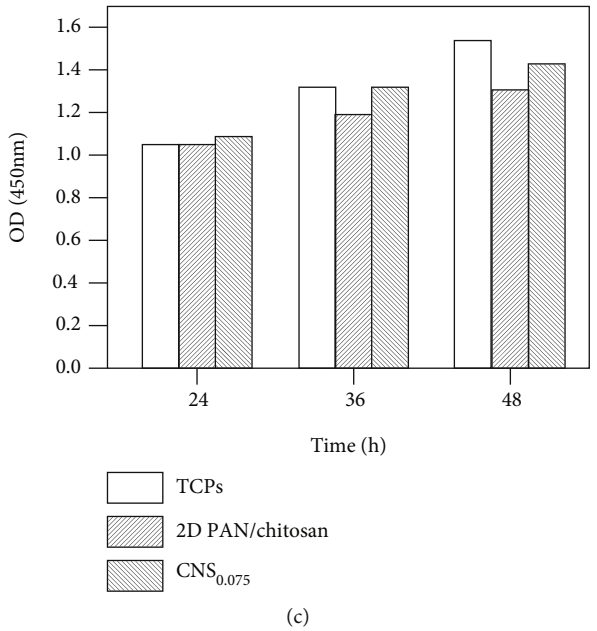
TCP, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ were examined in 5 groups. Before cell inoculation, all samples were immersed in 75% ethanol-water (*v*/*v*) solution for



(a)

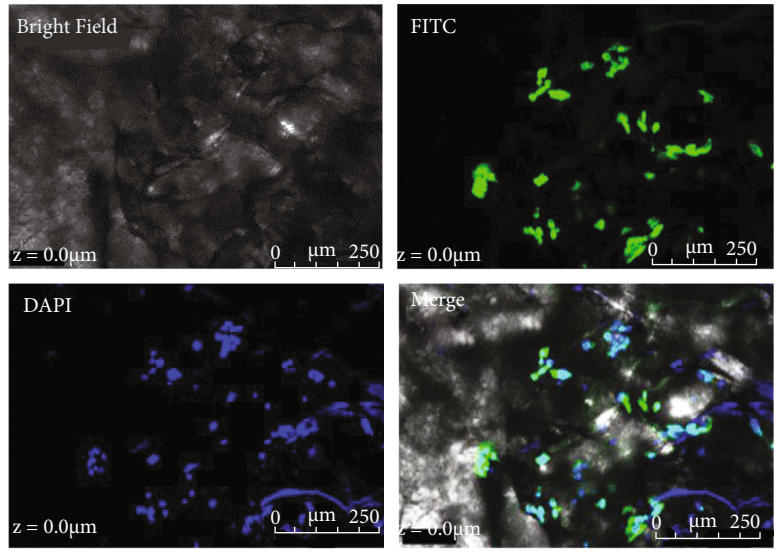


(b)



(c)

Figure 4: Continued.



(d)

FIGURE 4: (a) Cell viability and (b) fluorescence images of 3T3 fibroblast cells cultured on the TCPs, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and CNS_0; (c) the proliferation of 3T3 fibroblast cells cultured on the TCPs and $CNS_{0.075}$ (24 h, 36 h, 48 h); (d) fluorescence images of 3T3 fibroblast cells cultured on $CNS_{0.075}$.

30 min and treated by ultraviolet radiation for another 30 min in the 24-well microplates. Thereafter, 3T3 fibroblast cells were seeded onto CNSs at a density of 1.5×10^4 cells per well. 3T3 fibroblast cells were cultured in medium (DMEM, 10% *v*/*v* fetal bovine serum and 1% *v*/*v* penicillin/streptomycin solution) at 37°C under 5% CO_2 in a cell culture incubator for 2 days.

The cell samples were washed with PBS for three times. Then, the cells were fixed with a 4% formaldehyde solution and stained with a Fluo-4 AM (2.5 mM) solution. The standard MTT [22] assay was used to examine the viability of cells. Each sample was retested three times. The fluorescent images were taken by a fluorescence microscope (Olympus) configured with a Nuance CCD camera.

