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Electrospun chitosan/sericin composite nanofibers with antibacterial property as potential wound dressings

Rui Zhao[a], Xiang Li[a,b,*], Bolun Sun[a], Ying Zhang[b], Dawei Zhang[b], Zhaohui Tang[b], Xuesi Chen[b], Ce Wang[a]

[a] Alan G. MacDiarmid Institute, Jilin University, Changchun 130012, PR China
[b] Chinese Acad Sci, Changchun Inst Appl Chem, Key Lab Polymer Ecomat, Changchun 130022, PR China

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ABSTRACT

Chitosan and sericin are natural and low cost biomaterials. Both biomaterials displayed good compatibility to human tissues and antibacterial properties for biomedical application. In this study, we have successfully fabricated chitosan/sericin composite nanofibers by electrospinning. The obtained composite nanofibers were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FT-IR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA) studies. The composite nanofibers had good morphology with diameter between 240 nm and 380 nm. *In vitro* methyl thiazolyl tetrazolium (MTT) assays demonstrated that the chitosan/sericin composite nanofibers were biocompatible and could promote the cell proliferation. Furthermore, the composite nanofibers showed good bactericidal activity against both of Gram-positive and Gram-negative bacteria. Thus, the chitosan/sericin composite nanofibers are promising for wound dressing applications.

1. Introduction

Wound is the damage of normal skin tissue structure and function. Wound healing is a complex biological process and it is related to an integrate response of different cell types and growth factors [1–3]. In order to facilitate the wound healing, biomaterial dressings are often used [4–7]. An ideal wound dressing should maintain hemostasis, moist environment at the wound and prevent from external factors like dust and bacteria; allow water and air permeation and promote epithelization by releasing biological agents to the wounds [8,9]. Electrospinning is known to be a simple and versatile method to generate polymer or composite nanofibers [10,11]. Due to their high specific surface area, porous structure and high porosity, moreover these electrospun porous nanofibers can mimic the micromorphology of the native extracellular matrix (ECM) and enhance the cell migration and proliferation, the electrospun nanofibers have a wide range of applications in biomedical fields such as tissue engineering scaffolds, wound dressing materials and carriers for drug delivery [12–14].

Chitosan is a polysaccharide of N-acetyl-d-glucosamine and d-glucosamine produced by the deacetylation of chitin [15,16]. It is widely believed that chitosan is a good biomedical material because of its special characteristics, such as hemostatic, biodegradable, biocompatible, and non-toxic and it has extensive use in drug delivery systems, wound dressing materials, and tissue engineering scaffolds [17–19]. Much work has been done in the study of electrospun chitosan nanofibers. A pure chitosan fibers using trifluoroacetic acid (TFA) [20] or concentrated acetic acid [21] as a solvent and a bicomponent electrospinning system of chitosan with PEO [22], PLA [23], PCL [24], PVA [25], collagen [26], gelatin [27], alginate [28] and silk fibroin [29] have been widely investigated. Chitosan-based nanofibers received a great deal of attention in the field of wound dressing applications because of the special structure of electrospun nanofibers and special characteristics of chitosan. Chitosan with PLA nanofibrous mats have been successfully prepared by electrospinning, which showed that crosslinked electrospun chitosan/PLA mats occupied a high antibacterial efficacy and incorporation of chitosan into electrospun mats increases the anti-adhesive properties towards pathogenic bacteria *S. aureus*, rendering the bicomponent mats promising for wound dressing applications [30]. In a more recent report, chitosan/PEO blend solutions were electrospun to obtain nanofiber webs for wound-dressing applications. The results demonstrated that over 99% of the number of microorganisms

* Corresponding author at: Alan G. MacDiarmid Institute, Jilin University, Changchun 130012, PR China. Tel.: +86 431 85168292; fax: +86 431 85168292.
E-mail address: xiangli@jlu.edu.cn (X. Li).

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reduced with pure chitosan nanofibers and over 50% reduction with chitosan/PEO blend nanofibers [31].

In comparison with synthetic polymers, natural polymers usually exhibit better biocompatibility and low immunogenicity. Sericin is the outer coating of the silk fiber derived from *Bombyx mori* silkworm and it envelops the fibroin fiber with successive sticky layers that enables the formation of a cocoon. Sericin consists of 18 kinds of amino acids, such as serine, glycine, lysine, etc. Sericin is a biocompatible and biodegradable natural biopolymer exhibiting antioxidant, moisture absorption, antibacterial and UV resistance properties, and it is used in cosmetics and fabrics [32,33]. Recently, the electrospun sericin nanofibers are of great interest to be developed as biomedical materials. Nanoscale sericin electrospun fibers were produced from a sericin-hope cocoon, using TFA as a solvent. The availability of sericin nanofibers introduced a new set of possible uses in biomedical fields [34]. Moreover, a three-dimensional poly(epsilon-caprolactone)/sericin porous nanofibrous scaffolds were fabricated by electrospinning and the hydrophilic capability increased with the sericin addition, which improved human primary skin fibroblast cells adhesion and proliferation on the scaffolds [35].

