Biomaterials 40 (2015) 1–11

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Novel bilayer wound dressing composed of silicone rubber with particular micropores enhanced wound re-epithelialization and contraction

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A R T I C L E I N F O

Article history:
Received 8 September 2014
Accepted 23 October 2014
Available online 19 November 2014

Keywords:
Wound dressing
Silicone rubber
Bilayer
Micropore
Wound healing

A B S T R A C T

Wound dressing is critical important for cutaneous wound healing. However, the application of current products is limited due to poor mechanical property, unsuitable water vapor transmission rate (WVTR), poor anti-infective property or poor biocompatibility, etc. In the present study, a microporous silicone rubber membrane bilayer (SRM-B) composed of two layers with different pore sizes was prepared. The physical properties, the influences of pore structure on the bacterial penetration, the cell adhesion and proliferation were studied. Lastly, the effects of the SRM-B on the healing of a mouse full-thickness wound were examined. The data showed that the small pore upper layer of SRM-B could effectively prevent the bacterial invasion, as well as properly keep the water vapor transmission rate; the large pore lower layer of SRM-B could promote the cell adhesion and proliferation. The in vivo results showed that SRM-B could significantly enhance wound re-epithelialization and contraction, which accelerated the wound healing. Our data suggested that the SRM-B, with different particular pore sizes, could serve as a kind of promising wound dressing.

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1. Introduction

The skin is the largest organ in the human body and plays important roles in continuously maintaining life. For patients with serious skin defects such as burns or other extensive skin loss, this barrier, which protects the body from damage and microbial invasion and maintains body fluid, electrolytes and nutritional components, is destroyed. Thus, cutaneous wounds severely affect human life and health.

Wounds, especially the extensive full-thickness wounds, can not be immediately repaired. Therefore, a wound dressing that ideally meets the demands of rapid wound closure is needed. An ideal wound dressing should possess suitable mechanical property, satisfactory water vapor transmission rate (WVTR), anti-infective property and favorable histocompatibility, among other features. More importantly, medical practitioners prefer dressings that can supply a suitable moist microenvironment for wound healing or wound bed preparation [1–5]. However, the currently available products are not able to meet all the clinical needs due to their individual characteristics and shortcomings.

With the development of modern medical science, many novel wound dressings have been created. Chitosan, alginate, collagen, gelatin and polyurethane are widely used to prepare different kinds of dressings, e.g., films, foams, or hydrophilic gels [6,7]. These novel wound dressings exhibit some excellent properties that improve wound healing. However, these materials still have different limitations such as poor mechanical property, unsuitable WVTR, poor anti-infective property or others, which may lead to the infection, the dehydration or the maceration of the wounds [8,9]. On the other hand, the manufacturing processes for the existing modern dressing are relatively complicated. A better dressing for wound coverage is still a challenge.

As a polymer material, silicone rubber has excellent physical and chemical properties, such as good mechanical properties, chemical

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http://dx.doi.org/10.1016/j.biomaterials.2014.10.077
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stability and gas permeability [10]. In particular, silicone rubber has good biocompatibility. These excellent properties make it a potential ideal material for wound dressing. Actually, this material has already been approved by the Food and Drug Administration (FDA) for use in medical products. For example, Integra™, which was designed to rebuild the dermal substrate [11,12]. However, the main component of Integra was still the collagen/chondroitin-6-sulfate composite [13,14], the silicone sheet was just used to protect the collagen-based scaffold.

In our previous study [15], we developed a novel way of preparing a single layer silicone rubber membrane with a microporous structure. Pore size and thickness of the membrane were tunable with our method, and the procedure for preparing the membrane was relative simple.

Thus the current study focused on the preparation of a new type of wound dressing using the silicone rubber for meeting the requests of good physical and biological features for tissue regeneration. In this study, a microporous silicone rubber membrane bilayer (SRM-B) composed of two layers with different pore sizes was prepared. It was found that the SRM-B could temporarily reconstruct the mechanical barrier in a skin defect wound and protect a wound from infection, dehydration and mechanical damage. In this way, a SRM-B supplied a suitable moist microenvironment which enhanced wound re-epithelialization and contraction. Our results indicated that the prepared microporous SRM-B could serve as an ideal wound dressing for wound protection or wound bed preparation.

