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Research paper

Wound healing effect of electrospun silk fibroin nanomatrix in burn-model

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ABSTRACT

Silk fibroin has recently become an important biomaterial for tissue engineering application. In this study, silk fibroin nanomatrix was fabricated by electrospinning and evaluated as wound dressing material in a burn rat model. The wound size reduction, histological examination, and the quantification of transforming growth factor TGF-β1 and interleukin IL-1α, 6, and 10 were measured to evaluate the healing effects. The silk fibroin nanomatrix treatment exhibited effective performance in decreasing the wound size and epithelialization. Histological finding also revealed that the deposition of collagen in the dermis was organized by covering the wound area in the silk fibroin nanomatrix treated group. The expression level of pro-inflammatory cytokine (IL-1α) was significantly reduced in the injured skin following the silk fibroin nanomatrix treatment compared to the medical gauze (control) at 7 days after burn. Also, the expression level of TGF-β1 in the wound treated with silk fibroin nanomatrix peaked 21-days post-treatment whereas expression level of TGF-β1 was highest at day 7 in the gauze treated group. In conclusion, this data demonstrates that silk fibroin nanomatrix enhances the burn wound healing, suggesting it is a good candidate for burn wound treatment.

1. Introduction

Burn is one of the most widespread injuries in accidents and remains a global public health issue [1,2]. The burn wound is a continuous and severe threat against the rest of the body due to invasion of infectious agents, antigen challenge and repeated additional trauma caused by wound cleaning [3]. Although remarkable advances have been made in our understanding and treatment of burn injuries, most burn healing processes result in extensive scar formation, resulting in significant aesthetic disfigurement and dysfunction [4,5]. The healing process can be promoted by using biocompatible wound dressing material. An ideal wound dressing should prevent dehydration of the wound and retain a favorable moist environment at the wound interface allowing gas permeation as a barrier against dust and microorganisms. Furthermore, it should be non-adhesive and easily removed without trauma. Currently, wound dressings are fabricated with readily available biomaterials of nontoxic, non-allergenic, antimicrobial and wound healing properties [6].

Silk fibroin (SF) from *Bombyx mori* has been highlighted for diverse applications in the biomedical field due to its excellent mechanical property, controllable biodegradability, hemostatic properties, non-cytotoxicity, low antigenicity and non-inflammatory characteristics [7–10]. SF also exhibits exceptional compatibility with a variety of cells and tissues [11–13] Due to its ability to promote adhesion and proliferation of various cells including keratinocytes and fibroblasts, SF has been considered as potential biomaterial to fabricate wound dressings with various formulations [14–19].

SF can be fabricated into various forms through film casting, salt-leaching, lyophilizing, spinning, gelation, etc. Particularly, electrospinning has recently attracted interest for a wide variety of biomedical applications. Electrospun SF nanofibrous materials have thus far been studied with useful properties (i.e., high porosity,

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cytocompatibility, and less inflammation) for wound healing treatment. In a number of studies it has been shown that degradation of electrospun silk materials as an excellent wound dressing material [20,21]. It has also been reported that the incorporation of growth factors into electrospun silk mats accelerate wound healing process [22]. In spite of such advantages, conventional electrospun SF nanosheet has limitations for a wound dressing application. It is difficult to increase electrospun mat thickness (i.e., <1 mm) of SF nanosheet due to electric resistance and the inability to achieve a large pore structure. These structural limitations result in lower water absorbability and rapid drying of wound spot. Thus, SF nanosheet fabricated with a general electrospinning process has limitations for application in wound treatment compared to foam type dressings (e.g., alginate sponge, and polyurethane foam) used in clinical treatments.

In this study, we fabricated SF nanomatrix of bulk volume and large pores by using a modified electrospinning method combined with porogens (i.e., sodium chloride crystal) dispensing apparatus and evaluated burn wound healing effect using deep second-degree burn animal model in rats. The wound healing process was investigated by histological observations and RT-PCR assay was performed to elucidate the healing mechanism compared to a commercially available dressing (i.e., polyurethane foam and Medifoam®).

2. Materials and methods

2.1. Preparation of silk fibroin (SF) aqueous solution

Cocoons of *B. mori* silkworm were degummed by boiling in aqueous solution of 0.02 M Na_2CO_3 for 30 min and subsequently rinsed with distilled water to extract the sericin. The extracted SF was dried and dissolved in $CaCl_2$ solution in ethanol and water with molar ratio of (1:2:8) at 15 w/v%. The solution was then filtered through a miracloth (Calbiochem, San Diego, CA, USA) to remove the small aggregates present in solution. This solution was then dialyzed against de-ionized water using dialysis tubing with molecular weight cutoff 12,000–14,000 Daltons (spectra/Por®, Spectrum Laboratories, Inc CA, USA) for 3 days, and water was exchanged one a day.

