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Alginate/chitosan based bi-layer composite membrane as potential sustained-release wound dressing containing ciprofloxacin hydrochloride

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ABSTRACT

The aims of this research were to develop and evaluate a novel ciprofloxacin hydrochloride loaded bi-layer composite membrane based on alginate and chitosan. In vitro antimicrobial activity, drug permeation study, morphology, cytotoxicity, primary skin irritation and in vivo pharmacodynamics were investigated. Results showed that the membranes could inhibit the growth of microorganisms for longer than 7 days. And there was no significant decrease in the metabolic activity of the Hacat fibroblasts cells were treated with the membranes. No edema and erythema were observed after administration of membranes on the rabbit skin after 14 days. Moreover, the results of pharmacodynamics showed that the membranes were more effective in improving the wound healing process. In conclusion, a novel bi-layer composite membrane was developed and results suggested that it could be exploited as sustained-release wound dressings.

1. Introduction

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration [1]. It can be summarized into five independent and overlapping stages, including hemostasis, inflammation, migration, proliferation and maturation. The ideal wound dressing should have the ability to: (1) absorb exudates and toxic components from the wounds surface; (2) maintain a high humidity at the wound/dressing interface; (3) allow gaseous exchange; (4) provide thermal insulation; (5) protect the wound from bacterial penetration; (6) be nontoxic; and (7) be removed easily without trauma to the wound [2]. Among wound dressings, the bi-layer composite membranes which consist of dense outer layer and porous sub-layer have good ability to promote the healing process and were developed by a variety of biopolymers [3–5]. Usually, the outer layer is designed for the prevention of bacterial invasion, and possibly to act as rate-controlling layer for water vapor permeation. Meanwhile, the inner layer is designed for attachment to wound tissue and the drainage of wound exudates [6–8]. Based on these ideas, several bi-layer synthetic dressings such as sponge-like composite membranes have been developed in recent years [9–12].

Alginate (Alg) is an anionic linear polysaccharide containing 1,4-linked D-mannuronic acid and L-guluronic acid residues, which can form hydrogels in the presence of some multivalent metal ions. Because Alg-hydrogel not only can fit the contours of wound beds and provide a moist environment, but also can absorb a large amount of liquid so as to reduce the frequency of dressing changes, so it has been widely investigated. Chitosan is a copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranase and 1-amino-2-deoxy-D-glucopyranase.It possess many properties such as biocompatibility, biodegradability, hemostatic activity, anti-infection and wound healing acceleration properties, which are advantageous for wound dressing [13,14]. It is well known that under simultaneous electrostatic interaction between protonated amino groups of chitosan and carboxyl groups of alginate, chitosan can diffuse into three-dimensional gel network of alginate, which is very helpful for the formation of the bi-layer composite membranes [15].

Ciprofloxacin hydrochloride (CIP), a fluoroquinolone antibiotic, is one of the most widely used antibiotics in wound healing. CIP-loaded Alg/Chs sponge have been developed as a wound dressing to be used in wound and/or burn treatment, and morphology, water uptake, in vitro drug release behavior and antimicrobial activity were studied. Although results showed that the sponge could protect the wound from the infections by releasing CIP with desired

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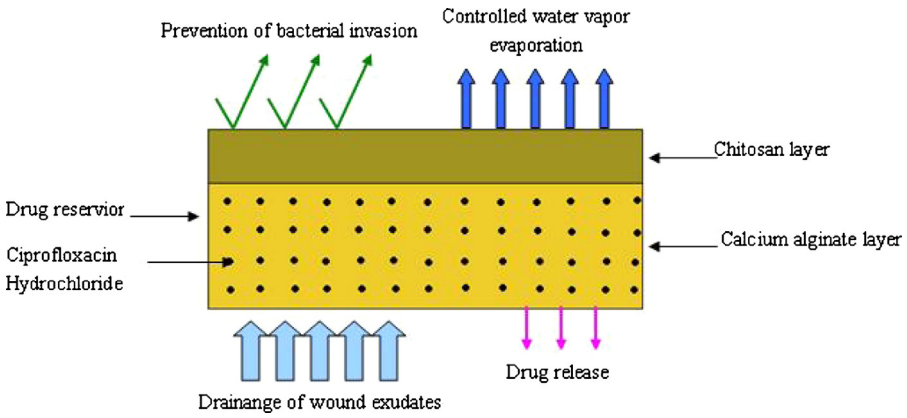

Fig. 1. Design of the Alg/Chs bi-layer composite membrane.

release rate, but it was only proved by in vitro antibacterial experiment and no in vivo research was carried out [16]. In order to better evaluate the potential and availability of the bi-layer composite membrane as a biomaterial for wound healing in vivo, a novel CIP-loaded Alg/Chs bi-layer composite membrane will be developed.

