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Preparation of chitosan-collagen-alginate composite dressing and its promoting effects on wound healing

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ABSTRACT

The present study aimed to prepare a composite dressing composed of collagen, chitosan, and alginate, which may promote wound healing and prevent from seawater immersion. Chitosan-collagen-alginate (CCA) cushion was prepared by paintcoat and freeze-drying, and it was attached to a polyurethane to compose CCA composite dressing. The swelling, porosity, degradation, and mechanical properties of CCA cushion were evaluated. The effects on wound healing and seawater prevention of CCA composite dressing were tested by rat wound model. Preliminary biosecurity was tested by cytotoxicity and hemocompatibility. The results revealed that CCA cushion had good water absorption and mechanical properties. A higher wound healing ratio was observed in CCA composite dressing treated rats than in gauze or chitosan treated ones. On the fifth day, the healing rates of CCA composite dressing, gauze, and chitosan were 48.49% ± 1.07%, 28.02% ± 6.4%, and 38.97% ± 8.53%, respectively. More fibroblast and intact re-epithelialization were observed in histological images of CCA composite dressing treated rats, and the expressions of EGF, bFGF, TGF-β, and CD31 increased significantly. CCA composite dressing showed no significant cytotoxicity, and favorable hemocompatibility. These results suggested that CCA composite dressing could prevent against seawater immersion and promote wound healing while having a good biosecurity.

1. Introduction

Wound dressing is an important component that contributes to the acceleration of wound healing and protection against bacterial infection. An ideal dressing should maintain a moist environment at the wound interface, allow gaseous exchanges, act as a barrier to microorganisms, and remove excess exudates [1,2]. Moreover, the materials should have powerful promoting effect on wound healing, possess a suitable mechanical property, be non-toxicity, have anti-infective properties, have a satisfactory water vapor transmission rate (WVTR) [3], and be easily removed without trauma [4]. To date, no single wound dressing can meet all of these requirements.

Many wound dressings have been created, such as alginate [5], collagen [6], and chitosan [7] in different kinds of forms, such as films, foams, fiber, or hydrophilic gel. Alginate is a biocompatible polysaccharide that is commonly used in the pharmaceutical, biomedical, cosmetic, and food industries. Though solid dressings composed of alginate can absorb water and promote wound healing, they are not effective hemostatic materials, particularly against massive hemorrhage [8]. Many collagen-related products have been developed in the past few years for wound healing purposes. Some of them are approved by drug-controlling authorities and are now commercially available [9]. In addition, collagen has low antigenicity and low inflammatory properties, good biocompatibility, and has the ability to promote cell attachment and proliferation. Chitosan is considered as a highly favorable material due to its excellent biocompatibility, nontoxicity, antibacterial

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properties, and hemostasis, whereas gelatin exhibits excellent film-forming properties and water-absorbing ability [10–12].

Simple materials may not satisfy to all of these requirements that an ideal dressing needs. We combined collagen, chitosan, and alginate dressing to produce a new type of dressing material that can promote wound healing efficiently. It was attached to an anti-seawater immersion PU membrane to form CCA composite dressing. This new type of dressing may be applied on wounded soldiers, seamen and sea-fishers who worked on sea.

2. Materials and methods

2.1. Materials

Collagen and chitosan (degree of acetylation was 85–95%) were kindly provided by Shanghai Qisheng Biologic Agent Co. Ltd. (China). Alginate dressing was kindly provided by Naiqier Biotechnology Co. Ltd. (China). SD male rats (200–250 g) were purchased from Sino-British SIPPR/BK Lab Animal Co. Ltd. (China).

2.2. Preparation of CCA composite dressing

The dressing was prepared by coating and vacuum freeze drying method. Briefly, 0.25 g of chitosan was dissolved in 100 mL of 0.4% acetic acid, and 50 g of collagen was added into the chitosan solution and mixed by mechanical stirring at 200 rpm for 2 h. 10 mL of the mixed chitosan-collagen solution was painted on a 10 cm × 10 cm alginate dressing which contained 1.5 g of alginate fibers, and freeze-drying at −50 °C for 24 h, then the CCA composite cushion was obtained. The cushion was attached to an anti-seawater immersion PU membrane (its prepared method will be presented in other paper) to form the CCA composite dressing.