2.6. Cell Proliferation Assay. According to the same operation for the cell cytotoxicity assays, 3T3 fibroblast cells were cultured on the standard tissue culture plates (TCPs), two-dimensional PAN/chitosan cellulose membrane, and $CNS_{0.075}$. The samples were taken for 24 h, 36 h, and 48 h. The wavelength of OD was measured at 450 nm. The standard MTT assay was used to examine the cell proliferation. The cells were fixed with a 4% formaldehyde solution and stained with a 4′,6-diamidino-2-phenylindole (DAPI) solution. Immunostaining the cytoskeleton of 3T3 fibroblast cells with FITC-labeled anti-Actin antibody was done as per the well-documented methods.

2.7. Antibacterial Experiment. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were selected and inoculated in 100 mL HS medium. The HS culture medium was composed of glucose 25, yeast extract 5, peptone 5, citric acid monohydrate 1.2, and Na_2HPO_4 2.7 ($g\,L^{-1}$). After inoculation, they were placed in the shaking table and shaken for 24 hours. 100 cfu mL^{-1} *E. coli* and *S. aureus* bacteria cells was grown in 50 mL liquid LB medium supplemented with 10 $\mu g\,mL^{-1}$ CNS_0, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$. The antibacterial activities of CNS_0, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ were determined by the shaking flask method. Growth rates and bacterial concentrations were determined by measuring the optical density (OD) of LB in broth medium at 600 nm every 6 hours.

3. Results and Discussion

3.1. Morphology of Fluffy Nanofiber Sponges (FNFSs). We successfully fabricated the fluffy nanofiber sponges (FNFSs) by the optimized electrospinning technique. The electrospinning process as shown in Scheme 1 was employed to fabricate the nanofibers in this study. 80% ethanol-water solution was used as the receiving device for electrospinning nanofibers to obtain the interconnected 3D (three-dimensional) structure. The SEM micrographs of three-dimensional FNFSs and two-dimensional fibrous membrane obtained from the aluminum foil are shown in Figure 1. From it, we can see that the fiber spacing of the two-dimensional fibrous membrane was less than 5 μm (Figure 1(a)), and fibers were accumulated and entangled mostly, with an average diameter of about 835 nm (Figure 1(b)). However, fiber spacing of the three-dimensional FNFSs could reach from 15 μm to 20 μm, and the fibers were obviously more dispersed from each other (Figure 1(c)) with an average diameter of about 808 nm (Figure 1(b)). The three-dimensional FNFS appeared as fibers with interconnected pores in the liquid environment (80% ethanol), presenting properties that were not available in two-dimensional fiber membranes (aluminum foil), which provided good application prospect in adsorption materials, tissue engineering, and other fields. This fact suggests that the three-dimensional FNFSs received by liquid are more stable, and the diameter distribution is narrower.

