

# 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  $221 \pm 105$  nm, the tensile strength was  $1.53 \pm 0.12$  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

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## 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

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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 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  $2.5 \times 10^4$  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  $1 \times 10^6$  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.