2.3. Assessment of physicochemical properties of CCA composite dressing

The swelling ratio of CCA composite dressing was detected by a gravimetric method [13]. Briefly, the CCA dressing pieces of same size and weight were immersed in physiological saline, deionized water and PBS at room temperature for 2 h, then took out the dressing pieces and removed the water adhered on the surface by gently blotting, and immediately weighted. The swelling ratio was calculated by the following equation.

$$\text{Swelling ratio}(\%) = (W_w - W_d)/W_d \times 100\%, \text{Swelling ratio}(\%) = (W_w - W_d)/W_d \times 100\%,$$

Here, W_d and W_w are the weight of the dressing before and after immersion, respectively. All samples were triplicate in the experiment.

The porosity of the prepared dressing was determined using the reported method [14]. The weighted dressing was immersed in absolute alcohol at room temperature until it was saturated (about 24 h), then taken out and the final weight was noted. The porosity was calculated using the following equation.

$$\text{Porosity}(\%) = [(Wt - Wd) \div \rho]/V \times 100\%\text{Porosity}(\%) = [(Wt - Wd) \div \rho]/V \times 100\%$$

Here, Wd and Wt indicate the weights of the dressing before and after immersion in absolute alcohol respectively, and ρ is the density of absolute alcohol, and V is the volume of the dressing before immersion. Experiment was done thrice and the average values were taken.

Degradation detection of CCA composite dressing was detected in wound exudate like solution. CCA composite dressing was dried to a constant weight (Wd), then immersed in 25 mL of wound exudate like solution which contained 8.298 g of NaCl, 0.368 g of $CaCl_2 \cdot 2H_2O$ in 1 L of distilled water. After soaking at 37 °C for 24 h, the dressing was taken out and blotted with filter paper to remove the surface solution. Then the dressings were freeze-dried and measured the dry weight (Wt). All the experiments were performed in triplicate. The degradation rate was calculated by the following equation.

$$\text{Degradationrate}(\%) = (Wt - Wd)/Wt \times 100\%.\text{Degradationrate}(\%) = (Wt - Wd)/Wt \times 100\%.$$

The tensile strength (MPa) and the percentage of elongation at break (%) of CCA composite dressings were measured by a universal testing machine (YG-B-026G-500, Huaheng Company, Chengdu China). The dressing specimens were in a rectangular shape with the dimensions of 100 mm × 15 mm. Three parallel tests were completed for each sample.

The morphology of the CCA composite dressing was studied by the scanning electron microscopy (Phenom Company, Netherlands).

2.4. Evaluation of the efficacy of CCA composite dressing on anti-seawater immersion and wound healing in vivo

The capacity of CCA composite dressing to heal full-thickness skin defects was evaluated in a rat model. The study was approved by the Ethical Committee of Naval Medical Research Institute and animals were treated according to the regulations. All rats were provided with environmental enrichment and food and water ad libitum. Twenty-four male rats weighing 220–250 g were randomly assigned to 3 groups, and each group for 4 time points (the fifth, the eighth, the 11th, and the 13th days) on the day of surgery. The first group was treated with sterilized gauze as the negative control, and the second group was treated with chitosan dressing as the positive control, and the third group was treated with CCA composite dressing. Prior to surgery, the dorsal hair was removed thoroughly with 8% sodium sulfide. Full-thickness skin wounds (diameter 0.8 cm) were created on each rat by excising the dorsum under sterile environment, and the dressing with anti-seawater immersion PU membrane were placed on the wound bed, respectively. After covering with the dressings the wounded rats were soaked in seawater at a constant temperature of 28 °C for 4 h. After the day of surgery, the rats were fed a standard diet and housed individually in a controlled temperature environment (22–23 °C), and were observed twice each day to ensure that the dressing on the wound was intact. The wounds were photographed on the first, the fifth, the eighth, the 11th, and the 13th days post-surgery. All images were adjusted to the same scale, and the sizes of the wounds were calculated according to the area of wound. The wound healing rate was calculated as the following equation.

$$\text{Woundhealingrate} = (S_0 - S_t)/S_0 \times 100\%, \text{Woundhealingrate} = (S_0 - S_t)/S_0 \times 100\%,$$

Here S_0 is the area of original wound and S_t is the area of the wound at the testing time.

2.5. Histological and immunohistochemistry analyses of wounded tissues treated by the dressings

After the efficacy evaluation of wound healing rate, the wound tissues of the three groups animals were collected on the fifth, eighth, 11th, and 13th days. The harvested wound tissue samples were fixed in 4% formaldehyde solution at 4 °C, dehydrated with a graded series of ethanol solutions, embedded in paraffin, and sequentially sectioned at 4 μm. Skin tissue sections were

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