

Development and Properties of Electrospun Collagen-chitosan Nanofibrous Membranes as Skin Wound Healing Materials

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Abstract

The objective of this study was to develop an original anti-bacterial material for skin wound healing. Collagen-chitosan nanofibrous membranes were fabricated by electrospinning. The morphology, tensile strength and contact angle of the membranes were measured. In addition, cell adhesion and proliferation on the membranes were evaluated. The antimicrobial property against *Staphylococcus aureus* of the membranes was also determined. The results indicated that the diameter of electrospun collagen-chitosan nanofibrous membranes was 221 ± 105 nm, the tensile strength was 1.53 ± 0.12 Mpa and the contact angle was $42.44 \pm 4.03^\circ$. Besides, the collagen-chitosan nanofibrous membranes promoted cell attachment and proliferation and also inhibited the growth of *Staphylococcus aureus*. In conclusion, these data suggest that electrospun collagen-chitosan nanofibrous membranes have potential to serve as skin wound healing materials, which might be ascribed to its favorable mechanical strength, excellent cell affinity, as well as good antimicrobial properties against *Staphylococcus aureus*.

Keywords: Collagen; Chitosan; Nanofibers; L929 Cell

1 Introduction

Wound dressing can be used to temporarily protect the wound and prevent bacterial infection. However, there are issues that have to be addressed such as its poor biocompatibility and ease of degradation. In order to solve the existing problems, natural materials such as collagen and chitosan have attracted much attention due to their excellent biological properties. It is known that collagen is one of the major components of extracellular matrix (ECM) and exhibits low immunogenicity and good biocompatibility [1, 2]. Chitosan is obtained by alkaline deacetylation of chitin and presents biodegradability, antibacterial and antifungal activity [3]. The degradation products of collagen and chitosan are non-toxic, non-antigenic, non-immunogenic and

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non-carcinogenic. In this study, the collagen-chitosan nanofibrous membranes were developed by electrospinning. The morphology, contact angle and tensile strength of membranes were investigated. Meanwhile, the cell viability and the antimicrobial properties against *Staphylococcus aureus* of the collagen-chitosan nanofibrous membranes were evaluated and collagen nanofibrous membranes were selected as control group.

2 Materials and Methods

2.1 Fabrication of Electrospun Nanofibrous Membranes

Porcine collagen type I was purchased from Sichuan Mingrang Bio-Tech Co. Ltd (China). Chitosan was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd (China). The collagen and chitosan was dissolved at the weight ratio of 80:20 and the nanofibers were formed under high voltage.

2.2 Characterization

The morphology of electrospun nanofibrous membrane was observed by Scanning Electron Microscope (SEM) (JSM-5600SLV) and the diameter of nanofibers was measured. Mechanical strength was determined by a universal materials testing machine (H5K-S, Hounsfield, England). Surface wettability of the electrospun fibrous membranes was characterized by water contact angle measurement (OCA40, Dataphysics, Germany).

2.3 Cell Adhesion and Proliferation

The fibrous membranes were prepared on 14mm diameter round slides. L929 cells were seeded on the fibrous membranes at a density of 2.5×10^4 per well for an hour, and the morphology of the cells were observed by SEM. The proliferation of cells after 1, 3 and 5 days post-seeding was evaluated by the 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay at an absorbance of 570 nm and 630 nm. Cells cultured on the cover slips served as control group.

2.4 Determination of Antimicrobial Efficiency Against *Staphylococcus Aureus*

The antimicrobial activity of fibrous membrane was tested against *Staphylococcus aureus* (ATCC43300). After the incubation of the bacteria at a concentration of 1×10^6 colony forming units (CFUs)/ml with fibrous membranes for 24 h, the bacteria dislodged and diluted up to 10^{-5} were plated on appropriate agar and incubated at 37 °C overnight to count CFUs. Reduction in the number of CFUs represented the antimicrobial activity.

3 Results

3.1 Morphology of Electrospun Nanofibrous Membranes

The morphology and diameter of electrospun collagen-chitosan nanofibrous membranes were shown in Fig. 1. The diameter of collagen-chitosan nanofibrous membranes is 221 ± 105 nm, similar to that of collagen nanofibrous membranes (245 ± 93 nm).

