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Preparation of chitosan/silk fibroin blending membrane fixed with alginate dialdehyde for wound dressing

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ABSTRACT

The objective of this work was to prepare chitosan/silk fibroin (CS/SF) blending membranes crosslinked with alginate dialdehyde (ADA) as wound dressings and to evaluate the physical properties and biocompatibility of the membranes. The morphology of membrane was observed by scanning electron microscopy (SEM) which showed that the well consistency of these two compositions. Further, the stability, water absorption and water vapor permeability of the ADA fixed CS/SF membranes could meet the needs of wound dressing. Furthermore, the biocompatibility of ADA fixed membranes was investigated by MTT assays and SEM in vitro, and the membranes were found to promote the cell attachment and proliferation. These results suggest that ADA fixed CS/SF blending membranes with a suitable ratio could be a promising candidate for wound healing applications.

1. Introduction

Burns are the serious trauma which often present in many serious accidents such as fires and war. It is very important to treat the burns effectively in most developed and developing countries [1–2]. Among these treatments of burns, wound dressings play a very important role [3]. Recently, there is an increasing interest in the development of wound dressings based on biopolymers as they are biocompatible, biodegradable and renewable. One material for wound dressing must be shown its importance and it is directly related to time and outcomes of skin's self-restoring [4]. An ideal wound dressing should economic and be required to provide optimal environment for wound healing process, such as maintaining a moist milieu, absorbing excess exudates, allowing gaseous exchange, being easy to apply and remove without causing new trauma as well as being biocompatible [5]. Considering these, each type of wound dressing requires a variety of sources, desirable physicochemical and nontoxic properties. Various wound dressings are available commercially in the markets, but there are still some problems for these wound dressings in respect of satisfying the all requirements mentioned above. Therefore, the researchers make an attempt to develop serials novel materials for wound healing.

As a kind of wound healing materials, chitosan (CS), a (1–4)-linked 2-amino-2-deoxy-β-D-glu-copyranose, is one of the most abundant natural polysaccharides [6]. CS has several excellent properties, such as biodegradability, biocompatibility, non-antigenicity, nontoxicity, biofunctional and antimicrobial properties, which could be beneficial for using in a wide variety of biomedical applications, such as drug delivery carriers, surgical thread, bone healing materials, especially wound dressings [7]. CS could achieve hemostasis and accelerate tensile strength of wounds by speeding the fibroblastic synthesis of collagen in the first few days of wound healing. Due to these reasons, CS has been used as one of the important biomaterials for wound managements in recent years [8]. However, as a semi-crystal polymer, pure CS membranes suffer from relatively poor mechanical properties resulted in their brittleness. Growing interests have been focused on CS-based materials to improve CS properties. Several blending biomaterials have been reported for biomedical applications which usually could achieve performances of their constituting ingredients synergistically. Therefore, CS has been selectively blended with some natural polymers to obtain several wounding dressing applications [9].

Silk fibroin (SF) is a natural polymeric biomaterial derived from silkworms and spiders [10], which is a linear polypeptide comprising 17 amino acids, mainly nonpolar ones such as alanine and glycine [11]. SF with β-sheet structure has been extensively utilized in wound dressings' areas owing to its oxygen transmission, mechanical properties, biocompatibility and biodegradability [12].

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In order to effectively combine the goodness of pure CS and SF, blending of both materials was attempted previously [13]. However, the biological stability of untreated CS/SF blending material is still not good enough for wound dressings. The CS could be modified in order to improve the stability and durability, and one of the most commonly modifications is chemically induced crosslinking. As an active research in recent years, alginate dialdehyde (ADA) is one of the effective cross-linking reagents which presents more reactive groups that can couple the active amino-group in protein [14]. Therefore, the aforementioned results promote us to evaluate the feasibility of CS/SF membranes using ADA as a crosslinking reagent [15].

In this study, ADA reagent was prepared under controlled conditions according to the reports [17]. And then CS/SF blending membranes fixed with ADA were prepared and the biological stability of these membranes was investigated. The water absorption rate, water vapor transmission rate and biocompatibility were also evaluated. This study would provide a novel membrane which could meet the requirements for wound dressing.

2. Materials and methods

2.1. Preparation of SF and ADA

In this experiment, The SF solutions were prepared by the method mentioned in the literature [16] and then lyophilized for about 48 h. Briefly, *bombyx morisilk* were treated twice with 0.5% (w/w) $NaHCO_3$ solution at 70 °C for 1 h and then rinsed with 70 °C distilled water to remove sericin. Degummed silk was dissolved in a mix solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (mole ratio, 1:2:8) at 70 °C for 6 h and filtered to get a SF solution. The SF solution was dialysis and then lyophilized. ADA was prepared with slight modification for better oxidation as the method in our previously study [17].

