Colloids and Surfaces B: Biointerfaces 143 (2016) 371–381

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Whey protein concentrate doped electrospun poly(epsilon-caprolactone) fibers for antibiotic release improvement

Said Mahmoud Ahmed[a], Hanaa Ahmed[a], Chang Tian[a], Qin Tu[b], Yadan Guo[b], Jinyi Wang[a,b,*]

[a] College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] College of Science, Northwest A&F University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form 18 March 2016
Accepted 20 March 2016
Available online 21 March 2016

Keywords:
Poly(epsilon-caprolactone)
Whey protein concentrate
Electrospinning
Nanofiber
Antibacterial activity

ABSTRACT

Design and fabrication of scaffolds using appropriate biomaterials are a key step for the creation of functionally engineered tissues and their clinical applications. Poly(epsilon-caprolactone) (PCL), a biodegradable and biocompatible material with negligible cytotoxicity, is widely used to fabricate nanofiber scaffolds by electrospinning for the applications of pharmaceutical products and wound dressings. However, the use of PCL as such in tissue engineering is limited due to its poor bioregulatory activity, high hydrophobicity, lack of functional groups and neutral charge. With the attempt to found nanofiber scaffolds with antibacterial activity for skin tissue engineering, in this study, whey protein concentrate (WPC) was used to modify the PCL nanofibers by doping it in the PCL electrospun solution. By adding proteins into PCL nanofibers, the degradability of the fibers may be increased, and this further allows an antibiotic incorporated in the fibers to be efficiently released. The morphology, wettability and degradation of the as-prepared PCL/WPC nanofibers were carefully characterized. The results showed that the PCL/WPC nanofibers possessed good morphology and wettability, as well as high degradation ability to compare with the pristine PCL fibers. Afterwords, tetracycline hydrochloride as a model antibiotic drug was doped in the PCL/WPC nanofibers. *In vitro* drug release assays demonstrated that PCL/WPC nanofibers had higher antibiotic release capability than the PCL nanofibers. Also, antibacterial activity evaluation against various bacteria showed that the drug-doped PCL/WPC fibers possessed more efficient antibacterial activity than the PCL nanofibers.

1. Introduction

In the last few decades considerable efforts have been devoted to the development of hybrid scaffolds that could recapitulate the extracellular matrix for tissue engineering [1]. Design and fabrication of scaffolds using appropriate biomaterials are a key step for the creation of functionally engineered tissues and their clinical applications [2]. Tissue engineering entails the basic approach of fabricating cell-contained polymeric scaffolds of biodegradable polymers to produce a three-dimensional functional tissue which is suitable for implantation, as well as a solution of critical medical problems such as tissue loss, organ failure and extensive loss/damage of skin [3,4]. Recently, fibrous scaffolds such as the system fabricated by electrospinning have been used in several biomedical applications [5]. Electrospinning is an efficient method for the fabrication of biomimetic scaffolds with micro to nanoscale topography to mimick the natural extracellular matrix for favorable cell attachment and proliferation [6]. The biomimetic scaffolds prepared by electrospinning could be also used as carriers for both hydrophilic and hydrophobic drugs, where the drug release profile can be finely controlled by the modulation of the scaffold's morphology, porosity and composition [7]. The main advantage of the system fabricated by electrospinning is that it offers site-specific delivery of any number of drugs from the scaffolds into the body and successfully incorporated and released from nanofibrous scaffolds without the loss of structural integrity or change in functionality. Compared with the conventional dosage forms such as improving therapeutic efficacy and reducing toxicity by delivering drug at a controlled rate [8], the nanofiber scaffolds, in particular, are considered a promising system for drug delivery due to their high specific surface area that could lead to efficient drug release [9]. To date, electrospinning has evinced more interest because of its versatility and potential for applications in various fields, as well as its simple

* Corresponding author at: Colleges of Veterinary Medicine and Science, Northwest A&F University, Yangling, Shaanxi 712100, China.
E-mail address: jywang@nwsuaf.edu.cn (J. Wang).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.059
0927-7765/

process operation, high performance in nanofiber fabrication and low in cost. Also, electrospun nanofibers could reproduce almost the same structure as the natural extracellular matrix [10].

In the skin tissue engineering, critical determinants in favorable wound healing outcomes are largely based on the physicochemical nature of scaffolds, cell types and cell-material interactions [11,12]. Recently, biodegradable natural and synthetic polymers with favorable mechanical properties and degradation ability have been extensively explored for therapeutic applications [13]. Poly(epsilon-caprolactone) (PCL), a biodegradable and biocompatible material with negligible cytotoxicity, is widely used to fabricate nanofibers by electrospinning for the applications of pharmaceutical products and wound dressings [14]. However the use of PCL as such in tissue engineering is limited due to its poor bioregulatory activity, high hydrophobicity, lack of functional groups and neutral charge. Therefore, co-polymerization or blending with other polymers allows the modification of its physical, chemical and mechanical properties [15]. It has been observed that co-polymerization could alter the chemical property that indirectly affects the other properties such as crystallinity, solubility and degradation pattern, resulting in a modified polymer with intended properties for drug delivery [16]. Generally, blending with other materials is an important method in nanofibrous scaffold manufacturing and has received increasing attention because of the strong economic incentives arising from the use of polymer blends [17]. These blends can combine the advantages of both components and have better properties than either component [18].

