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Preparation, characteristics and assessment of a novel gelatin–chitosan sponge scaffold as skin tissue engineering material

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ABSTRACT

In order to develop a skin tissue engineering material for wound dressing application, a novel gelatin–chitosan sponge scaffold was designed and studied. The effect of chitosan and gelatin ratio on the morphology, pore size, porosity, water uptake capacity, water retention capacity and the degradation behavior were evaluated. Biocompatibility was investigated by both MTT method and AO/EB staining method. Antibacterial assessment and in vivo pharmacodynamic was also studied to evaluate the potential for wound healing. Results showed the sponge scaffold have uniform porous structure with pore size range between 120 and 140 μm, high porosity (>90%), high water uptake capacity (>1500%), high water retention capacity (>400%), and degradation percent in 28 days between 38.3 and 53.9%. Biocompatibility results showed that the activity of cells could not be affected by the nature of the sponge and it was suitable for cell adhesion and proliferation for 21 days. In vivo evaluation indicated that the sponge scaffold could offer effective support and attachment to cells for skin wound healing. In conclusion, the developed sponge scaffold was a potential skin tissue engineering material with appropriate physical properties and good biocompatibility.

1. Introduction

In tissue engineering, a porous scaffold is required to function as a template and guide in cell adhesion, extension, proliferation, and differentiation. A target tissue would be in growth when the scaffold is biomimetic for the physiological need of the regenerating tissue. Therefore, controlling the pore size and structure of scaffold is crucial to host tissue formation (Moore and Lemischka, 2006; Yaniv et al., 2006; Tai et al., 2007). As we know, the skin tissue engineering materials are more suitable for large wound dressing applications. Particularly in cases of full thickness of skin or flash missing during serious accidental injury or burning, immediate coverage of wound surface with dressings is necessary before skin grafting (Denkbas et al., 2004). Generally, an ideal skin tissue engineering material should have high liquid absorbing capacity, proper gas permeation, biocompatibility and antibacterial properties to protect the skin defect from infections, dehydration and subsequently tissue damaging.

Chitosan (Chs) has been popular in tissue engineering application as a tissue culture scaffold and wound dressing (Florence and Christine, 2013). It has many properties such as biocompatible, biodegradable, nontoxic and antibacterial capabilities (Jayakumar et al., 2011; Muzzarelli, 2009). Chitosan based scaffolds and sponges have been investigated for wound care in different situations. Chitosan sponges can meet the requirements mentioned above and prevent the wound from infection and dehydration (Dutta et al., 2011; Olteanu and Enescu, 2007; Drury and Mooney, 2003; Seol et al., 2004). Recently, Anisha also prepared chitosan based sponges for wound care (Anisha et al., 2013). These sponges had a typical porous structure and exhibited enhanced liquid absorbing capacity and cell interaction. However, one major limitation for chitosan materials is its brittle behavior. Blends with other synthetic materials are believed to be an effective way to develop a suitable tissue engineering material.

Gelatin (Gel) is one of the well known biomaterials, a polypeptide commercially derived from the hydrolysis of collagen with triple helix structure. Gelatin and their derivatives are non-toxic, biocompatible and biodegradable. Therefore, it has potential in synthesizing biocomposites with various substances incorporated, such as organic molecules, drugs and nanoparticles. Especially, drugs, DNA and cells have also been incorporated in the gelatin/collagen matrix in attempts to develop bactericides, artificial skin substitutes, wound healing dressing, drug and cell carriers, and bio-responsible sensors (Tseng et al., 2007).

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In this work, gelatin was blended with chitosan to develop a novel Gel/Chs sponge scaffold as skin tissue engineering material. As shown in Fig. 1, the outer 3 M tape layer with suitable permeability was designed for prevention of bacterial invasion, controlling water vapor permeation and retaining a favorable moist environment at the wound interface. Meanwhile, the inner Gel/Chs sponges prepared by freeze drying was conceived not only as an absorption system with excellent water uptake capacity for wound exudates but also as a template and guide in cell adhesion, extension, proliferation and differentiation. Concretely, the effect of Gel and Chs ratio on the morphology, pore size, porosity, water uptake capacity, water retention capacity, the degradation behavior and biological compatibility of the Gel/Chs sponges were studied and presented. Compared with the previous studies which were focused on the physical properties, mechanical properties and degradability of sponge scaffolds, it should be pointed out that the biological compatibility of the Gel/Chs sponge scaffold was successfully proved by the AO/BE staining method in this paper (Huang et al., 2005; Deng et al., 2007). And then, ciprofloxacin hydrochloride (CIP) was selected as model drug and antibacterial property of sponge scaffold was preliminary studied in vitro to evaluate the potential for wound healing. Moreover, the preliminary safety and effectiveness of sponge scaffold were also demonstrated via in vivo pharmacodynamic evaluation.