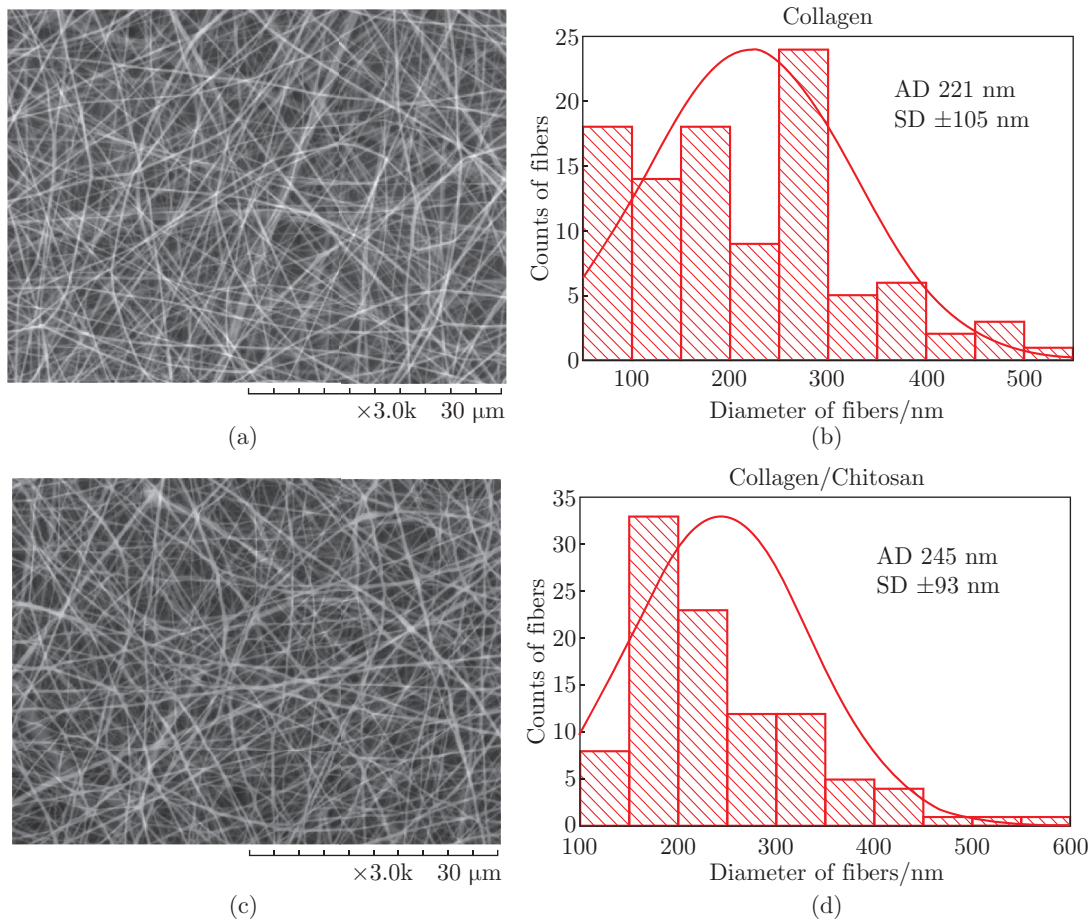


Fig. 1: SEM micrographs of nanofibrous membranes. (a) Collagen; (c) Collagen-chitosan. Fiber diameter distributions of nanofibrous membranes. (b) Collagen; (d) Collagen-chitosan

3.2 Tensile Testing

The tensile strength of electrospun collagen-chitosan nanofibrous membranes was respectively 17.02 ± 1.03 MPa and 1.53 ± 0.12 Mpa in the dry and hydrous conditions, while the elongation percentage was respectively $2.93 \pm 0.28\%$ and $70.5 \pm 3.25\%$. Compared with the collagen nanofibrous membranes, the tensile strength of collagen-chitosan nanofibrous membranes was better than that of collagen nanofibrous membranes (5.37 ± 0.63 MPa), and the elongation percentage was less than that of collagen nanofibrous membranes ($4.32 \pm 0.44\%$) in dry conditions. In the hydrous condition, the situation of collagen-chitosan nanofibrous membranes was similar to that of the collagen nanofibrous membranes (1.89 ± 0.38 MPa, $70.83 \pm 3.02\%$) (Fig. 2).

