Acta Biomaterialia 10 (2014) 3167–3176

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Therapeutic efficacy of antibiotic-loaded gelatin microsphere/silk fibroin scaffolds in infected full-thickness burns

Yong Lan [a,b], Weichang Li [a,c], Yanpeng Jiao [a,b], Rui Guo [a,d,*], Yi Zhang [a,d], Wei Xue [a,d], Yuanming Zhang [a,c,*]

[a] Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, People's Republic of China
[b] Department of Materials Science and Engineering, Jinan University, Guangzhou 510632, People's Republic of China
[c] Department of Chemistry, Jinan University, Guangzhou 510632, People's Republic of China
[d] Department of Biomedical Engineering, Jinan University, Guangzhou 510632, People's Republic of China

ARTICLE INFO

Article history:
Received 25 November 2013
Received in revised form 24 March 2014
Accepted 26 March 2014
Available online 3 April 2014

Keywords:
Silk fibroin
Gentamycin sulfate
Gelatin microspheres
Full-thickness burn infection
Wound healing

ABSTRACT

Despite advances in burn treatment, burn infection remains a major cause of morbidity and mortality. In this study, an antibacterial silk fibroin (SF) scaffold for burn treatment was designed; gelatin microspheres (GMs) were impregnated with the antibiotic gentamycin sulfate (GS), and the GS-impregnated GMs were then embedded in a SF matrix to fabricate GS/GM/SF scaffolds. The developed GS/GM/SF scaffolds could serve as a dermal regeneration template in full-thickness burns. The average pore size and porosity of the GS/GM/SF scaffolds were 40–80 μm and 85%, respectively. Furthermore, the drug release rate of the scaffolds was significantly slower than that of either GS/GM or GS/SF scaffolds. And the composite scaffold exhibited stronger antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Hence, we evaluated the wound-healing effects and antibacterial properties of the GS/GM/SF scaffolds in a rat full-thickness burn infection model. Over 21 days, the GS/GM/SF scaffolds not only significantly reduced burn infection by *P. aeruginosa* but also accelerated the regeneration of the dermis and exhibited higher epithelialization rates than did GS/SF and SF scaffolds. Thus, GS/GM/SF scaffolds are potentially effective for treatment of full-thickness infected burns, and GS/GM/SF scaffolds are a promising therapeutic tool for severely burned patients.

1. Introduction

Burn infection has been a constant threat to human health throughout history, because infection prevents and delays wound healing and can lead to death [1,2]. It is reported that 50–75% of the morbidity in burn patients is related to infection [3]. The most common pathogens responsible for serious infections in burn patients include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) [4]. Complications associated with infected burns constitute serious problems in the care of severely burned patients [5]. Treatment of these infections is frequently accomplished by debridement to remove as much of the source of infection as possible [6]. However, full-thickness burns are particularly difficult to heal and pose a high risk of bacterial infection. Therefore, development of a dermal regeneration template to reduce the incidence of burn infection and promote full-thickness burn healing is desired.

Dermal regeneration templates for treatment of full-thickness burns should exhibit several characteristics, including excellent biocompatibility in terms of a lack of toxicity and immunogenicity, and a microstructure that promotes burn healing. Various materials have been used to fabricate dermal regeneration templates, such as collagen, chitosan, chondroitin sulfate and silk fibroin (SF) [7–10]. These scaffolds significantly improve skin recovery from full-thickness defects. In particular, SF, which is derived from the silkworm *Bombyx mori*, is a structural polymer possessing unique physical properties, including good biocompatibility, low immunity, non-toxicity and biodegradability [10,11]. Therefore, SF is currently being investigated for a number of biomedical applications [12]. For example, SF has been exploited as a biomaterial for in vitro cell culture and in vivo tissue engineering [13]. Furthermore, due to its peptide constituents, SF promotes the proliferation of human skin fibroblasts [14] and can be used as a wound dressing. Sugihara et al. examined the influence of silk film on full-thickness skin wounds; the silk film promoted greater skin regeneration than Alloask D, which is a commonly used dressing for burns and ulcers [15]. However, SF itself

* Corresponding authors at: Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, People's Republic of China. Tel./fax: +86 20 85222756.
E-mail addresses: guorui@jnu.edu.cn (R. Guo), tzhangym@jnu.edu.cn (Y. Zhang).

http://dx.doi.org/10.1016/j.actbio.2014.03.029
1742-7061/

does not exhibit antimicrobial properties, which are crucial for the prevention of bacterial infection of burn wounds.

To introduce antibacterial activity in a SF scaffold, we developed a composite SF scaffold using gelatin microspheres (GMs) loaded with vancomycin (Vm) as the antimicrobial agent [16]. The use of the GMs for Vm encapsulation improved the drug-release properties; GMs are widely accepted as efficient drug carriers for various administration routes, including nasal, gastrointestinal and rectal delivery [17]. The Vm-impregnated GM/SF (Vm/GM/SF) scaffold exhibited strong antimicrobial activity against *S. aureus* in vitro. However, Vm is active only against Gram-positive bacteria, such as *S. aureus* [18], which restricts the application of composite Vm/GM/SF scaffolds in burn treatment. Furthermore, the therapeutic efficacy of antibiotic-loaded GM/SF scaffolds in infected full-thickness burns has not been evaluated. Because of its broad activity against both Gram-negative and -positive bacteria, gentamycin sulfate (GS) has been used as a topical antibiotic in the treatment of superficial skin infections and sepsis [19].