2. Materials and methods

2.1. Materials and animals

The liquid silicone rubber (LSR) precursor used in this study was a medical-grade product purchased from Shenzhen Kuwart Silicone Materials Co., Ltd., China. Analytical-grade liquid paraffin (LP), hexane and sorbitan monooleate (Span-80) were obtained from Kelong Chemical Reagent Factory, Chendu, China.

Green fluorescent protein (GFP) transgenic neonatal mice and BALB/c mice (male, 18–20 g) were purchased from the Experimental Animal Department of the Third Military Medical University. All of the animals were used in accordance with ethical standards, and the animal protocols were approved by the Institutional Animal Care and Use Committee of the Third Military Medical University. The animals were individually raised in plastic cages under standardized conditions (room temperature: 25 °C; relative humidity: 50%; and circadian rhythm: 12 h). The animals were fed autoclaved standard rodent chow and water ad libitum and were adaptively bred for 1 week in the facility before the experiments.

2.2. Preparation of the microporous silicone rubber membrane

2.2.1. Preparation of the single layer microporous membranes with different pore sizes

The dry-cast technique used to prepare the single layer microporous membrane was described in our previous study in detail [15]. To prepare the single layer silicone rubber membrane with small pores (SRM-SP), i.e. the pore size of the membrane was less than 10 μm, a solution of the LSR precursor/LP/hexane/Span-80 (25/5/68/2 w/w/w/w) solution was mixed evenly and cast in a polytetrafluoroethylene (PTFE) mold. The PTFE mold was kept in an oven for 60 min at 30 °C to allow the evaporation of the hexane, then kept at 100 °C for 20 min, the liquid silicone rubber precursor turned into the solid membrane and this cured membrane was obtained. The obtained membrane was immersed in hexane at 60 °C for 4 days (the hexane was refreshed every day) to remove the liquid paraffin rich phase. Consequently, the desired micropores were formed. After that the membrane was sequentially immersed in ethanol and deionized water for 60 min to completely remove the hexane. Finally the membrane was dried in the oven at 50 °C for 6 h and SRM-SP was obtained.

To prepare the single layer silicone rubber membrane with large pores (SRM-LP), i.e. the pore size of the membrane was larger than 100 μm, a solution of LSR precursor/LP/hexane (25/20/55 w/w/w) was prepared and mixed evenly. The other procedures used to prepare the SRM-LP were as the same as those for the SRM-SP.

2.2.2. Preparation of the SRM-B

Finally, to simulate the structure of human skin, the SRM-B was prepared. The SRM-B was designed to be composed of two layers, and the pore sizes of two layers were quite different, as mentioned above. Briefly, a solution of LSR precursor/LP/hexane (25/20/55 w/w/w) was mixed evenly and cast in the PTFE mold to form the 1 mm-thickness lower layer first. To ensure the uniformity of the casting thickness, the PTFE mold was maintained horizontally using a spirit level (Jinhua epoch tools, China). The mixture was put into an oven for 60 min at 30 °C. Lastly, the membrane was kept at 100 °C for 20 min to obtain a cured membrane. After cooling at room temperature for approximately 1 h, the surplus liquid paraffin on the surface of the membrane was removed using filter paper.

To prepare the upper layer of SRM-B, the cast LSR precursor/LP/hexane/Span-80 (25/5/68/2 w/w/w/w) solution for the upper layer was prepared, and the mixture was cast directly onto the lower layer, with a casting thickness of 0.5 mm. The PTFE mold was then kept in the oven at 30 °C for 60 min, followed by 100 °C for 20 min. The bilayer membrane was then released from the mold and soaked in hexane at 60 °C for 4 days. Lastly, the membrane was sequentially immersed in ethanol and deionized water for 60 min. After drying in the oven at 50 °C for 6 h, the SRM-B was obtained.