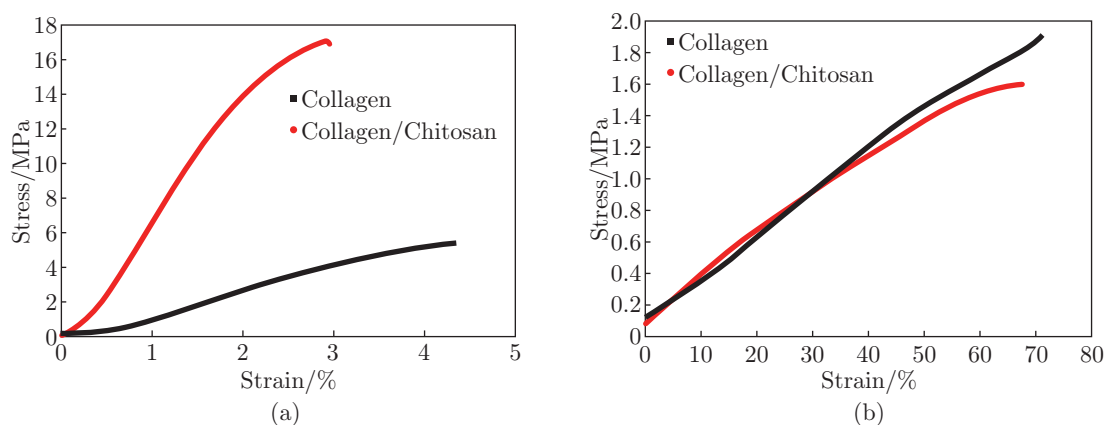


Fig. 2: Mechanical property of collagen-chitosan nanofibrous membranes and collagen nanofibrous membranes. (a) The stress-strain curves of nanofibrous membranes in dry condition; (b) The stress-strain curves of nanofibrous membranes in hydrated condition ($N = 6$).

3.3 Contact Angle

The contact angle of electrospun collagen-chitosan nanofibrous membranes was $42.44 \pm 4.03^\circ$, which showed good hydrophilicity although it was slightly larger than that of the collagen nanofibrous membranes ($22.09 \pm 2.03^\circ$) (Fig. 3).



Fig. 3: Images of the static contact angles of nanofibrous membranes measured by a contact angle goniometer ($n = 6$). (a) Collagen; (b) Collagen-chitosan

3.4 Cell Adhesion and Proliferation

The morphology of L929 cells adhered on collagen-chitosan nanofibrous membranes for an hour was shown in Fig. 4 (a, b). It indicated that L929 cells spread well on the membranes. The results were similar to those of collagen nanofibrous membranes. The proliferation of L929 cells on 1, 3, and 5 days after seeding on the nanofibrous membranes was shown in Fig. 4 (c). It can be seen that both the nanofibrous membranes promoted cell growth and proliferation compared with cover slips.

3.5 Antimicrobial Effect Against Staphylococcus Aureus

The number of viable Staphylococcus aureus in a 24 h suspension was determined by the spread plate method (Fig. 5). Compared to the collagen nanofibrous membranes, lower numbers of live

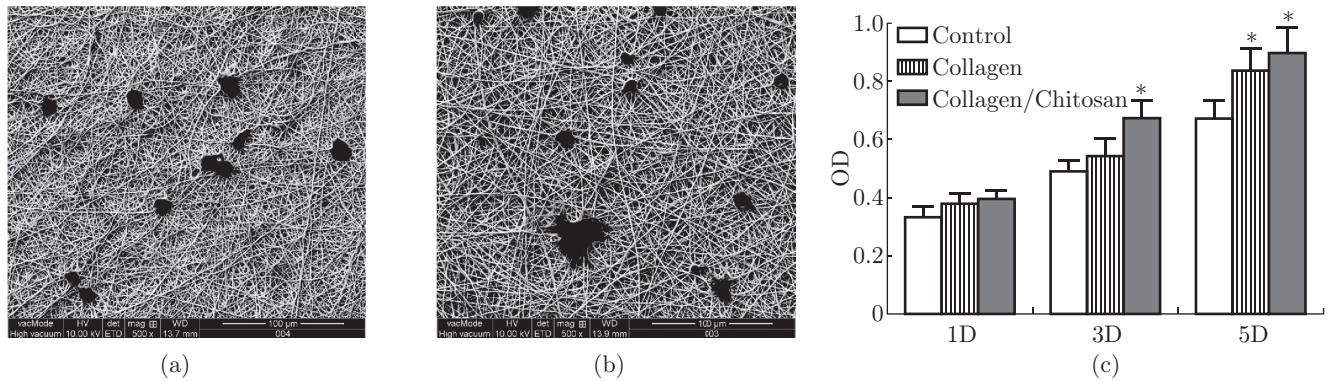


Fig. 4: Cell viability of L929 cells cultured on fibrous membranes. (a) SEM micrographs of cells adhered on collagen nanofibrous membranes for one hour; (b) SEM micrographs of cells adhered on collagen-chitosan nanofibrous membranes for one hour. (c) Proliferation of L929 cells cultured on nanofibrous membranes and cover slips for 1, 3, 5 days. Statistical difference between groups was indicated ($*p < 0.05$)

