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Physico-chemical properties and efficacy of silk fibroin fabric coated with different waxes as wound dressing

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ABSTRACT

Silk fibroin (SF) has been widely used as a wound dressing material due to its suitable physical and biological characteristics. In this study, a non-adhesive wound dressing which applies to cover the wound surface as an absorbent pad that would absorb wound fluid while accelerate wound healing was developed. The modification of SF fabrics by wax coating was purposed to prepare the non-adhesive wound dressing that is required in order to minimize pain and risk of repeated injury. SF woven fabrics were coated with different types of waxes including shellac wax, beeswax, or carnauba wax. Physical and mechanical properties of the wax-coated SF fabrics were characterized. It was clearly observed that all waxes could be successfully coated on the SF fabrics, possibly due to the hydrophobic interactions between hydrophobic domains of SF and waxes. The wax coating improved tensile modulus and percentage of elongation of the SF fabrics due to the denser structure and the thicker fibers coated. The *in vitro* degradation study demonstrated that all wax-coated SF fabrics remained up to 90% of their original weights after 7 weeks of incubation in lysozyme solution under physiological conditions. The wax coating did not affect the degradation behavior of the SF fabrics. A peel test of the wax-coated SF fabrics was carried out in the partial- and full-thickness wounds of porcine skin in comparison to that of the commercial wound dressing. Any wax-coated SF fabrics were less adhesive than the control, as confirmed by less number of cells attached and less adhesive force. This might be that the wax-coated SF fabrics showed the hydrophobic property, allowing the loosely adherence to the hydrophilic wound surface. In addition, the *in vivo* biocompatibility test of the wax-coated SF fabrics was performed in Sprague-Dawley rats with subcutaneous model. The irritation scores indicated that the carnauba wax-coated SF fabric was not irritant while the shellac wax or beeswax-coated SF fabrics were slightly irritant, comparing with the commercial wound dressing. Therefore, SF fabrics coated with waxes, particularly carnauba wax, would be promising choices of non-adhesive wound dressing.

1. Introduction

Wound healing process is complex and involved with many different cell–cell and cell–matrix interactions to establish complete tissue regeneration [1,2]. Wound dressings which are applied to cover and protect the wound as well as accelerate natural wound healing should meet several criteria: (1) biocompatibility, (2) prevent dehydration of the wound and retain a favorable moist environment, (3) physically protect the wound against dust and microorganisms, (4) allow gas permeability, (5) promote epithelization, and (6) non-adherent property which can be easily removed without trauma. Wound dressings currently used are mostly made of readily available biomaterials that require minimal processing, possess non-toxic, non-allergenic, and antimicrobial properties, as well as promote wound healing [3].

Among biologically derived materials, silk fibroin (SF) has been explored as a biomaterial in biomedical applications for centuries. SF, derived from the *Bombyx mori* silkworm, is a fibrous protein in which the main components (*i.e.* glycine and alanine) are specific sequence of non-polar amino acids. SF has unique physical, mechanical and biological properties, including strength, toughness, elasticity, lightweight, biocompatibility, biodegradability, minimal inflammatory reaction, capability to promote wound healing, and easy chemical modification to suit the applications [4]. Due to the ability to promote adhesion and proliferation of various cells including keratinocytes and fibroblasts, SF has been used as a biomaterial to fabricate bioactive wound dressings in various formulations [5–11]. It was reported that the

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wound dressings made of SF could successfully heal the wounds due to their favorable physical and biological properties [5,8–11]. Baoyong et al. have investigated the feasibility of recombinant spider silk protein as a wound dressing material for coverage of deep second-degree burn wounds in Sprague-Dawley (SD) rat model [5]. It was found that the recombinant spider silk protein membrane promoted the recovery of wound skin by increasing the expression and secretion of basic fibroblast growth factor and hydroxyproline. The effects of PVA/chitosan/fibroin-blended spongy sheets on wound healing in rats were investigated [8]. It was reported that the wounds treated with SF-containing spongy sheets showed accelerated regeneration due to the increase of vascular in growth and the absence of inflammatory cells. Vasconcelos et al. have fabricated the novel SF/elastin scaffolds for the treatment of burn wounds [11]. Wound healing assessed through the use of human full-thickness skin equivalents (EpidermFT®) showed the good re-epithelialization and fast wound closure in the wounds treated with SF/elastin scaffolds. Although the successful use of SF wound dressings was reported, only a few researches focus on the adhesive property of the dressings to the wound bed. After the wound is healed, the dressing must be removed, therefore, the non-adhesive wound dressing is required in order to minimize pain and risk of repeated injury. In this study, we have established the new types of non-adhesive wound dressings which are the SF fabrics coating with various biocompatible waxes.

