Materials Science and Engineering C 77 (2017) 739–747

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

The repairing of full-thickness skin deficiency and its biological mechanism using decellularized human amniotic membrane as the wound dressing

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ARTICLE INFO

Article history:
Received 18 October 2016
Accepted 25 March 2017
Available online 27 March 2017

Keywords:
Decellularized human amniotic membrane (dHAM)
Wound healing
Mechanism
Skin regeneration

ABSTRACT

Human amniotic membrane (HAM) was a biocompatible scaffold with advantages of anti-inflammatory, low antigen, feasibility, tolerance and low cost. In our previous work, HAM was treated to be decellularized using surfactant, lipase and DNAase methods and the efficacy as an implantable biological mesh was verified after decellularization treatment. In this work, we used the previous protocol to decellularize the fresh HAM, and applied it to repair full-thickness skin defects with Sprague-Dawley rats as the test animals. The wound healing progress was followed in the duration of 8 months, and the biological repairing mechanism was explored. From the wound area alteration, white blood cell (WBC) measurements and H&E staining, dHAM was detected to promote the wound healing, comparing with the traditional clinic treatment. Immunohistochemical analyses of the bio-factors involved in the wound healing, vascular endothelial growth factor (VEGF), alpha-smooth muscle actin (α-SMA) and transforming growth factor beta-1 (TGF-β1), exhibited that dHAM enhanced VEGF and α-SMA secretion but reduced TGF-β1 expression at early stage, which alleviated the wound inflammation, promoted the tissue regeneration and relieved the scar formation.

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1. Introduction

As the largest protective organ, skin plays an important role in defending against harms from external environment [1]. Skin is also the first safeguard of human body, which provides the functions of sensation and water equilibrium [2,3]. Any skin wounds such as burns, chemical damage and tearing apart will cause the body infections, ulcers and ultimately scars after wound repairing [4]. The necessary treatment is to find a wound dressing as early as possible, aiming at healing the defects and restoring the functions of the skin [5]. Currently the traditional methods include autologous skin graft, allogeneic skin transplantation, xenogeneic graft and artificial synthetic skin substitutes [6]. However, due to the donor deficiency, immunological rejection and the possibility of carrying disease, the application of those methods is still limited. Seeking an ideal skin substitute is an urgent task for skin regeneration in clinic [7].

Human amniotic membrane (HAM) is a translucent membrane with the thickness of 0.02–0.5 mm. It consists of three main components, i.e. epithelial layer, basement layer and connective tissue layer [8]. It is increasingly applied to wounds, burns and ophthalmology in recent years due to its advantages of anti-inflammatory, low antigen, feasibility, tolerance and low cost [9,10]. In our previous work, HAM has been decellularized using surfactant, lipase and DNAase methods, where surfactant and lipase were used to denature cell membrane and DNAase was used to enzymolyze DNA content. The decellularization efficiency and the biocompatibility as an implantable biological mesh after treatment were verified [11]. The decellularized HAM contains only basement layer with the components of collagen types I, III and IV, laminin (LN), fibronectin and a variety of growth factors [12,13]. The LN contains a variety of protein molecules, which composes extracellular matrix (ECM) as non-collagen glycoprotein. It combines with collagen IV to form the ECM skeleton [14,15]. Thus, the decellularized human amniotic membrane (dHAM) becomes more biocompatible and is thought to be more suitable as a biological substitute than HAM [11]. On the other hand, the dHAM still contains a variety of growth factors such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and transforming growth factor (TGF) alpha and beta etc. [16,17]. All these growth factors have powerful abilities in promoting cell or/and tissue regeneration.

As literatures reported, the progress of wound healing is complex, continuous and dynamic. It usually includes three phases (inflammation, proliferation and remodeling), and involves various cells, cytokines and growth factors which interact with each other to regenerate the whole layers of skin [18,19]. Some researches have been conducted

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http://dx.doi.org/10.1016/j.msec.2017.03.232
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with HAM as the wound dressing and the healing effect was evaluated [20,21]. In this work, we treated the fresh HAM with our previous protocol to get decellularized HAM. This dHAM was applied to cover the wound as the animal skin was defected. The results displayed that dHAM promoted skin repairing at early stage and advanced the skin regeneration in the whole stage. The molecular mechanism was also explored in the animal model, via tracing the wound evolution and detecting the alterations of some growth factors. We thought that the dHAM might influence one or two or all three phases to benefit the healing conditions.

2. Materials

Fresh HAM was obtained from Obstetrics Department of Ningbo First Hospital, China. Triton X-100 (Solarbio, China), penicillin and streptomycin (Beyotime Institute of Biotechnology, China), lipase (Solarbio, China) and DNAase (Sinopharm Chemical Reagent Co., China) were utilized in decellularizing process of HAM. Primary antibodies, anti-α-SMA (Abcam, China), anti-VEGF (Boster, China) and anti-TGF-β1 (Boster, China), goat anti-rabbit IgG (ZSBio PV-6001, China) and 3,3′-diaminobenzidine (DAB, ZSBio, China) were used in immunohistochemical staining.

