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Green electrospun Manuka honey/silk fibroin fibrous matrices as potential wound dressing

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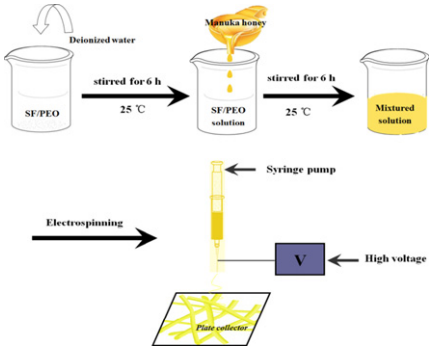
HIGHLIGHTS

- Manuka honey/silk fibroin (MH/SF) fibrous matrices were prepared by green process.
- MH/SF fibrous matrices favor cell attachment and growth.
- MH/SF fibrous matrices demonstrated high antibacterial ability.
- MH/SF fibrous matrices showed good performance on improving wound healing.

GRAPHICAL ABSTRACT



Schematic illustration for the fabrication of Manuka honey-loaded silk fibroin composite fibrous mats.

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ABSTRACT

Wound dressing plays an irreplaceable role in preventing infection and accelerating healing of wounds, especially the chronic non-healing wounds. Recently, the electrospun silk fibroin (SF) fibrous matrices is regarded as an ideal candidate for wound healing in virtue of its excellent skin affinity and good permeability for air and water. Manuka honey (MH) has demonstrated its unique properties in wound healing, including anti-inflammatory and anti-bacterial function as well as promoting tissue growth and reducing pain of patients. Here we report the feasibility of developing MH/SF composite fibrous matrices as antimicrobial wound dressing. SF fibrous matrices loading different amount of MH were manufactured by green electrospinning. The FTIR spectra indicated that MH was successfully loaded into the SF fibers. The composite fibers show smooth morphology and their diameter increases with MH content increased. Interestingly, the incorporation of MH significantly improved the antimicrobial activity of SF fibrous matrices, without negative effect on the excellent biocompatibility of SF. Moreover, the MH/SF composite fibrous matrices showed good performance on improving wound healing according to the data of animal experiment. Our findings suggest as-prepared natural green composite matrices combining the merits of both SF and MH could be a promising candidate for wound dressing.

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1. Introduction

Wound care and management are challenged by increasing wound infection which is caused by bacteria and other microorganisms and often delays wound healing [1,2]. Generally, chronic non-healing wounds make the skin frequently expose to bacteria and microorganisms which may interrupt the natural physiological healing process by inducing strong inflammatory response [3,4]. Therefore, the wound dressing against infection is desired for wound healing. Moreover, a successful candidate should also possess excellent skin compatibility and is able to accelerate the healing process [5,6]. Application of traditional wound dressings mainly made of cotton or linen are limited due to their unsatisfactory biocompatibility and high risk of infection. Many novel biomedical antibacterial wound dressings have been achieved in recent years [4,7]. Some of them are able to reduce the adhesion and proliferation of bacteria in the wound bed by incorporating antifouling or antimicrobial agents [8–10].

In particular, electrospun fibrous matrices have been widely investigated for wound healing application recently due to their specifically high surface area and porosity [11–13]. The electrospun chitosan nanofibrous matrices is one of the most popular candidate materials as antibacterial dressing for their ability to induce the death of bacteria by the disorganization of the cell wall [14,15]. However, as a result of its poor solubility in most common solvents, toxic organic solvents such as trifluoroacetic acid (TFA) were involved in the electrospinning of chitosan [16], the residual of which is harmful to humans and environment. In addition, sliver ions and sliver nanoparticles were also widely used as additive agent in different electrospun dressings [17–19] for their excellent antimicrobial properties. Unfortunately, the cell toxicity of silver make it limited in further applications [20]. Thus, it is highly desirable to develop green dressings with favorable antimicrobial properties and outstanding skin compatibility.