2.2.3. Preparation of the nonporous silicone rubber membrane (nSRM)

Nonporous silicone rubber membrane was prepared by casting the LSR precursor in a PTFE mold directly, with a casting thickness of 1.5 mm. The PTFE mold was kept in an oven for 60 min at 30 °C, and then kept at 100 °C for 20 min to obtain the nSRM.

2.3. Detection of the physical properties of the prepared silicone rubber membranes

2.3.1. Observation of the porous and two-layer structure of the SRM-B

The porous structure of the upper layer and lower layer of the prepared SRM-B was observed under a scanning electron microscope (SEM, Inspect F, Philips, Netherlands). Briefly, the SRM-B was cut into small pieces (3 × 3 mm), coated with gold–palladium under a vacuum atmosphere and observed under a SEM. The average pore size ($n = 50$ pores) and the pores' surface density were measured with Image-Pro Plus 6.0 (IPP 6.0) software (Media Cybernetics, USA).

The two-layer structure of the SRM-B was observed using a super depth of field microscope (VHX-1000, KEYENCE, Japan).

2.3.2. Measurement of the mechanical properties of the membranes

The mechanical properties of the SRM-B and the nSRM were measured by tensile testing. Briefly, Samples were cut into a dumbbell shape and tested by the Instron 5567 materials testing system (Instron, USA). The samples were clamped, oriented vertically and stretched to failure. The velocity of stretching was 50 mm/min, and the results were recorded automatically. Three specimens were tested for each group ($n = 3$).

2.3.3. Determination of the WVTR of the prepared membranes

To determine the moisture permeability of the SRM-B and the nSRM, the WVTR was measured according to the American Society for Testing and Materials (ASTM) standard [16]. A sample was cut into a disc with a diameter of 35 mm and mounted on the mouth of a cylindrical cup with a diameter of 34 mm containing 10 ml of water. The sample was sealed with teflon tape across the edge and then placed into an incubator kept at 37 °C and 50% relative humidity. The assembly was weighed every 2 h for 24 h, and the results were recorded automatically by using the water vapor transmission rate tester (W3/030, Labthink, China). All measurements were repeated three times ($n = 3$).

2.4. Effects of the pore size on the bacterial permeation and the cell adhesion

2.4.1. Bacterial permeation test

The membranes were sterilized by irradiation with a ^{60}Co source and then washed with phosphate-buffered saline (PBS; pH 7.4) three times before experiments. *Escherichia (E.) coli* (K12MG1655) was cultured aerobically at 37 °C and shaken at 150 rpm overnight. Sterilized samples of the SRM-LP, the SRM-SP, the nSRM and the vaseline gauze were cut into circular discs (diameter 10 mm) and placed on the agar dish. Next, the bacterial suspension was diluted to 1×10^9 colony-forming units (CFU)/ml and 50 μl of suspension was instilled into the center of each sample, and the inoculum was uniformly plated on the surface of each sample. After incubation at 37 °C for 24 h, the agar under each sample was cut with a knife and placed into a tube containing 2 ml of PBS. The bacteria on the agar were detached using an ultrasonic cleaner, and the number of bacteria was determined by routine CFU analysis on an agar dish with different dilutions.

2.4.2. Cell adhesion test

GFP transgenic fibroblasts were isolated from GFP transgenic neonatal mice as previously described [17]. Briefly, the obtained skin tissue was washed with PBS three times and cut into approximately 0.5 cm^3 pieces. The epidermis and dermis were separated after digestion with 0.5 mg/ml Dispase II (Sigma, USA) at 4 °C overnight. The dermal tissue was minced and washed with PBS three times. The dermis pieces were then digested in 2 ml of 2.5 mg/ml trypsin (Boster, China). After incubation for 10 min at 37 °C, the digestion was discontinued by adding Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (Gibco, USA). After being filtered through a cell strainer (200 meshes), the suspension was centrifuged at 1000 rpm for 10 min, and the fibroblasts were collected. The

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