Sprague-Dawley rats (2 months-old, male) were ordered from Experimental Animal Center of Zhejiang Province and kept in the experimental animal center of Medical School of Ningbo University.

3. Methods

3.1. Decellularization of HAM

Decellularization of HAM was performed with our previous protocol [11]. HAM was peeled from placenta by mechanical treatment after blood on the fresh placenta was washed away using phosphate buffered saline (PBS). The clean HAM was put into 75% alcohol/water for 10 s and rinsed with PBS containing penicillin-streptomycin (200 U/mL) immediately. It was then immersed in PBS (penicillin-streptomycin, 200 U/mL) with shaking for 2 d and changing the solution for each 12 h. Finally, treatments with chemicals in sequence included 1% Triton X-100 PBS for 4 h, lipase PBS (2000 U/L) for 10 h and DNAase PBS (2000 U/L) for 4 h at 37 °C.

The whole performance was conducted under the sterile state. The decellularized HAM (dHAM) was cut into pieces around 2.0 × 2.0 cm and stored at 4 °C until the surgery was operated.

3.2. Full-thickness skin defects on rats' back

25 rats were collected to be anesthetized with 3% pentobarbital sodium (intra-peritoneal injection, 30 mg/kg). After shaving off the fur and disinfecting the surface, two full-thickness defects at two sides of the rat's back were symmetrically made with the wound size of 1.3 cm × 1.3 cm (Fig. 1a). One piece of 2.0 cm × 2.0 cm dHAM was used to cover the left wound (Fig. 1b, arrow, named as dHAM group) entirely but not the opposite wound which was cleaned by saline (Fig. 1b, named as control group). Both wounds were sterilized with iodine, covered with gauze and dressed with medical tapes. All rats were fed normally and kept in cages individually. Each rat was given penicillin (160,000 U/mL) through intra-peritoneal injection once a day at the initial three days after operation. The gauze was removed next day and iodine was applied to disinfect wounds each day within the first week. After some days, skins at the wound site were cut off and stored at −80 °C for the following tests.

The animals used in this study were treated in accordance with the ethical committee of Ningbo University and NIH's Principles of Laboratory Animal Care.

3.3. Tracing the wound areas

12 rats were processed as above, aiming to trace the wound area alteration with the time past. The wound areas were recorded at the operation day (1 d) and the following each day postoperation. 12 repeats with each group at the each time point were performed and averaged. The healing ratios, wound area at different time/original defect area, were thus calculated. The curves to display the healing tendency were then schematically drawn and the statistical analyses were performed.

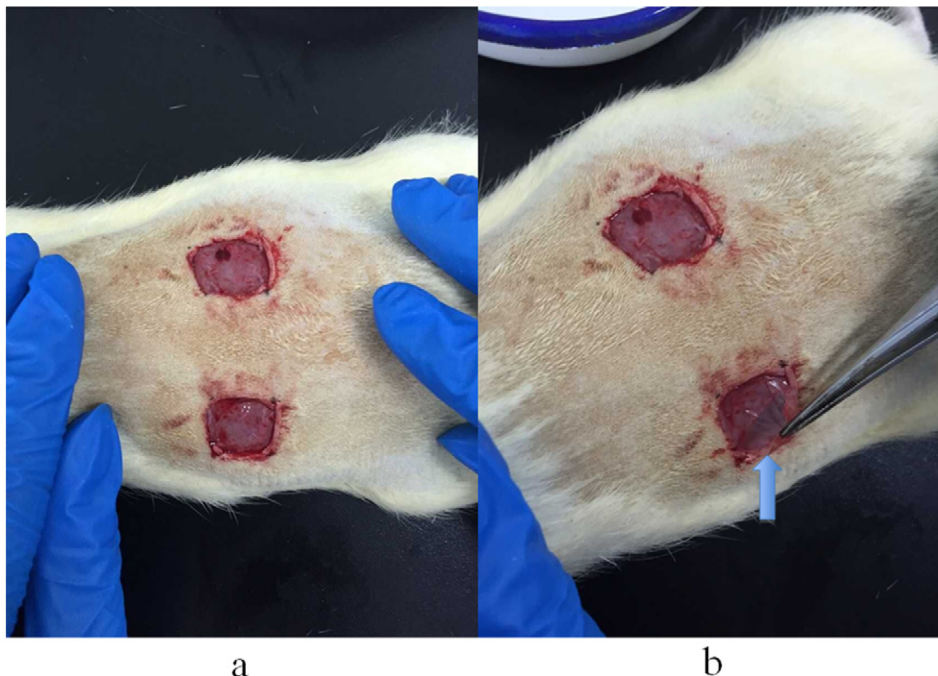

Fig. 1. Full-thickness skin defects on the back of SD rats. (a) Wound appearance immediately after surgery. (b) dHAM adhered onto the left wound (arrow); the right wound was set as the control.

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