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Green electrospun pantothenic acid/silk fibroin composite nanofibers: Fabrication, characterization and biological activity

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ABSTRACT

Silk fibroin (SF) from *Bombyx mori* has many established excellent properties and has found various applications in the biomedical field. However, some abilities or capacities of SF still need improving to meet the need for using practically. Indeed, diverse SF-based composite biomaterials have been developed. Here we report the feasibility of fabricating pantothenic acid (vitamin B_5, VB_5)-reinforcing SF nanofibrous matrices for biomedical applications through green electrospinning. Results demonstrated the successful loading of D-pantothenic acid hemicalcium salt ($VB_{5\text{-hs}}$) into resulting composite nanofibers. The introduction of $VB_{5\text{-hs}}$ did not alter the smooth ribbon-like morphology and the silk I structure of SF, but significantly decreased the mean width of SF fibers. SF conformation transformed into β-sheet from random coil when composite nanofibrous matrices were exposed to 75% (v/v) ethanol vapor. Furthermore, nanofibers still remained good morphology after being soaked in water environment for five days. Interestingly, as-prepared composite nanofibrous matrices supported a higher level of cell viability, especially in a long culture period and significantly assisted skin cells to survive under oxidative stress compared with pure SF nanofibrous matrices. These findings provide a basis for further extending the application of SF in the biomedical field, especially in the personal skin-care field.

1. Introduction

Silk fibroin (SF) from domestic silkworm (*Bombyx mori*) cocoons is a natural biological protein polymer. It has many established excellent properties: good biocompatibility and biodegradability, tunable mechanical properties, controllable biodegradable rate *in vivo* and easily processing properties in aqueous environment [1–6]. Indeed, SF has received a great deal of attention from biomedical researchers since clinically used as surgical suture. It has been widely processed into particles, fibers, films, and gels for a variety of applications [2,4,7–9]. Recently, the research and application of SF nanofibers from electrospinning has become a hot topic in the biomedical field due to some unique properties of as-spun nanofibrous matrices. The specifically high surface-area-to-volume ratio and inherent porous structure of electrospun nanofibrous matrices closely mimics the natural extracellular matrix (ECM), making them very suitable for use as tissue regeneration and skin care matrices [2,6,10–16]. However, some performances of electrospun SF nanofibrous matrices (ESFNM) such as antibacterial activity, bone-induced ability and antioxidation capacity still need further enhancing for special benefits. A good strategy is to develop SF-based hybrid or composite nanofibrous matrices with improved function [2,17,18].

Pantothenic acid, usually termed as vitamin B_5 (VB_5), is an important component of coenzyme A. It plays a key role in numerous physiological responses and thus its deficiency often leads various diseases or disorders [19–21]. In particular, a wide range of studies have demonstrated the skin benefit of VB_5. VB_5 can assist mammalian cells to survive in oxidative stress by increasing the level of glutathione, as revealed by Wojtczak et al. [22]. Aprahamian et al. reported that VB_5 supported fibroblast immigration and proliferation, and therefore promoted wound healing [23]. The clinical study from Leung et al. has also demonstrated that VB_5 can reduce sebum secretion, cure acne vulgaris and make smooth skin [24]. Therefore, VB_5 is a desired bioactive factor for personal skin care or tissue engineering products.

This work focuses on investigating the feasibility of fabricating VB_5-reinforcing SF nanofibrous matrices via a green electrospinning process for biomedical applications (Scheme 1). The morphology of composite nanofibers was shown using SEM

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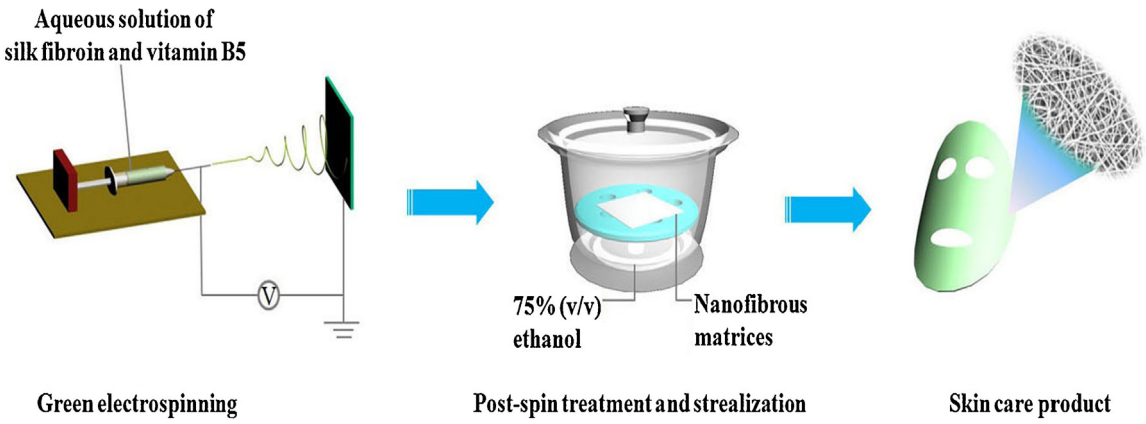

Scheme 1. Green process of electrospinning pantothenic acid/silk fibroin composite nanofibers-based personal skin care products.