Overall, the yield of aqueous SF solution was calculated to be 6 wt%, which was determined by weighing the remaining solid weight after drying. To fabricate the SF electrospun solution, 200 mL of SF solution was concentrated to 8 wt% by adding in dialysis tubing with a molecular weight cutoff of 6000–8000 Daltons, and the tubing was sprinkled with 40 g of poly(ethylene glycol) (PEG, MW 20,000) powder covering the tubing membrane and stored in refrigerator for overnight resulting in concentrated 100 mL viscous solution that equaled 12 wt% aqueous SF solution. Finally, this 12 wt% aqueous SF solution was diluted to 10 wt% solution and used for fabricating scaffolds (Fig. 1). The aqueous SF solutions were stored at 4 °C and used within 15 days of time, to avoid denaturation and precipitation.

2.2. SF nanosheet and SF nanomatrix fabrication

SF solution was blended with poly(ethylene oxide) (PEO) to obtain desirable viscosity and spinnability. Silk/PEO (mixture ratio: 4:1) aqueous solution was prepared by adding PEO (200,000 MW) directly into the SF aqueous solution of 10 wt.%. For electrospinning, the silk/PEO solution was placed in a 10 mL plastic syringe with a 22 needle gauge (0.7 mm OD × 0.4 mm ID) at a constant flow rate of 1.2 mL/h, maintained using a syringe pump to keep the solution drop at the tip of the needle without dripping [23]. The positive electrode was used to apply a voltage of 20 kV to the needle tip through an alligator clip, while the negative electrode was set to an applied voltage of 2 kV to the collector. Four syringes were mounted in parallel to produce nanofibers. The needle tip-to-collector distance was 15 cm. Such processing parameters were accurately adjusted so that stable jets could be obtained.

To achieve a highly porous structure in the nanosheet, sodium chloride crystals (with a diameter of <180 μm) were dispensed onto a rotating drum above the collector during the electrospinning (Fig. 2). The dispensing rate was approximately 35 g/h [24].

After the electrospinning, the silk nanosheet and sodium chloride crystal embedding SF nanomatrix was dried and subsequently immersed in ethanol 100% for 1 h for re-crystallization of SF. Then, the embedded sodium chloride particles and PEO were leached out by soaking the nanomatrix in distilled water for one day; the water was changed three times. The SF electrospun nanomatrix was lyophilized for 48 h (Fig. 2).

2.3. Characterizations

Scanning electron microscopy (SEM) (SUPRA55 V VP-FESEM, Carl Zeiss) was performed to investigate SF nanosheet and SF nanomatrix morphology. The samples were coated with a thin 10 nm layer of gold/palladium for 120 s at 15 mA discharge current (Ion Sputter 1010, Hitachi, Japan). After coating, the micrographs were taken at an accelerating voltage of 1.2–1.3 KV. The average pore sizes were determined by using software (INNERVIEW 2.0) after measuring the average size of random pores (50 individual pores per sample) from SEM images (Fig. 3).

Water uptake of swollen SF nanosheet and SF nanomatrix, Medifoam® were measured by immersing them in distilled water for 24 h at room temperature and determined by following Equation [25]: (Fig. 4A).

$$\text{Water uptake}\,(\%) = \frac{W_s - W_d}{W_s} \times 100 \quad (1)$$

whereas W_s and W_d are wet and dry weight of sample, respectively. The dry weight was measured after drying in vacuum oven of 60 °C. Porosity of sample was measured by a liquid displacement method. Ethanol was used as displacement liquid as it permeates through sample without swelling or shrinking the matrix. Sample (dry weight, W) was immersed in a known volume (V_1) of ethanol in a graduated cylinder for 5 min. The total volume of ethanol and the ethanol-impregnated sample was recorded as V_2. The ethanol-impregnated sample was then taken from the cylinder and the residual ethanol volume was recorded as V_3. The porosity of sample (P) was determined by following Equation [26]. (Fig. 4B).

$$\text{Porosity}\,(\%) = \frac{V_1 - V_3}{V_2 - V_3} \times 100 \quad (2)$$

2.4. Animal model

The animal studies were carried out in accordance with guidelines and approval of the Institutional Animal Care and Use committees (IACUC) of Hallym University in Korea. Thirty male Sprague-Dawley rats (obtained at 250–270 g) were housed in separate cages with free access to laboratory rodent food and water under standardized air and light conditions at a constant temperature of 25 °C ± 1 °C with a 12 h light/day cycle.

2.5. Measurement of burn wound healing

To examine the effects of SF nanomatrix on the burned wound-healing process, burn wounds were created on the back of rat under anesthesia with single intraperitoneal dose of 0.15 mL of Tiletamine/Zolazepam HCl (Zoletil, Virbac, France) and 0.1 mL Xylazine HCl (Rompun, Bayer, Korea). After anesthetizing rats, fur on the back skin was removed. The back of the rat was exposed for 30 s