In this study, we explored the applications of GS-impregnated GM/SF (GS/GM/SF) scaffolds for treatment of infected full-thickness burns. The morphology and properties of GMs, SF and GS/GM/SF scaffolds were investigated, and the release behaviour of GS from GS/GMs, GS/SF scaffolds and GS/GM/SF scaffolds were evaluated. The antimicrobial activities of the SF scaffolds, GS/SF scaffolds and GS/GM/SF scaffolds were investigated using the Kirby–Bauer (KB) test. Next, we prepared a wound-infection model based on full-thickness burns in male Sprague–Dawley (SD) rats, in which the dorsal skin was artificially burned and infected with *P. aeruginosa*. Thereafter, the burns were treated with the different SF scaffolds. The dynamic wound healing of the full-thickness skin defects was then investigated.

2. Materials and methods

2.1. Materials

Bovine gelatin (isoelectric point of 5.0) was purchased from Acros Organics (Geel, Belgium). *B. mori* silkworm cocoons used in the experiment were kindly donated by Sijia Min from Zhejiang University. GS and pentobarbital sodium were obtained from Sigma–Aldrich (St. Louis, MO, USA). *E. coli* (ATCC8099), *S. aureus* (ATCC6538) and *P. aeruginosa* (ATCC9027) were obtained from the Department of Biomedical Engineering of Jinan University and were maintained on solid agar medium at 4 °C. All other reagents were of analytical grade and used without further processing.

2.2. Preparation of the GS-impregnated GMs (GS/GMs)

An emulsion solvent evaporation method was used to prepare the GMs [20]. GS was incorporated into the GMs according to a method reported previously [16,21], with slight modification. Briefly, 4 μl of GS solution (25 mg ml^{-1}) was added dropwise to 1 mg of freeze-dried GMs that were cross-linked with glutaraldehyde, and then the GMs were maintained at 4 °C overnight. Because the volume of GS solution that was added to the GMs was considerably less than the theoretical swelling saturation volume of the GMs, the GMs absorbed all of the GS solution [22]. Therefore, the incorporation efficiency of GS into GMs was 100%. The composite microspheres were then freeze-dried for 24 h to remove any remaining solvent.

2.3. Preparation of the composite SF scaffolds

SF solution was prepared according to a method established previously [23]. The final concentration of the SF solution was ~4% (w/v), which was determined gravimetrically after freeze-drying for 24 h to remove any remaining solvent. The GS/GM/SF scaffold was produced by mixing 1 ml of 4% (w/v) SF solution and 10 mg of GS/GMs, and then pouring the solution into a 24-well plate and freeze-drying for 24 h. The GS/SF scaffold was fabricated by mixing 1 ml of 4% (w/v) SF solution and 4 μl of GS solution (25 mg ml^{-1}) and then pouring the mixture into a 24-well plate and freeze-drying for 24 h. Pure SF scaffolds were also prepared. All SF-based scaffolds were immersed in 90% (v/v) methanol aqueous solution for 30 min to induce a structural transition that generated water-insoluble SF scaffolds [17,24]. This methanol treatment has little effect on GS, due to GS being insoluble in most organic liquids, including acetone and methanol [25].

2.4. Swelling of the GMs

Dried and wet (saturated with deionized (DI) water for 4 h at room temperature) microspheres were observed under a microscope (Axio Scope A1 FL; Carl Zeiss, Wetzlar, Germany). At least 100 of both dried and wet microspheres were viewed. Their diameter was measured, from which the volume of respective microspheres was calculated [21]. The swelling ratios were then calculated using the following formula:

$$\text{Swelling ratio} = \frac{\text{Volume of wet microsphere}}{\text{Volume of dried microsphere}}$$

2.5. Fourier transform infrared spectroscopy

The infrared (IR) spectra of GMs, GS and GS/GMs were obtained using a Fourier transform infrared (FTIR) spectrometer (Vertex 70; Bruker, Billerica, MA). The IR spectra in the absorbance mode were recorded using a diamond crystal plate and obtained in the spectral region 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and 20 scans per sample.

2.6. Scanning electron microscopy

The morphologies of GMs, SF scaffolds and GS/GM/SF scaffolds were characterized by scanning electron microscopy (SEM; LEO1530 VP, Philips, Amsterdam, the Netherlands). The pore sizes of the scaffolds were evaluated by measurement of 25 random pores in SEM images of the same sample using ImageJ software (NIH, Bethesda, MD, USA). The porosity of the SF scaffolds was measured according to a method published previously [26].

2.7. In vitro release of GS

The release profiles of the GS/GMs, GS/SF scaffolds and GS/GM/SF scaffolds were determined by immersion in 10 ml of phosphate-buffered saline (PBS) and incubation at 37 °C [27]. At pre-set time intervals, 1 ml of supernatant was collected and replaced with an equal volume of fresh PBS. The amount of GS in the supernatant was determined spectrophotometrically at 248 nm using an ultraviolet/visible (UV/Vis) spectrophotometer (UV-2550; Shimadzu, Otsu, Japan) and calculated using a standard curve, which was obtained in previous experiments. Quintuplicate experiments were carried out. The percentage of drug released was calculated using the following formula:

$$\text{Release } (\%) = \frac{\text{Released Gentamycin sulfate}}{\text{Total Gentamycin sulfate}}$$

2.8. Antibacterial activity

The antimicrobial activity of the free GS and GS-containing SF scaffolds were investigated using the Kirby–Bauer (KB) test against