(scanning electron microscopy). The structure of nanofibrous matrices was analyzed by ATR-FTIR (attenuated total reflectance Fourier transform infrared spectroscopy). The loaded VB_5 within SF nanofibers was investigated using XPS (X-ray photoelectron spectroscopy). The cytocompatibility of VB_5/SF nanofibrous matrices was shown through *in vitro* MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) viability assay and microscopic imaging of cells cultured on the composite fibrous matrices. The skin benefit of nanofibrous matrices was demonstrated through the viability of L929 cells grown on nanofibers under oxidative stress induced by *tert*-butyl hydroperoxide (*t*-BHP) *in vitro*.

2. Materials and methods

2.1. Materials

Cocoons from domestic silkworm (*B. mori*) were kindly provided by Jiaxing Silk Company (China). D-Pantothenic acid hemicalcium salt: $C_9H_{16}NO_5 \cdot 1/2Ca$ ($VB_{5\text{-hs}}$) (purity ≥ 95.0%, HPLC) was purchased from Sigma–Aldrich (China). Mouse fibroblast L929 cells (L929 cells) were from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). All other reagents in this work were of analysis grade or higher. Ultrapure water used throughout this study was produced with a purification system (Rephile Shanghai Bioscience & Technology Co., Ltd., China) with a resistivity around 18 MΩ cm.

2.2. Preparation of regenerated silk fibroin (RSF)

RSF was fabricated as described in a previous study [2]. Briefly, cocoons were degummed in a boiling aqueous solution of 0.5% (w/v) Na_2CO_3 three times (30 min each time) and then rinsed thoroughly with warm ultrapure water to remove the sericin. After being dried, degummed 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. The resulting solution was dialyzed against ultrapure water with cellulose tube (molecular weight cutoff 14 kDa, Jing Ke Hong Da Biotechnology Co., Ltd., China) at ambient temperature for three days. Finally, RSF was obtained by lyophilizing the filtered SF solution.

2.3. Preparation of $VB_{5\text{-hs}}$/SF composite nanofibrous matrices and post-spin treatment

$VB_{5\text{-hs}}$ powder and SF were respectively dissolved in ultrapure water. In this work, the amount of $VB_{5\text{-hs}}$ is 4 wt% based on the total weight of $VB_{5\text{-hs}}$ and SF sponge. The $VB_{5\text{-hs}}$/SF aqueous solution was obtained by adding SF solution into $VB_{5\text{-hs}}$ solution under uninterrupted agitation (the final concentration of SF solution is 30 wt%). The amount of $VB_{5\text{-hs}}$ was selected according to the previous report [25,26]. Then, the solution was filled into 2.5 mL plastic syringe capped with blunt needle. The syringe was located in syringe pump (Model 789100C, Cole-Parmer Instrument Co., USA) with a rate of 0.3 mL/h. A voltage of 20 kV generated with a high voltage power supply (BGG6-358, BMEICO, Ltd., China) was applied to the needle, a grounded aluminum foil as the collector and the distance between tip and collector was 20 cm. The electrospinning was performed at ambient temperature. After being dried, the resulting composite nanofibrous matrices were treated by placing them in a sealed desiccator saturated with 75% (v/v) ethanol vapor at ambient temperature [6].

2.4. Characterization

The morphology of nanofibers was observed with SEM (JEOLJSM-5600LV, Japan) after samples were sputter-coated with gold. The width distribution of as-spun fibers was determined using Image-J 1.34 software (National Institutes of Health, USA). At least 90 nanofibers from different SEM images for each sample were randomly measured.

To confirm the loading of $VB_{5\text{-hs}}$, pure SF nanofibers and $VB_{5\text{-hs}}$/SF composite nanofibers were respectively scanned using XPS (Escalab 250, Thermo Scientific Electron, East Grinstead, UK) with Mg K at 1486.6 eV and 150 W power at the anode, and the relative content of chemical elements was calculated from the peak height.

ATR-FTIR was carried out using an Avatar 380 FTIR instrument (Thermo Electron) in a wavenumber range of 1300–2000 cm^{-1} and a resolution of 4 cm^{-1}.

2.5. Cytocompatibility assessment

2.5.1. Cell culture

L929 cells were incubated in a humidified incubator at 37 °C with 5% CO_2 using RPMI 1640 medium (Gibco) containing 10% fetal bovine serum (Gibco). Before cells seeded, pure and composite SF nanofibrous matrices were deposited on circular cover slips (14 mm in diameter). After being treated with 75% (v/v) ethanol vapor and dried in a sterilized fume hood, cover slips with nanofibrous matrices were directly placed into a 24-well cell culture plate without further sterilizing, and fixed with autoclaved stainless steel-rings. In this work, L929 cells were seeded at the density