International Journal of Pharmaceutics 473 (2014) 345–355

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Fabrication and structure analysis of poly(lactide-co-glycolic acid)/silk fibroin hybrid scaffold for wound dressing applications

Sheida Shahverdi [a], Mirhamed Hajimiri [a,b], Mohammad Amin Esfandiari [a], Bagher Larijani [c], Fatemeh Atyabi [a,d], Afsaneh Rajabiani [e], Ahmad Reza Dehpour [f], Ali Akbar Gharehaghaji [g], Rassoul Dinarvand [a,d,*]

[a] Nanomedicine and Biomaterial Lab, Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[b] Nano Alvand Co., Avicenna Tech Park, Tehran University of Medical Sciences, Tehran, Iran
[c] Endocrinology and Metabolism Research Center (EMRC), Tehran University Medical Sciences, Tehran, Iran
[d] Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[e] Department of Pathology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[g] Department of Textile Engineering, Amirkabir University of Technology, Tehran, Iran

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 16 July 2014
Accepted 16 July 2014
Available online 19 July 2014

Keywords:
Electrospun scaffold
Silk fibroin
PLGA
Wound dressing
Diabetic wounds

ABSTRACT

Silk fibroin (SF) and poly(lactide-co-glycolic acid) (PLGA) have been proved to be invaluable polymers in the field wound healing. This study aims at optimizing the electrospinning process of those polymers to make a hybrid membrane as a chronic wounds dressing. After characterizing the scaffolds, PLGA/SF (2:1), and PLGA scaffolds were selected for further study according to their superior tensile mechanical properties. The attachment and proliferation of mouse fibroblasts (L929) on scaffolds were measured using colorimetric assay and scanning electron microscopy. Furthermore, to evaluate the wound healing effect of the scaffolds in comparison with gauze and Comfeel® dressings, an excision wound model was conducted on diabetic rats. On the postoperative days of 3, 6, 9, 12, and 15, residual wound area was calculated using macroscopic data. *In vitro* results showed that the attachment and proliferation of L929 were significantly increased on PLGA/SF (2:1) hybrid scaffold. Animal study and histopathological evaluation outcomes confirmed the *in vitro* results as well. On day 15, the residual wound area in PLGA/SF (2:1) hybrid membrane group was significantly smaller than PLGA and control groups. This promising scaffold has the potential to be used for the upcoming development of wound dressings with or without biological drugs.

1. Introduction

Chronic wounds, including diabetic foot ulcers, pressure sores, and other types of wounds, curing by secondary purpose are common in both acute and community settings. Diabetic foot problems which are common worldwide cause major social, medical and economic outcomes for the patients, their families, and society. Foot ulceration is one of the major complications in diabetes mellitus with a 15% life time risk in all diabetic patients (Rathur and Boulton, 2007; Ranjbar, 2008; OMeara et al., 1999). So, wound healing has been a challenging subject for medical teams and researchers. Natural or synthetic bands, cotton, and linen gauze and sterilized bandage have been used as a conventional method of dressing for many years. However, nowadays various novel types of wound healing products are available all over the world to prevent and treat chronic wounds (O'Meara et al., 1999).

Silk fibroin (SF), the protein of silk worm *Bombyx mori* filaments, is considered to be helpful material that facilitates collagen synthesis and re-epithelialization in the treatment of skin injuries (Roh et al., 2006; Chen et al., 2006). It has been also

Abbreviations: FDA, food and drug administration; DM, diabetes mellitus; STZ, streptozotocin; DMF, dimethyl formamide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; PBS, phosphate buffer saline; DMEM, dulbecco's modified eagle's medium; PLGA, poly lactic-co-glycolic acid; SF, silk fibroin; SEM, scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; ECM, extracellular matrix; NWC, negative wound control; PWC, positive wound control; HWC, hybrid wound control; PLWC, PLGA wound control.

* Corresponding author at: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran P.O. Box 1417614411, Iran. Tel.: +98 2166959055; fax: +98 2166959052.
E-mail address: dinarvand@tums.ac.ir (R. Dinarvand).

http://dx.doi.org/10.1016/j.ijpharm.2014.07.021
0378-5173/

reported that SF has a promising function as wound dressing due to its good dissolved oxygen permeability characteristic in wet situation, comparable to human healthy skin. In addition, β-sheet structure is necessary to make SF insoluble for the biomedical uses. For this purpose, the role of formic acid is significant as a solvent in induction of this conformation (Um et al., 2003).

The potential advantages of naturally derived polymers facilitate specific cell interactions agent to their hydrophilic nature but they suffer from poor tensile mechanical properties. In contrast, the hydrophobic properties of scaffolds which are derived from synthetic polymers may block cell seeding. These scaffolds have not enough performance for cell-recognition signals, too (Park et al., 2012).

Poly(lactide-co-glycolic acid) (PLGA) is a known polymer for various applications in biomedical and pharmaceutical fields because of its good biocompatibility and biodegradability. The use of biocompatible and biodegradable materials reduces the risk of undesirable toxicities and adverse effects (Kumbar et al., 2008; Makadia and Siegel, 2011; Almería et al., 2010; Hong et al., 2012; Lü et al., 2009). It has also received FDA approval for therapeutic devices and is simply formed to most wanted shapes with good mechanical strength (Katti et al., 2004).