2. Materials and methods

2.1. Materials

Gelatin (Gel) and Chitosan (Chs) were supplied by Rousselot France SAS and Hangzhou Golden-Shell Biochemical, respectively. Ciprofloxacin hydrochloride (CIP) was bought from Henan Topfond Pharmaceutical Co., Ltd. The human skin fibroblast (HSF) and the human keratinocyte (HaCaT) cell lines were bought from Biosis Biological Technology Co., Ltd. (Shanghai, China). Water used in experiment was distilled. All other chemicals and solvents were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of Gel/Chs sponge scaffold

Firstly, 0.3 g Gel and 0.3 g Chs were mixed and added in 27 mL HAc solutions (1%, v/v) at 40 °C. And then 3 mL glutaraldehyde solutions (0.25% w/v) were added in the HAc solutions and mixed homogenously for 2 h. The mixed solutions were placed into 24-well cell culture plate and then the samples were freeze dried using a tray freeze dryer under −20 °C. According to this procedure, these sponge scaffolds were expressed as Chs Gel/Chs 37, Gel/Chs 55, Gel/Chs 73 and Gel corresponding to No. 1–5 in Table 1, respectively.

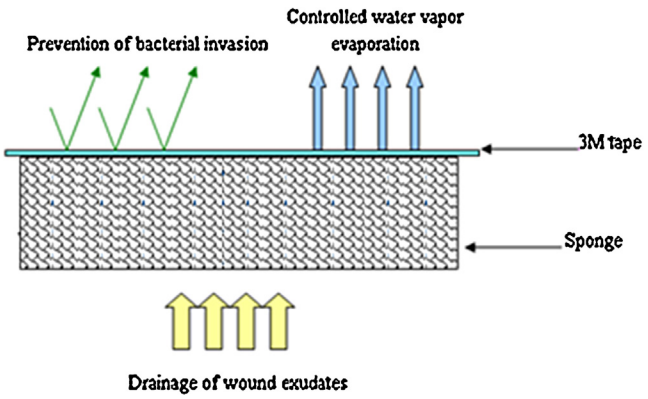

Fig. 1. Design of the Gel/Chs sponges scaffold.

Table 1
Formulations of sponges scaffold.

No.	Formulations	Ratio of Gel to Chs	Total amount of Gel and Chs (g)
1	Chs	–	0.6
2	Gel/Chs 37	3:7	0.6
3	Gel/Chs 55	5:5	0.6
4	Gel/Chs 73	7:3	0.6
5	Gel	–	0.6

2.2.2. Morphology study using scanning electron microscopy

The morphology study of the selected formulations was performed on a SEM (HITACHI S-3400N, Hitachi, Japan) at 5 kV for surface of the Gel/Chs sponge scaffold. Samples were examined after the specimens were coated with an ultra-thin layer of gold in a coating apparatus.

2.2.3. Pore size and porosity

The porosity of the sponge scaffold was measured by liquid displacement method (Nazarov et al., 2004; Zhang and Ma, 1999). Absolute ethyl alcohol was used as the displacement liquid as it permeates through sponge scaffold without swelling or shrinking the matrix. The sponge scaffold was immersed in a known volume (V_1) of absolute ethyl alcohol in a graduated cylinder for 5 min. The total volume of absolute ethyl alcohol and the absolute ethyl alcohol-impregnated scaffold was recorded as V_2. The absolute ethyl alcohol-impregnated scaffold was then removed from the cylinder and the residual volume of absolute ethyl alcohol was recorded as V_3. The total volume of the scaffold was obtained by Eq. (1):

$$V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3 \quad (1)$$

where $V_2 - V_1$ is the volume of the scaffold and $V_1 - V_3$ is the volume of absolute ethyl alcohol within the scaffold. The porosity of the scaffold (P) was obtained by Eq. (2):

$$P(\%) = \frac{V_1 - V_3}{V_2 - V_3} \times 100 \quad (2)$$

2.2.4. Water absorption capacity and water retention capacity

2.2.4.1. Water absorption capacity. Water absorption capacity test were conducted according to the method used by Lee and Chen, 2001. A known weight of dry sponge scaffold (W_0) was immersed in a beaker containing 50 mL of 0.1 M PBS at a pH of 7.4 at 32 °C and shaken for 24 h. And then, the samples were removed from the beaker and placed on a wire mesh rack. Excessive water was drained for 1 min and the sample was weighed (W_1). The weight of water absorbed at 24 h was found as a fraction of the dry weight as shown in Eq. (3). The values were expressed as mean ± standard deviation ($n = 6$):

$$W_s = \frac{W_1 - W_0}{W_0} \times 100 \quad (3)$$

where W_s is the water absorption capacity (%), W_1 is the weight of the wet scaffold at equilibrium (g), W_0 is the weight of the dry scaffold (g).

2.2.4.2. Water retention capacity. The water retention capacity was measured in a procedure as follows. A piece of a Gel/Chs sponge scaffold in dryness was accurately weighed and recorded as W_{dry}.