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Study of multi-functional electrospun composite nanofibrous mats for smart wound healing

Lin Tan [a], Jinlian Hu [a,b,*], Huahua Huang [a], Jianping Han [a], Huawen Hu [a]

[a] Institute of Textiles and Clothing, The Hong Kong Polytechnic University, Hong Kong, China
[b] PolyU Shenzhen Base, Nanshan District, Shenzhen, China

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ABSTRACT

Composite nanofibers derived from synthetic and natural polymers normally show desirable characteristics in biomedical applications. In this study, composite nanofibrous mats (denoted as CNMs) with diameters of around 300 nm were fabricated facilely using blends of chitosan, gelatin and shape memory polyurethane (SMPU) by electrospinning and subsequent post-treatment with a silver nitrate solution. The obtained CNMs have shape memory effect and show desirable water vapor transmission ratio, surface wettability, satisfactory biological properties including antibacterial activity against the common Gram-negative and Gram-positive bacteria, cytocompatibility demonstrated to fibroblast, and the hemostatic property through a whole-blood clotting test. In addition, such CNMs can possibly benefit the wound healing through shape fixation-assisted easy processing and shape recovery-assisted closure of cracked wounds, which can be fine-tuned by pre-programming. Therefore, the CNMs presented in this study can be used as potential smart wound dressings.

1. Introduction

Nanofibers are fast-developing nano-materials which are typically prepared through electrospinning technology and have found a wide range of biomedical applications such as wound dressing [1], drug delivery [2], tissue scaffold [3], biosensor [4], and gene vector [5].

Wound healing is a complicated physiological process which involves the performance of cell growth, proliferation and migration leading to tissue regeneration [6,7]. A qualified wound dressing should be an effective matrix with required performances that should include: (1) preventing the bacterial infection during wound healing; (2) providing the microenvironment for the growth, proliferation and migration of cells, especially the fibroblasts and keratinocytes; (3) being semi-permeable itself for ensuring sufficient gas and nutrient exchange for wound healing. Substantial efforts have been devoted to exploring new materials for accelerating wound healing. Fortunately, composite nanofibrous mats can be well adjusted to satisfy the requirements of wound healing due to their unique characteristics, including high similarity to natural extracellular matrix in terms of structure, chemical composition, mechanical property, high surface to volume ratio, porosity, and stretchability [8–10].

As a biocopolymer consisting of glucosamine and N-acetylglucosamine, chitosan has been widely applied to various biomedical applications, such as drug delivery carrier, surgical thread, bone healing material and wound dressing [11,12]. Chitosan has also been reported to improve the tensile strength of wounds by speeding up the fibroblastic synthesis of collagen in the first few days of wound healing [13]. Furthermore, it possesses the potential to prevent the bacteria penetration and promote the repair of damaged tissue [14]. In view of these merits, chitosan has been regarded as an important biopolymer for wound management in recent years [15]. Nevertheless, due to the strong hydrogen bonding interaction within chitosan molecules, it is necessary to blend with other polymers to improve the spinnability for yielding the chitosan electrospun fibers [16]. Gelatin, a natural protein, is derived from the partial and irreversible hydrolysis of collagen. It contains the same amino acid sequences as those in collagen, and shows almost the same remarkable biological properties as those of collagen. As indicated by a previous report, collagen nanofibers obtained through the electrospinning technique could readily lose its original structure and activity [17]. Hence, gelatin nanofibers can be a promising alternative to collagen nanofibers partially because gelatin is less expensive and easier to preserve. Currently, several studies have applied electrospun gelatin nanofibrous mats as wound dressing with silver composition for antibacterial

* Corresponding author at: Institute of Textiles and Clothing, The Hong Kong Polytechnic University, Hong Kong, China. Tel.: +852 27666437.
E-mail address: tchujl@polyu.edu.hk (J. Hu).

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function [18,19]. Meanwhile, in order to avoid disintegrating into fluids, or improve their water-resistance and thermo-mechanical performance for potential biomedical applications, the electrospun gelatin nanofibers were normally crosslinked by saturated glutaraldehyde (GTA) vapor [20], and genipin [21]. In addition, gelatin has been demonstrated as a hemostatic agent which can effectively prevent bleeding after surgery [22]. However, poor mechanical properties, like tensile deformation, limit the application of this natural polymer. In contrast, synthetic polymers such as polyurethane, with good mechanical properties, can be used to offset such disadvantages.

Shape memory polyurethane (SMPU) is currently one of the most widely reported smart synthetic polymers which can impart the nanofibers with good mechanical properties, such as stretching capability [23]. In addition, SMPU can show good controllability on deformation and force because of its shape memory function and has been studied for wound-care products such as suture [24–26]. However, insufficient biological properties limit its application as wound dressing materials. Therefore, it is necessary to modify SMPU with other compatible biopolymers so as to achieve desirable properties, such as cell attachment, proliferation and blood blotting. Composite structures have been reported to combine the advantages of different components and hence enhance the final properties as compared with the pristine material. Recently, multifunctional composite nanofibrous mats derived from biocompatible synthetic polymers, natural proteins and polysaccharides, start to draw attention for wound healing applications [27,28].