There are different methods for fabrication of micro- or nanofibrous scaffolds. Among them electrospinning, patented Formhals by Anton in 1934 (Formhals, 1934), is a variation of the electrospray process, which happens when the surface tension force of a polymer solution is overcome by an applied electrical force, and tiny droplets or fibers are extruded from every soluble or fusible polymer (Luu et al., 2003; Zhao et al., 2008). It is also an interesting and simple technique for producing a fibrous scaffolds with high porosity and surface area to volume ratio. This structure can simulate the topographic facial appearance of the extracellular matrix (ECM). Electrospinning of the mixture of synthetic and natural polymer blends in the same syringe-pumps has been studied in order to overcome the disadvantages of synthetic and natural polymers, which have resulted in new types of scaffolds with improved tensile mechanical properties and enhanced biocompatibility (Duan et al., 2006). Ding et al. designed a multi jet electrospinning device together with a rotatable tubular collector to obtain a uniform thickness of blended nanofibrous scaffolds with good dispersions of the two components in the membranes. This device can be used to fabricate poly blend nanofibrous scaffolds. However, the electrospun parameters such as flow rate, tip-to-collector distance, and voltage have to be the same for different polymer solutions forming the nanofibrous scaffolds (Ding et al., 2004). Also, Min et al. built a setup for electrospinning of two polymer solutions delivered by two syringe-pumps at different flow rates (Min et al., 2004).

In the present study, a dual source electrospinning setup was designed to fabricate multicomponent nanofibrous membranes of controlled compositions. It was demonstrated that by using this experimental setup, PLGA and SF nanofibers could be electrospun separately by selecting optimal electrospinning parameters for the two polymers independently. The fabricated nanofibrous PLGA/SF scaffolds were then subjected to physical, mechanical, cytocompatibility and *in vivo* evaluations to examine their potential applications in wound healing.

2. Materials and methods

Silk worm cocoons (*B. mori*) were kindly supplied from Abrisham Guilan Co. (Rasht, Iran). *N*,*N*-Dimethylformamide (DMF) was purchased from Duksan (Korea) and formic acid was obtained from Janssen Pharmaceuticals (NJ, USA). PLGA (75:25, RESOMER® RG 756 S) with an inherent viscosity of 0.7–1.0 dl/g was supplied by Evonik Industries (Germany). Streptozotocin (STZ) was purchased from Enzo Life Science (UK). Dulbecco's modified eagle's medium (DMEM) and trypsin for cell culture were from Sigma–Aldrich (MO, USA). Fetal bovine serum (FBS) was purchased from Life Technologies (NY, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma–Aldrich (MO, USA). Dodecyl sulfate, sodium carbonate, calcium chloride, chloroform and other reagents were purchased from Merck (MO, USA).

2.1. Scaffolds fabrication

2.1.1. Preparation of SF fibroin and PLGA solutions

At first stage, silk worm cocoons were chopped into small pieces, cleaned from larvae and then the sericin was removed *via* degumming process as previously reported (Altman et al., 2009). Briefly, sodium dodecyl sulfate (0.25% w/v) and sodium carbonate (0.25% w/v) were dissolved and heated to 100 °C. Pieces of cocoons were added at 1:100 (w/v) and boiled for 1 h. Degummed SF was rinsed in warm running distilled water, air-dried and then dissolved in a ternary solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (1:2:8 molar ratio) at 70 °C at a 10% w/v concentration for 2 h. This solution was then passed through a 5 μ pore-size syringe filter (Minisart®, Sartorius stedim biotech GmbH, Germany) and dialyzed (SPECTRA/POR®3, MWCO 3500, USA) against excess of deionized water for a period of 72 h. The regenerated SF fibroin sponge was obtained by lyophilization.

PLGA was dissolved in a 4:1 chloroform:DMF mixed solvent at a 15–20% w/v concentration. The mixture was consequently stirred for at least 72 h at room temperature in order to complete the dissolution.

2.1.2. Optimizing of SF and PLGA solution concentrations for electrospinning

Two hours before spinning of SF, formic acid (99%) was added to each SF vial in concentrations of 10%, 13%, 15%, 17%, and 20% with constant stirring. The solution was transferred to a 1 ml syringe which was fitted with a 25 G needle and placed in the electrospinning set up. A programmable syringe pump (Medifusion, Ms-2200, South Africa) was used for the controlling of the polymer solution flow from the syringe into the needle. Scaffolds were electrospun at 25 kV voltage with a stable solution flow rate of 0.2 ml/h under ambient conditions (25 ± 2 °C and 16% relative humidity). The collector drum (10 cm in diameter), covered with aluminum foil, was placed in a tip to collector distance of nearly 7 cm. The PLGA solutions (15, 17, and 20%) were transferred to separate 1 ml syringes which were fitted with a 22 G needle and were placed in the electrospinning apparatus. The solution flow rate was 1 ml/h under the same condition as SF and the tip to collector distance was 15 cm. The electrospinning voltage was fixed on 25 kV.

2.1.3. Dual source electrospinning

A dual source electrospinning setup comprised of a high voltage power supply, two programmable one-single syringe pumps which were located opposite each other. The grounded collector drum was placed in the middle of the two sets of syringe pumps and rotated directly at 10 rpm which is schematically shown in Fig. 1. After selecting optimized solution concentrations for SF and PLGA electrospinning separately, the hybrid scaffolds were prepared.

2.2. Physical and mechanical characterization

Electrospun fibers were gold-coated and examined by scanning electron microscopy (SEM) (XL-30, Philips, Netherland) to