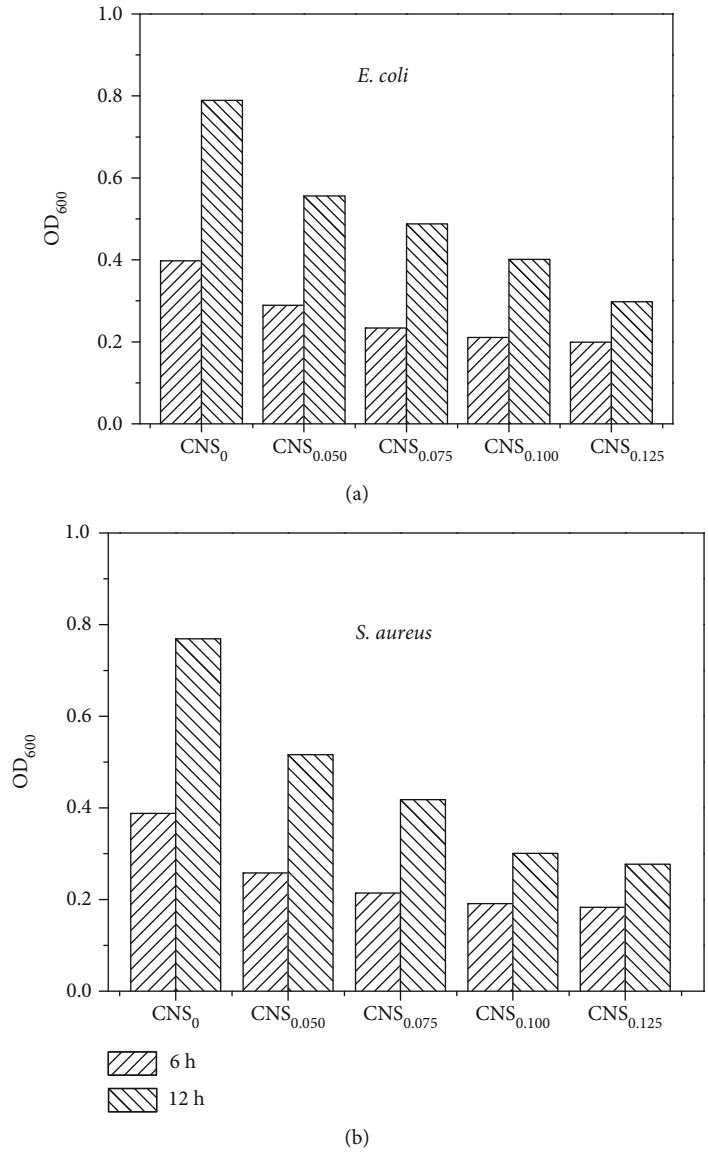


Figure 5: Bacteria suspension concentration (OD_{600}) of CNS_0, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ by *E. coli* (a) and *S. aureus* (b).

3.2. Characters of Chitosan-Coated Nanofiber Scaffolds (CNSs). The SEM micrographs of CNSs with different chitosan concentrations are shown in Figure 2. The chitosan looks like a layer of membranes at the surface of the CNSs, which do not affect the shape of the CNSs or the three-dimensional mesh structure. With the increase of the concentration of chitosan from 0.050% to 0.125% (*w*/*v*), the chitosan nanofibers loaded on CNS increased obviously. On the one hand, the incorporation of chitosan with synthetic CNS scaffolds had shown vital advantages in improving scaffold biocompatibility, maintaining morphology and structural stability of three-dimensional scaffolds under low pressure. On the other hand, scaffolds with enough interconnected space were favorable for cell growth, migration, and proliferation.

Figure 3 exhibits the FT-IR spectra of PAN and CNSs. Three intense peaks at 2933 cm^{-1}, 2242 cm^{-1}, and 1450 cm^{-1} belong to C-H bands, C≡N bands, and -CH_2 bands, respectively [23, 24]. The characteristic structures of PAN are observed both in the spectra of PAN and CNSs, showing the characteristic structures of PAN. There were three new peaks in the spectrum of CNSs appeared at 3368 cm^{-1}, 2870 cm^{-1}, and 1559 cm^{-1}, which could, respectively, be due to the telescopic vibration of the O-H, the telescopic vibration of the C-H, and the bending vibration of the N-H in molecular chitosan [25]. Taking these results into consideration, it was clear that the combined electrospinning scaffolds (CNSs) contained both PAN and chitosan. Figure 3(b) displays the FT-IR spectra of $CNS_{0.01}$ before (i) and after (ii) crosslinking. After crosslinking, the characteristic N=C telescopic vibration peak at 1656 cm^{-1} becomes stronger and the characteristic N-H bending vibration absorption peak becomes weaker. Combining the photos of CNS with the photos of CNS after crosslinking, it can be found that due to the formation of N=C bands, CNS becomes slightly yellow.

3.3. Cytotoxicity and Cell Proliferation of CNSs. The 3T3 fibroblast cells as model cells were cultured on CNSs with different concentrations of chitosan solution for 2 days. The cytotoxicity of different samples was evaluated by MTT cell viability assay. As shown in Figure 4(a), 3T3 fibroblast cells maintained nearly 104% and 92% of cell viability on the substance of $CNS_{0.050}$ and $CNS_{0.075}$, respectively, but only 87% and 67% of $CNS_{0.100}$ and $CNS_{0.125}$, respectively. The blank control experiments were included using the standard tissue culture plates (TCPs) for comparison with normalization. It suggests that both $CNS_{0.050}$ and $CNS_{0.075}$ exhibit very low cytotoxicity for 3T3 fibroblast cells. 3T3 cells were imaged with a fluorescent microscope after they were cultured on TCPs and CNSs with different concentrations of chitosan for 2 days. As shown in Figure 4(b), the number of cells cultured on $CNS_{0.050}$ and $CNS_{0.075}$ are similar to that on TCPs. It also confirmed the low cytotoxicity of $CNS_{0.050}$ and $CNS_{0.075}$ for 3T3 fibroblast cells. Meanwhile, it was obvious that the cells growing on smooth TCP were better distributed because of the freedom of cell migration, while the growth of the cells cultured on CNS presented local directivity, indicating that the cells basically grew towards the direction of the fibers. These results suggested that $CNS_{0.050}$ and $CNS_{0.075}$ could provide good biocompatibility and well-controlled microenvironments for the growth and proliferation of 3T3 fibroblast cells.