In this paper, the objective of the current study is to construct novel composite nanofibrous mats (CNMs) consisting of chitosan, gelatin and SMPU through electrospinning. Among the three polymers, SMPU is the main component which performs as a mechanical matrix, and chitosan and gelatin serve to improve the hydrophilicity and biological properties of the composite materials. Furthermore, to enhance the antimicrobial properties of the present composite material, e.g., to prevent the infection against Gram-positive and Gram-negative bacteria more effectively, a silver nitrate ($AgNO_3$) solution with safe concentration was employed to treat the composite by soaking [29]. Finally, the functional CNMs were obtained and their structure and properties were systematically studied, including shape memory effect (SME), controllability on the working force under different strains, water vapor transmission ratio (WVTR), surface wettability, antibacterial activity, cytocompatibility and hemostatic performance. With these properties, the present CNMs can be highly recommended as novel materials for wound healing applications.

2. Experimental

2.1. Materials

Chitosan was purchased from Aqua-Chem (UK), and the viscosity in acetic acid (20 °C) 1% was 400–800 mPa s; gelatin (Type A, bloom index 300) from porcine skin was purchased from Sigma–Aldrich (USA), and the molecular weight ranging from 40 kDa to 250 kDa was measured through sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE); SMPU was synthesized according to our previous study [30], its weight-average molecular weight ($M_w \approx 506{,}000\ g\ mol^{-1}$) was confirmed by the gel permeation chromatography (GPC, Waters), and its thermal transition temperature was about 40 °C investigated by differential scanning calorimeter (DSC, Perkin-Elmer Instruments); glutaraldehyde (GTA) solution (25%) was purchased from Sigma–Aldrich (USA), and silver nitrate ($AgNO_3$) was purchased from Sinopharm Chemical Regent Co., Ltd.; the test strains, *Escherichia coli* (Gram −ve), *Pseudomonas aeruginosa* (Gram −ve), *Staphylococcus aureus* (Gram +ve), and mouse fibroblast cell line, L929, were obtained from local labs. All other reagents were local commercial products with analytical grade.

2.2. Synthesis of shape memory polyurethane (SMPU)

Generally, the obtained SMPU composed of PCL, MDI and chain extenders EDA. The reaction to prepare prepolymer was carried out in a 500 mL conical flask equipped with a mechanical stirrer. PCL mixed with MDI for 2 h at 80 °C, and followed by the additional reaction with EDA for another 2 h with same temperature. The whole isocyanate group content was excessive by 3 mol%. All the chemicals used in the synthesis process should be dehydrated with 4 Å molecular sieves for at least 2 days in advance.

2.3. Preparation of solution of chitosan/gelatin/SMPU

In the course of producing electrospun nanofiber, it is preferable to apply only one solvent which is volatile and relatively less toxic, in order to obtain a homogeneous solution. In this study, formic acid (FA) was employed because of its higher dissolving capacity, higher volatility and lower toxicity as compared to the solvents containing fluorine, such as hexafluoroisopropanol and trifluoroethanol. Particularly, the solution was prepared by blending chitosan, gelatin and SMPU simultaneously in FA at 50 °C with magnetic stirring for 3 h. The final mass ratio of chitosan, gelatin and polyurethane was fixed at 1.5:1.5:7.0, and the solid concentration of the mixture was 5.0 wt%.

2.4. Preparation of CNMs

The above solution was subsequently processed *via* an electrospinning system (Kato-Tech Co., Japan). The electrospinning apparatus consists of a 25 mL syringe that connects to a syringe pump, the voltage gradient of 2.0 kV/cm was applied, and the solution feeding rate was maintained at 0.05 mm/min. The formed fibers were collected on an aluminum foil. After that, the as-fabricated mats were treated with a 1.0×10^{-4} M $AgNO_3$ solution by soaking method for 1.0 h, and the final CNMs were air-dried overnight and stored in a desiccator before the following characterizations and tests. For comparison, pure gelatin, thermoplastic polyurethane (TPU) and SMPU nanofibrous mats were also prepared using the similar method, and gelatin nanofibrous mats were crosslinked by GTA overnight followed by placing in a vacuum desiccator to remove the residual GTA.

2.5. Characterizations

The morphology of samples was observed by scanning electron microscope (SEM, Hitachi S450, Japan) with an accelerated voltage of 20 kV and working distance of 10 mm, and the diameter of the obtained nanofibers was measured by the software of *ImageJ*; the wettability was investigated by applying a contact angle equipment (Contact Angle System SCA 20, DataPhysics Instrument GmbH, Germany) using the sessile drop method under ambient condition, and the deionized water (2.0 μL) was dropped onto the CNMs surface, and the pictures were captured immediately once the droplet escaped from the needle. All water contact angle (WCA) data represent the averages of at least three individual samples. The chemical structure and conformation of CNMs were analyzed by Fourier transform infrared (Perkin-Elmer Spectrum 100 FT-IR Spectrometer, USA) spectroscopy in the range of 650~4000 cm^{-1} at room temperature; thermal decomposition was investigated by thermogravimetric analysis (Mettler Toledo TGA/DSC 1 Simultaneous Thermal analyser, Switzerland) with a heating scan from room temperature to 700 °C, and the flow rate of nitrogen gas