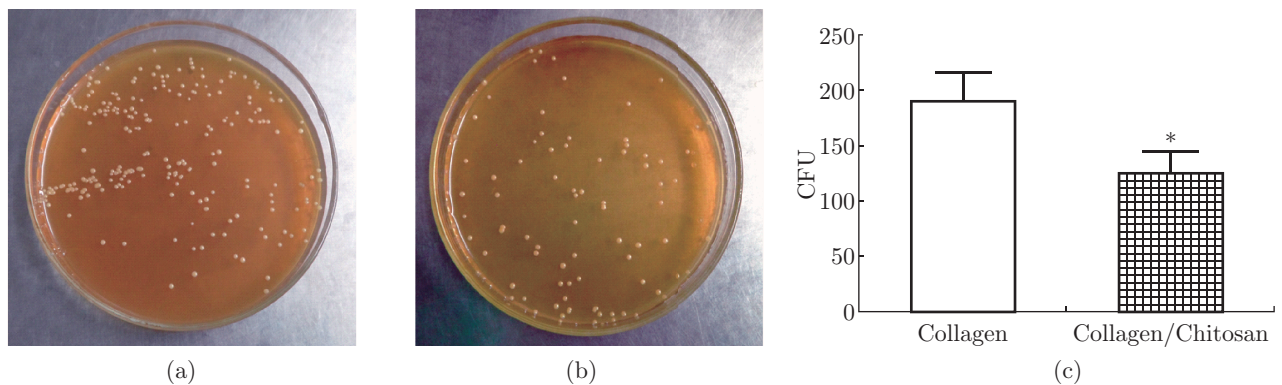


Fig. 5: Recultivated bacteria colonies were collected from (a) Collagen and (b) Collagen-Chitosan nanofibrous membranes. (c) Counts of CFU on agar. Statistical difference between groups was indicated ($*p < 0.05$)

bacteria were observed on the collagen-chitosan nanofibrous membranes ($P < 0.05$).

4 Discussion

In order to develop an original skin wound healing material with antibacterial properties, many researchers have paid much attention to collagen and chitosan. It was reported that electrospun collagen-chitosan nanofibrous membranes possessed the best mechanical strength with the ratio of 80/20 [4]. However, few studies have attempted to investigate the hydrophilicity, cellular biological effect and antibacterial property of the membranes. Therefore, we developed electrospun collagen-chitosan nanofibrous membranes according to this ratio. The results showed that the diameter of collagen-chitosan nanofibers was small, and the membranes exhibited high porosity. When an ideal biomaterial was selected for skin wound healing, the following requirements must be considered: favorable tensile strength, hydrophilicity, biocompatibility and antibacterial properties [5, 6]. As shown in Fig. 2, the elongation percentage of collagen-chitosan nanofibrous membranes was improved in the hydrous condition compared with that in the dry condition,

while tensile strength decreased. Besides, the tensile strength of collagen-chitosan nanofibrous membranes was better than that of collagen nanofibrous membranes in the dry state, however, it was similar to that of collagen nanofibrous membranes in hydrous condition. The above results indicated that the mechanical strength of nanofibrous membranes was not reduced by the addition of chitosan. What's more, some studies have reported that it is favorable for cells to adhere and proliferate on the scaffold with a hydrophilic surface [7]. In agreement with these reports, L929 cells were found to be spread well and grown fast on collagen-chitosan nanofibrous membranes in our study. Meanwhile, collagen-chitosan nanofibrous membranes displayed excellent antibacterial property against *Staphylococcus aureus*. The antibacterial effect of the nanofibrous membranes was derived from chitosan due to its ability to destroy the bacteria membranes. It has been reported that the antibacterial effect of chitosan against *Staphylococcus aureus* can be enhanced with the increased content [8].

5 Conclusion

In the present study, the collagen-chitosan nanofibrous membranes were developed by electrospinning. Our results showed that electrospun collagen-chitosan nanofibrous membranes possessed favorable tensile strength with hydrophilic surfaces. In addition, the membranes promoted cell adhesion and growth, and displayed excellent antibacterial property against *Staphylococcus aureus*. Overall, it is demonstrated that electrospun collagen-chitosan nanofibrous membranes have potential application as skin wound healing materials.

Acknowledgments

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