As shown in Fig. 1, the outer Chs layer was designed for prevention of bacterial invasion, controlling water vapor permeation and retaining a favorable moist environment at the wound interface. Meanwhile, the inner Alg layer was conceived as an absorption system with excellent water uptake capacity for wound exudates. More innovatively, the inner layer was also conceived as a drug reservoir for drug sustained-release and sustained antimicrobial efficacy. And our previous study showed that the CIP was found to be released from the composite membrane for 48 h and the membrane was found to control the bacterial growth persistently [17]. In this study, the biological compatibility and in vivo pharmacodynamic evaluation will be carried out to evaluate its potential as wound dressing.

2. Materials and methods

2.1. Materials

Alginate and chitosan were supplied by Qingdao Crystal Rock Biology Development Company and Hangzhou Golden-Shell Biochemical, respectively. Ciprofloxacin hydrochloride (CIP) was bought from Henan Topfond Pharmaceutical Co., Ltd. Water used in experiment was distilled. Bactroban (Mupirocin Ointment, GlaxoSmithKline, Tianjin) was chosen as the positive control drug in the pharmacodynamic evaluation. All other chemicals and solvents were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of Alg/Chs bi-layer composite membrane

Briefly, 100 mL solutions containing Alg (1.8%, w/v), glycerol (5%, w/v) and different content of CIP were poured into a Teflon plate (15 cm × 15 cm) and allowed to dry at 40 °C for 24 h, respectively, according to Table 1. 1.2 g Chs was dissolved in 60 mL HAc solutions (1%, v/v), and then the solutions were separately cast onto the Alg layer homogenously and allowed to dry at 40 °C. These composite membranes were firstly immersed in 10% sodium tripolyphosphate solution for 1 min, and then were separately immersed in various $CaCl_2$ solutions for different times according to Table 1. According to this procedure, these membranes were expressed as 'CA-*a*-*b*-*c*', respectively, where *a* represented the content of CIP, *b* represented the concentration of $CaCl_2$ solution and *c* represented the crosslinking time. As shown in Table 1, formulations No. 1–5 containing different CIP were prepared for antibacterial activity assessment. Formulations No. 6–11 with different crosslinking time were prepared for evaluating the effect of crosslinking time on drug release. And formulations No. 12–17 with same crosslinking time (5 min) were prepared to evaluate the effect of concentration of $CaCl_2$ solution on drug release. It should be noted that No. 5 and No. 15 were the same formulation which expressed as CA-0.1-10-5, and No. 1 was the blank membrane which expressed as CA-0-5-3.

2.2.2. Antibacterial activity assessment

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Escherichia coli* were used to evaluate the antibacterial activity. One loopful of the bacteria was inoculated in a test tube and then it was shaken in an air-bath shaker at 37 °C for 24 h. The cultures of three strains containing approximate 108 CFU/mL were prepared and used for the evaluation of antibacterial activity, and then conducted as the agar diffusion method according to Chinese Pharmacopoeia 2010. Alg/Chs bi-layer composite membranes were sterilized by Co60 before investigation. In order to evaluate the effect of drug loading on the antibacterial activity, the bi-layer membranes with different drug content were all cut into small circular discs of diameter around 5 mm each and denoted as CA-0-5-3, CA-0.02-5-3, CA-0.1-5-3, CA-0.5-5-3, and CA-0.1-10-5, respectively. These discs were put on the surface of the petri dishes. The antibacterial activity plates were incubated at 37 °C and the inhibition zones were measured in diameter with transparent ruler and estimated once daily for 7 days.

2.2.3. Franz cell drug permeation studies

Vertical Franz-type diffusion cells were used to evaluate the drug release from the developed formulations. The composite membrane (No. 6–17) was mounted on vertical Franz-type diffusion cells with the inner layer facing the receptor compartment, respectively. The thickness of membrane was about 100 ± 20 μm and the surface area was 0.785 cm^2. The temperature of the assay was accurately controlled at 37 ± 0.5 °C. The acceptor medium was composed of 12 mL PBS (pH = 7.4) and it was withdrawn at suitable time intervals over 48 h using a syringe needle, and the same volumes were replaced with freshly prepared acceptor medium. The CIP content in the samples was determined spectrophotometrically at 273 nm (Shimadzu, UV2550) and the cumulative amount of CIP was plotted against time. The average values were calculated from Franz cell experiments ($n = 6$). In order to optimize the preparation technology, effect of crosslinking time (No. 6–11) and concentration of $CaCl_2$ solution (No. 12–17) on drug release from the composite membranes were studied, respectively.

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