In this work, we present a novel way to prepare chitosan/sericin composite nanofibers for wound dressing applications. To our knowledge, it is the first time to fabricate chitosan/sericin blend non-woven via electrospinning. We blend the bi-natural-polymers, chitosan and sericin, that both have good biocompatibility and antibacterial properties, hoping that it can improve the biological response ability through their synergistic biological effects. The physical–chemical properties of the composite nanofibers were investigated. Furthermore, the biocompatibility and antibacterial property were estimated. Our results demonstrated that the as-prepared composite nanofibers would be a good candidate for wound dressing materials.

2. Materials and methods

2.1. Materials

Chitosan (viscosity = 50–800 mPa s) was purchased from Sinopharm Chemical Reagent Corporation. Sericin was purchased from Huzhou Aotesi Biotechnology Co., Ltd. Trifluoroacetic acid (TFA) was purchased from Aladdin Industrial Corporation. Tryptone, yeast extract, agar and NaCl were obtained from GL Biochem (Shanghai) Ltd. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco (Solon, Ohio, USA). Dimethyl sulfoxide (DMSO) was purchased from Tianjin Yongda Chemical Reagent Co., Ltd. All reagents were of analytical grade and used without further purification.

2.2. Preparation of chitosan/sericin blend solutions

The chitosan/sericin blend solution was prepared by dissolving chitosan and sericin in the TFA at total solid concentration of 3 wt% with different mass ratios (1/1, 2.5/1, 4/1, and 5/1). Above solutions were under magnetic stirring for 8 h at room temperature. These preparations were then used for electrospinning.

2.3. Preparation of the chitosan/sericin composite nanofibers

Electrospinning was performed at room temperature. The as-prepared solution was loaded into a 2 mL glass needle and connected to a high-voltage power supply. An electric potential of 18 kV was applied between the needle tip and the collector with a distance of 15 cm. The received nanofibers were dried at 100 °C in vacuum for 6 h.

2.4. Characterizations

The morphology of the composite nanofibers was observed by scanning electron microscopy (SEM, SHIMADZU SSX-550). The mean diameter and diameter distribution of the composite nanofibers were calculated from measuring the different parts of the nanofibers using the commercial software package, Image-Pro Plus. IR spectras were obtained on a Fourier transform infrared spectrometer (FT-IR, BRUKER VECTOR 22). The fibers and raw materials were characterized by X-ray diffraction (XRD; Scintag XDS 2000 diffractometer with a Cu Kα, radiation) in the scan range 2θ between 5° and 80°. Thermogravimetric analysis (TGA) was obtained in an air atmosphere at a heating rate of 10 °C/min between 50 °C and 700 °C using a Perkin-Elmer PYRIS 1 TGA. Zeta potential measurements of the composite nanofibers in aqueous phase were made on ZEN3600 Zetasizer (Malvern Co., Ltd. UK).

2.5. In vitro cytotoxicity

The cell viability of the composite nanofibers was examined by the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay in L929 fibroblasts. The cells were cultured in DMEM medium supplemented with 10% FBS and 1% antibiotic (streptomycin at 3×10^{-4} mol/L and penicillin at 5×10^{-4} mol/L). After 90% cell confluence, the cells were removed with trypsin (0.05%) and seeded in wells of 96–well plate at a density of 10^4 cells per well. The composite nanofibers were dissolved in PBS (pH = 7.4), making that the concentration was 5 mg/mL. Defined amount of the above solution was added to each well to make that the final nanofibers concentrations were 250, 125, 62, 32, and 16 μg/mL, respectively. After the above operation, the cells were incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. After 24, 48, and 72 h of incubation, the supernatant of each well was replaced with MTT diluted in serum-free medium and the plates were incubated at 37 °C for another 4 h. After aspirating the MTT solution, dimethyl sulfoxide (150 μL) was added to each well to ensure solubilization of the formazan crystals. The absorbency of the solution was measured on a Bio-Rad 680 microplate reader at 492 nm. Cell viability was calculated based on the following equation [36]:

$$\text{Cell viability}\,(\%) = \frac{OD_N}{OD_C} \times 100$$

where OD_N is the absorbance of cells cultured in the presence of different concentrations of the composite nanofibers and OD_C is the absorbance of control group (incubated with culture media without the composite nanofibers). All experiments were performed three times with six replicate wells for every sample and control per assay. Data were analyzed by student's *t*-test and differences at the 95% confidence level were considered to be significant.

2.6. Antibacterial assessment

The Gram-negative bacteria *Escherichia coli* and the Gram-positive bacteria *Bacillus subtilis* were cultivated in the Luria-Bertani (LB) nutrient broth that contained tryptone (50 mg), yeast extract (25 mg) and NaCl (50 mg) in 5 mL sterile distilled water at pH 7.0 in a CO_2 incubator overnight, followed by dilution with LB nutrient broth. The above diluted bacteria suspension was cultured in the vials containing different amounts of the sterilized nanofibers making the concentration of the nanofibers 0, 0.1, 0.2, 0.4 mg/mL, respectively. The vials were incubated at 37 °C for 12 h, and then the bacteria collected from each vial were plated onto the solidified Luria-Bertani agar medium that contained tryptone (50 mg), yeast extract (25 mg), agar (75 mg) and NaCl (50 mg) in 5 mL sterile distilled water at pH 7.0. After incubation at 37 °C for 24 h, the