As a natural biopolymer, SF has been extensively applied in biomedical field owing to its satisfied biocompatibility, low inflammatory response and all-aqueous fabrication process [21,22]. Especially, SF is extraordinarily beneficial to simulate skin microenvironment, eliminate scarring and alleviate atopic dermatitis [23,24]; it has evoked the interest of researchers to develop wound dressings based on SF [25–28]. Disappointingly, most SF-based antimicrobial dressings were fabricated by simply blending SF with chitosan or silver, which involved some unfavorable factors as mentioned above. Alternatively, honey, an ancient pharmaceutic nutrition, has exhibited excellent antimicrobial, anti-inflammatory and antioxidant activity [29,30], which could be used as a potential substitute for sliver to manage various infected wounds. Brudzynski et al. found that the dose of honey plays a vital role in its antibacterial efficiency [31]. Among eight kinds of honey studied, Manuka honey (MH) originated from New Zealand showed antibacterial activity at a wide range of concentrations from 6.25% to 50% (v/v). Interestingly, it can efficiently inhibit bacteria at a high concentration, but without causing any toxicity in skin cells [32]. Inspired by these studies, we hypothesized that the combination of MH and SF nanofibers could provide a potential option for developing green functional antimicrobial wound dressings.

In our previous study, we successfully fabricated SF nanofibrous matrices loading vitamins or grape seed extract by green electrospinning [24,33–35], which showed great benefit to skin cells. In this study, novel MH-loaded green SF fibrous matrices were prepared by electrospinning. The morphology, secondary structure, cytocompatibility and antibacterial activity of the resulting materials were investigated by scanning electronic microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), cell viability, bacteriostatic assays and animal healing assays, respectively.

2. Experimental

2.1. Materials

Cocoons of *Bombyx mori silkworm* were purchased from Jiaxing Silk Co. (China). Manuka honey (UMF® 5^+) was from Comvita New Zealand LTD. Poly(ethylene oxide) (PEO, Mw = 900.000) was purchased from Sigma-Aldrich China Inc. L929 cells were provided by Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). *Methicillin-resistant Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were cultured and stored in our own laboratory. BALB/c mice were purchased from Shanghai SLAC Animal Laboratory Co. Ltd. Other chemicals were all of analytical grade or higher and used as received. Ultrapure water was used throughout the whole study.

2.2. Preparation of regenerated SF

SF was prepared using the method described in our previous study [36]. Briefly, cocoons were boiled three times (30 min/time) in a 0.5% (wt/v) Na_2CO_3 aqueous solution, and then sericin was removed by rinsing with ultrapure water. The degummed and dried silk was dissolved in a ternary solvent system of $CaCl_2/H_2O/CH_3CH_2OH$ solution (1:8:2 in molar ratio) at 65 °C for 2 h. Small molecular impurity was eliminated by dialyzing this solution for 3 days using a cellulose dialysis tubing (molecular weight cutoff = 14.000 Da) against distilled water changed every 6 h. After filtering, the SF solution was lyophilized for three days to obtain the regenerated SF sponges.

2.3. Preparation of MH-loaded SF fibrous matrices

1.0 g SF, 0.1 g PEO and certain amounts of MH were dissolved together in 5 mL ultrapure water and the mixture was stirred over 12 h to obtain stable spinning solutions with a determined concentration of MH (0, 10%, 30%, 50% and 70% (wt/v)), respectively. These solutions were then electrospun at a stable extruding rate of 1.0 mL/h under a voltage of + 12 kV and the collect distance was 15 cm. The resulting MH-loaded SF fibrous matrices were dried and stored under vacuum at room temperature prior to use.

2.4. Characterization of MH-loaded SF fibrous matrices

Fiber morphology was observed using SEM (TM-1000, Japan) after sputter-coating with gold. The average fiber diameter was determined from 100 random measurements on a typical SEM image using Image-J 1.34 software (National Institutes of Health, USA).

Secondary structure of the MH, SF and MH-loaded SF fibrous matrices was detected by FTIR (Avatar 380, USA) from 4000 to 600 cm^{-1} wave numbers.

2.5. Cell culture and analysis

L929 cells were used to assess the biocompatibility of the MH-loaded SF fibrous matrices using MTT assay. L929 cells were cultured in DMEM (high glucose) medium (HyClone) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco) and 1% (v/v) penicillin/streptomycin (Invitrogen) and incubated in a humidified incubator at 37 °C with 5% CO_2. The fibrous matrices were collected on circle glasses (14 mm in diameter) for cellular study. After treated with 75% (v/v) ethanol vapor, the fiber-deposited glasses were placed into a 24-well culture plate without further sterilizing, and fixed with autoclaved stainless steel rings, with fiber-free glasses as controls. L929 cells were seeded on the fibrous matrices at a density of 1.0×10^4 cells/well.

The cells were cultured for 1, 3 and 5 days, and the medium were changed every two days. At the time point of 1, 3 or 5 days, the old medium was abandoned and wells were rinsed with PBS three times. Then,