In order to investigate the proliferation of 3T3 fibroblast cells cultured on $CNS_{0.075}$, the control experiments were included using the standard tissue culture plates (TCPs) and the two-dimensional PAN/chitosan cellulose membrane for comparison with normalization. Figure 4(c) shows the proliferation of cells which were cultured for 24 h, 36 h, and 48 h. After 24 h in culture, the proliferation of cells on these three materials was almost the same. After 36 h in culture, the proliferation of cells on the two-dimensional PAN/chitosan cellulose membrane and $CNS_{0.075}$ began to be lower than that of TCPs. After 48 h in culture, the proliferation of cells on the CNS was about 90% of TCPs but higher than that on the two-dimensional PAN/chitosan membrane. Taken together, these results suggest that CNS three-dimensional scaffolds can provide good biocompatibility and well-controlled microenvironments for cell growth and proliferation, which can be applied to wound healing in the future.

From Figure 4(d), we can see that the 3T3 fibroblast cell growth forms between the multilayered structures of the materials. The cellular skeleton structure was immunostained with the primary antibody (Rabbit, antitubulin antibody) and the secondary antibody (Goat anti-Rabbit IgG, FITC-labelled by Phalloidin-Oregon). Cell nuclei were stained by Hoechst (DAPI). The images displayed that 3T3 fibroblast cells grew very well in the 3D space of $CNS_{0.075}$ substrates. This preliminary experiment suggests that the 3D-CNS composite has a good biocompatibility in terms of cell culture, resulting in good 3D microenvironments for cell culture.

3.4. Antibacterial Performance. The antibacterial performance of the CNS_0, $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ nanofibers was investigated by both Gram-negative bacterial strains, *E. coli*, and Gram-positive bacterial strains, *S. aureus* [26]. As shown in Figure 5, the bacterial OD_{600} values of $CNS_{0.050}$, $CNS_{0.075}$, $CNS_{0.100}$, and $CNS_{0.125}$ were lower than that of CNS_0 (the control group) in the experiments of relatively short incubation time (6 h, 12 h), on both *E. coli* and *S. aureus*, due to the antibacterial properties of chitosan [27]. Furthermore, the OD_{600} percentage of *S. aureus* bacterial suspension with CNS after 12 h cultivation is lower than that of *E. coli*, which indicated that CNS has better antibacterial properties for Gram-positive bacteria than Gram-negative bacteria [28].

4. Conclusions

Here, the fluffy nanofiber sponge was successfully prepared by controlling the process parameters of liquid-assisted electrospinning. Compared with the traditional electrospinning technology, the nanofiber spacing can reach to 15-20 μm, forming an interconnected pore structure. Then, chitosan was loaded on the prepared nanofibers and crosslinked. The composite scaffolds were used as three-dimensional scaffolds for 3T3 fibroblasts in vitro, and a series of cell experiments and in vitro antibacterial experiments were carried out. The results show that these composite three-dimensional scaffolds have low cytotoxicity, providing a good biological microenvironment for cell adhesion, migration, and proliferation, and are conducive to self-repair of damaged tissue in the wound. At the same time, these three-dimensional composite scaffolds have good antibacterial properties due to chitosan loading, which can effectively prevent wound infection in practical application, where traditional medical dressings cannot achieve. In addition, chitosan itself has excellent loading capacity, which is often used to make a carrier. The addition of chitosan provides great help for loading new drugs into wound dressings and simplifies the process. In the future, we believe that the excellent properties of this kind of CNS material will be used in biology, adsorption materials, tissue engineering, and other fields.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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