2.2. Preparation of CS/SF blending membranes

Membranes were prepared via blending CS and SF and crosslinked with ADA. CS and SF with different weight ratios (60%CS/40% SF and 80% CS/20% SF) were dissolved in acetic acid aqueous solution. CS/SF was dissolved completely and then divided into two groups: one group was added with a corresponding ratio of ADA (1ADA:4CS/SF, w/w), the other was not. In the process of adding with ADA, the samples were deaerated and dried in a vacuum drying oven at 40 °C for about 4 h and then sprayed ADA solution on the surface of the membranes. Finally, the samples were dried thoroughly. Pure CS film was served as control. Samples were stored in a refrigerated desiccator until used.

2.3. Morphology and chemical analysis of CS/SF blending membranes

The surface morphology of the CS/SF blending membranes was observed with a scanning electron microscope (SEM) (Hitachi S3000N, Hitachi, Ltd., Chiyoda, Tokyo, Japan). All specimens were coated with a conductive layer of sputtered gold for enhancing surface conductivity before scanning. Chemical structure of the membranes was investigated by Fourier transform infrared (FTIR) spectrometer using a spectrophotometer (Spectrum One, Perkin-Elmer Inc., Waltham, MA, U.S.A.) over a wavenumber range between 400 and 2000 cm^{-1} with a resolution of 0.125 cm^{-1}.

2.4. Stability in normal saline solution

The samples stability in normal saline solution was determined by measurement of loss of weight of the dried samples (initial weight M_1) in normal saline solution at 37 °C about 24 h Then, each sample was dried to constant weight (M_2) at 40 °C. The weight loss ratio = $(M_1 - M_2)/M_1 \times 100\%$. All experiments were done in quintuplicate.

2.5. Water absorption rate

The water absorbing capacity of a wound dressing is a key design criterion for providing and maintaining a moist environment over the wound bed. It was determined by a gravimetric method. The samples were weighted (M_1) and immersed in distilled water at room temperature for 1 h. Afterward, they weighted after removing the water from the surface with blotting paper (M_2). The percentage water absorption of samples (B) in the distilled water was then calculated from the formula:

$$B = \frac{M_2 - M_1}{M_1} \times 100\%.$$

All samples were done in quintuplicate and the average value was taken as the percentage water absorption.

2.6. Water vapor permeability

Water vapor permeability was determined according to the ASTM E96/E96M-10 [ASTM, 2010]. The glass bottles (1.35 cm inside diameter, D) were filled with a normal saline solution and then the test membranes were fixed onto its opening, respectively. An open bottle was used as the control ($n = 5$). Put the bottles (known weight M_1) into the drying oven at 37 °C. Evaporation of water through the test membrane was monitored by measurement of loss of weight of the bottles. After 24 h weighed the bottles on an electronic balance (M_2). The water vapor transmission rate (WVTR) was then calculated from the formula:

$$A = \frac{4(M_1 - M_2)}{\pi D^2},$$

where A is air permeability 24 h/m^2.

2.7. Cells culture and analysis

Cell proliferation on the surface of blending membranes was assessed by colorimetric assay, which detected the conversion of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) to formazan. Briefly, 1 cm × 1 cm test samples made from the membranes were sterilized by ethylene oxide vapor and immersed in RPMI 1640 medium with 10% FBS for 24 h ($n = 3$). Then, the activity L929 fibroblasts were cultured on each test sample and determined after two, four and six days. 20 μl/well of MTT solution (5 mg/ml in phosphate buffered saline (PBS)) was added in each well and the plate was incubated at 37 °C to allow the formation of formazan crystals. After 4 h the dimethylsulfoxide (1.5 ml/well) was added to all wells after aspirating the culture medium and mixed thoroughly to dissolve the dark blue crystals at room temperature. Ten minutes later, the optical density (OD) at 492 nm was measured with a microplate reader (Model550, Bio Rad Corp. USA).

In order to observe the cellular morphology on membranes, cell-seeded membranes were pre-treated by washing with PBS three times and immersing in PBS containing 3% glutaraldehyde (pH 7.4) for 2 h. They were then dehydrated in increasing concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) twice. The critical point drying of samples was undertaken with liquid CO_2. The samples were sputter coated with gold and examined by SEM (Japan Electronics Co., Ltd.). The growth of cells on the membranes was observed on the 4th day.