Shellac is a natural polymer that is obtained by refining the secretion of *Kerria lacca*, a parasitic insect found on trees in Southeast Asia. Shellac wax is known as enteric material that provides a protective layer for active substances as well as a good moisture barrier and desirable gloss [12]. It is widely used either alone or in mixture with lipid compounds for fruit coating [13,14]. Beeswax is a natural wax produced in the bee hive of honey bees of the genus *Apis*. It is mainly esters of fatty acids and various long chain alcohols. Beeswax is one of the oldest commercial products in man's history, and its chemistry has been extensively studied [15,16]. Purified and bleached beeswax is used in the production of food, cosmetics, and pharmaceuticals [17]. Carnauba wax is a natural edible coating material, which is recovered from the underside of the leaves of a Brazilian palm tree (*Copernica cerifera*). It is mainly used to retard moisture loss and impart glossiness [18]. The beneficial role of carnauba wax is well known for enhancing shelf life and maintaining post-harvest quality of fruits [19].

In this study, a non-adhesive wound dressing which applies to cover the wound surface as an absorbent pad that would absorb wound fluid while accelerate wound healing was developed. The modification of SF fabrics by wax coating was purposed to prepare the non-adhesive wound dressing that wound be easily removed without trauma. In addition, our wax-coated SF fabrics would be advantageous in terms of the reduction of friction and the prevention of dehydration for the wound. SF woven fabrics were coated with different types of waxes including shellac wax, beeswax, or carnauba wax. Physical and mechanical properties of the wax-coated SF fabrics were characterized. *In vitro* degradation test was performed in lysozyme solution. In addition, a peel test of the wax-coated SF fabrics was carried out in the partial- and full-thickness wounds of porcine skin, comparing with the commercial wound dressing mesh "Sofra-tulle®". The *in vivo* biocompatibility test of the wax-coated SF fabrics was also performed in Sprague-Dawley rats with subcutaneous model. The irritation scores were reported compared with those of the "Sofra-tulle®". Hematoxylin and eosin (H&E) staining was carried out to evaluate the infiltration of inflammatory cells including macrophages and giant cells which indicated the extent of foreign body reaction.

2. Materials and methods

2.1. Materials

Silk fibroin (SF) woven fabric was purchased from Chul Thai Silk Co., Ltd., Phetchabun province, Thailand. Shellac wax, beeswax, and carnauba wax were purchased from Sigma–Aldrich Laborchemikelien, Germany. Other chemicals were analytical grade and used without further purification.

2.2. Differential scanning calorimetric (DSC) analysis

Thermal characteristics of shellac wax, beeswax, and carnauba wax were analyzed by differential scanning calorimeter (DSC) using Perkin Elmer Diamond DSC model. The sample weight was about 10 mg. All samples were performed in the scanning mode from 35 to 180 °C at the heating rate of 10 °C/min. Dry nitrogen gas was introduced into the DSC cell as the purging gas.

2.3. Fabrication of wax-coated SF fabrics

Shellac wax, beeswax, and carnauba wax were dissolved in morpholine solution (0.1% w/v). SF fabric (5 × 5 in.) was stretched, immersed completely in the wax solution, and left to dry quickly at room temperature to obtain the wax-coated SF fabrics. The fabrics before and after wax coating were weighed. Percentage of weight increased was calculated ($n = 3$).

2.4. Mechanical test

The tensile test was performed on the wax-coated SF fabrics (150 mm in length and 25 mm in width) at room temperature using a universal testing machine (Instron, No. 5567) at a constant rate of 300 mm/min. The curves of force as a function of deformation (mm) were automatically recorded by the software. The tensile modulus (MPa) and elongation at break were calculated according to the ASTM D638-01 method ($n = 6$).

2.5. Scanning electron microscope (SEM) observation

Surface appearance of the wax-coated SF fabrics was observed on a scanning electron microscope (SEM, JSM 5400, JEOL) at an accelerating voltage of 12–15 kV after sputter-coating with gold.

2.6. In vitro degradation test

A known weight of wax-coated fabrics was subjected to the degradation in PBS (pH 7.4) containing lysozyme (1.6 μg/mL) at 37 °C. For the comparison between the wax-coated fabrics and the commercial wound dressing mesh "Sofra-tulle®", accelerated study of degradation was conducted in PBS (pH 7.4) containing lysozyme (1.6 μg/mL) at 70 °C [20]. At each time point, the remained fabric was collected, washed with DI water, freeze dried, and weighed. Percentage of weight remaining was calculated ($n = 3$).

2.7. Peel test with porcine skin

Porcine skin was used within 2 h after sacrifice. Partial-thickness wounds were prepared by scraping the outer skin layer while full-thickness wounds were prepared by cutting the skin at 1 cm in depth. The wounds were randomly dressed with wax-coated fabrics or the commercial wound dressing mesh "Sofra-tulle®" (Patheon UK Limited, Swindon, UK) as a control. After 12 h, the dressings were removed and fixed in 2.5% (v/v) GA solution at 4 °C for 1 h. Then, the dressings were dehydrated in serial dilutions of ethanol (50, 70, 80, 90, 95, 99 and 100% v/v, respectively) for