Over the past several decades, proteins have been growing as a class of therapeutic molecules [19]. Whey proteins, one of the two milk protein groups along with the caseins, have been found to be a valuable dietary supplement and a functional food enhancer. Furthermore, whey proteins have been evaluated and recognized for their antimicrobial, antiviral and anticarcinogenic effects [20]. In the study of the skin tissue engineering, nanofibrous scaffolds with antimicrobial ability would be the most promising materials, since the injured part of skin is an ideal environment for microbial growth [21–23]. In addition, by adding proteins into PCL nanofibers, the degradability of the fibers may be increased, and this further allows an antibiotic incorporated in the fibers to be efficiently released. With the attempt to found nanofiber scaffolds with antibacterial activity for skin tissue engineering, therefore, in the current study we used whey protein concentrate (WPC) to modify PCL nanofibers by doping it in the PCL electrospun solution. The morphology, wettability and degradation of the as-prepared PCL/WPC nanofibers were carefully characterized. Afterwards, tetracycline hydrochloride as a model antibiotic drug was doped in the PCL/WPC nanofibers. Meanwhile, the *in vitro* release of tetracycline from the electrospun fibers was investigated. Also, the antibacterial activity of the drug-doped nanofibers was evaluated against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* CMCC 54004, as well as the Gram-negative bacteria *Escherichia coli* ATCC 25922, *E. coli* HB101 and *Salmonella typhimurium* SL1344.

2. Experimental

2.1. Materials

Whey protein concentrate (WPC) was purchased from Davisco Foods International Inc. (Eden Prairie, MN, USA). The WPC contained 81.4% protein, 5.4% fat, 2.6% ash and 8.0% lactose (data was supplied by the manufacturer). Poly(epsilon-caprolactone) (PCL, M_n = 70–90 kDa) was obtained from Sigma Aldrich (MO, USA) and used as received. Tetrahydrofuran (THF), dichloromethane (DCM) and N, N-dimethylformamide (DMF) were obtained from Shanghai Fine-Chemicals Co., Ltd. (Shanghai, China) and were used as solvents. The antibiotic drug tetracycline hydrochloride used in this study was supplied by a local commercial supplier. Yeast extract and tryptone were obtained from Oxoid Ltd. (Basingstoke, Hampshire, England). *E. coli* ATCC 25922 (*E. coli* ATCC 25922), *E. coli* HB101 (*E. coli* HB101) and *S. aureus* ATCC 25923 (*S. aureus* ATCC 25923) were supplied by Prof. Peng Chen at College of Life Science, Northwest A&F University (Yangling, China). *S. typhimurium* SL1344 (*S. typhimurium* SL1344) and *L. monocytogenes* CMCC 54004 (*L. monocytogenes* CMCC 54004 were supplied by Prof. Jinyou Duan at College of Science, Northwest A&F University. All of the solutions were prepared using ultrapurified water (18.4 MΩ cm) supplied by a Milli-Q water system (Millipore, Billerica, MA).

2.2. Preparation of PCL solutions for electrospinning

To investigate the effects of the solvent types and polymer concentrations on the formation and morphology of nanofibers, polymer solutions in two solvent systems with different PCL concentrations (4%, 6% and 9%, w/v) were prepared. Briefly, polymer solutions were prepared by dissolving PCL powder in each solvent and stirring overnight using a magnetic stirrer at room temperature. The first polymer solution was prepared by dissolving PCL powder into a mixed solvent of THF/DMF (7:3, v/v). The other polymer solution was prepared by dissolving PCL powder into a mixed solvent of DCM/DMF (8:2, v/v).

2.3. Doping of WPC in PCL electrospinning solutions

In this study, the protein WPC was doped in the nanofibers by using the blend electrospinning technique [24]. Selected solutions of PCL were first prepared and then, 5 wt.% and 10 wt.% WPC were respectively added to these solutions and stirring overnight at room temperature to obtain homogeneous electrospinning solutions.

2.4. Fabrication of PCL/WPC nanofibers by electrospinning

An electrospinning workstation (Ucalery, Beijing, China SS-25344, UC120815) was used to fabricate PCL/WPC nanofibers (Fig. 1). Briefly, the spinning solution was first loaded in a 10-mL NORM-JECT Luer Lok plastic syringe having a 21-gauge stainless-steel needle. An aluminum foil covered on a laboratory produced roller with the diameter of 15 cm was used as a collector to collect the nanofibers. During electrospinning, a positive high voltage of 14 kV was applied to the needle, and the solution feed rate was set at 0.1 mm/min. The distance of the needle tip to the collector was settled at 15 cm. The electrospun PCL/WPC nanofibers were peeled off from the aluminum foil, kept in vacuum at room temperature for 1 h and then kept in a dry place at room temperature for several days to remove the residual solvent.

2.5. Characterization of the electrospun nanofibers

2.5.1. Morphology

The morphology of the prepared nanofibers was observed by a field emission scanning electron microscopy (FE-SEM S-4800, Hitachi Ltd., Japan) at the accelerating voltage of 10 Kv. The specimens for SEM observation were prepared by cutting an aluminum sheet covered with the as-spun products and the cut section was carefully affixed on a copper stub. Each specimen was gold-coated by a Balzers Union SCD 040 sputtering device. The obtained images were further analyzed to examine fiber diameters by Image-Pro Plus 6.0 (Media Cybernetics, Inc., USA). At least 30 fibers were measured for their fiber diameters and the average and standard deviations were plotted.