International Journal of Biological Macromolecules 76 (2015) 94–101

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Characterization of polycaprolactone/collagen fibrous scaffolds by electrospinning and their bioactivity

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 27 January 2015
Accepted 28 January 2015
Available online 20 February 2015

Keywords:
Collagen
Nile tilapia skin
Tissue engineering

ABSTRACT

Fibrous scaffolds for tissue engineering were fabricated using collagen extracted from Nile tilapia skin and polycaprolactone (PCL) by electrospinning. The scaffolds were characterized by scanning electron microscopy (SEM), ATR-Fourier transform infrared, X-ray diffraction, and differential scanning calorimetry. Diameters of PCL/collagen fibrous scaffolds (PCFSs) decreased from 987 ± 274 to 689 ± 299 nm with an increase in collagen content, crystallinity was low, and crystal size was small. All of the characteristic bands of PCL and collagen could be observed in PCFSs. Furthermore, PCFSs had a higher dehydration temperature (50–60 °C) than native collagen (32.5 °C). The ultimate tensile strength of PCFSs increased with an increase in collagen content. Circular dichroism and a degradation assay in vitro indicated that PCFSs had good stability and a low degradation rate. Cellular behavior on PCFSs was investigated by a MTT assay, SEM, and laser scanning confocal microscopy. The results indicated that the PCFSs could provide a suitable environment for the growth and viability of L929 fibroblasts, maintain good cell adhesion, and retain good biocompatibility. It implied the possibility of using PCFSs as a promising candidate for tissue engineering.

1. Introduction

Tissue loss or organ failure due to severe disease or trauma is a major healthcare problem, as the transplantation of the tissue or organ is not economical and limited by the accessibility of a compatible donor [1]. Tissue engineering, an application of biological science and engineering to regenerate biological substitutes for repairing/replacing a damaged organ or tissue, involves three important components: cells, scaffold (3D polymeric matrix), and growth factors [2]. Among these three components, the scaffold is important it interacts with the cells and growth factors to regenerate a specific tissue [3]. For successful application, the scaffold needs particular characteristics, e.g., it should be biocompatible and biodegradable, have mechanical properties that are similar to the tissue it is replacing, and support cell attachment and growth [4]. Above all, it should mimic the morphological structure and chemical composition of the extra cellular matrix (ECM), so that cells can adhere to the scaffold surface, proliferate, and differentiate into new tissue [2].

However, the major barrier in tissue engineering is to create an excellent scaffold. Until now, various polymers have been employed for scaffold fabrication, such as poly-L-lactide (PLLA) [5], poly(trimethylene carbonate) (PTMC) [6], polyethylenimine (PEI) [7], and polycaprolactone (PCL) [7–9]. PCL is commonly used to engineer tissue scaffold because of its biocompatibility, biodegradability, structural stability, and mechanical properties [10]. Though PCL has many advantages, it shows poor cell adhesion [11,12], low bioactivity, and low surface energy (high hydrophobicity) [10]. A scaffold, if prepared from a single polymer, cannot impart all of the desired properties, but by taking two or more polymers in combination, it is possible to tailor a scaffold with the proper characteristics [13].

Collagen, the principal structural protein of the extracellular matrix (ECM), plays a dominant role in maintaining biological and structural integrity of ECM and undergoes constant remodeling for physiological functions [14]. Collagen has been widely used as a biomaterial in the pharmaceutical and medical fields for drug delivery [15], dressings for wound healing [16], filters [17], nanocomposites [18], and for tissue scaffolds [19], because of its biological origin, non-immunogenicity, excellent biocompatibility, and biodegradability.

Commonly, the principal sources of collagen are bovine skins and bones. However, there has been a growing concern about

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http://dx.doi.org/10.1016/j.ijbiomac.2015.01.063
0141-8130/

transmissible diseases, especially bovine spongiform encephalopathy (BSE) [20]. As a consequence, alternative collagen sources are sought from fish skin or bone, which are byproducts from the processing of aquatic products.

There is increasing interest toward employing electrospinning for scaffold fabrication because the mechanical, biological, and kinetic properties of the scaffold are easily manipulated by altering the polymer solution composition and processing parameters [21]. Another advantage of using the electrospinning process is the ability to produce a non-woven nanofibrous structure, which has morphological and architectural features similar to that of the natural ECM in tissue [22]. Additionally, the scaffold structure changes dynamically over time as the polymer nanofibers degrade, allowing the seeded cells to proliferate and produce their own ECM [23]. These outstanding properties make polymer nanofibers an optimal candidate for tissue engineering applications.

Fabricating and characterizing PCL/collagen composite scaffolds by electrospinning have shown good mechanical properties as well as good cell adhesion and proliferation [24–27]. However, collagen derived from fish skin has rarely been used in fabricating scaffolds. In this study, PCL/collagen fibrous scaffolds (PCFSs) with different contents of Nile tilapia skin collagen for tissue engineering applications were fabricated using an electrospinning process. The characterization and the biological activities of these scaffolds to L929 mouse fibroblasts were investigated.

2. Experimental

2.1. Materials

Nile tilapia (*Oreochromis niloticus*) skin was obtained from Mingji Aquatic Product Company (Yunfu, Guangdong Province, China). L929 mouse fibroblast cell line was purchased from Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (Shanghai, China). Eagle's alpha minimum essential medium (α-MEM), fetal bovine serum (FBS), 3-(4,5-dimethyl-2-thiazoly (2.5-diphenyl-2H-terazolium bromide)) (MTT) and penicillin–streptomycin solution were obtained from Sigma Aldrich (Milwaukee, WI, USA). Phosphate-buffered saline (PBS, 0.0067 M, pH 7.4) was purchased from HyClone (Thermo Scientific Fisher Inc., Beijing, China). Polycaprolactone (PCL) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and has an average molecular weight (M_n) of 80,000. 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Glacial acetic acid, chloroform, and methanol were of high-performance liquid crystallography grade. All other reagents used were of analytical grade.

2.2. Isolation of acid-soluble collagen from Nile tilapia skin

Collagen was isolated from Nile tilapia skin following the procedure described by Senaratne et al. [28] with slight modification. All procedures were performed at 4 °C. The Nile tilapia skin was treated with 0.1 M NaOH (solid/solvent ratio of 1:10) for 1 h to remove noncollagenous proteins and pigments, and then washed with distilled water until the pH was neutral. To remove fat, the skins were treated with cyclohexane for 6 h. Defatted skins were washed with distilled water, and then lyophilized. The lyophilized skins were extracted with 60 volumes of lactic acid of pH 2.3 for 46 h, and the extract was centrifuged at 10,000 × *g* for 30 min (CT17RT, Techcomp, China). The supernatants were salted-out by adding NaCl to produce a final concentration of 0.9 M. The solution was left overnight, and the resultant precipitates were collected by centrifuging at 10,000 × *g* for 30 min. The precipitates were dissolved in 10 volumes of 0.5 M acetic acid. The resulting solution was dialyzed against 0.1 M acetic acid for 1 day, distilled water for 2 days, and then the dialyzed sample was lyophilized.

2.3. Preparation of PCL/collagen fibrous scaffolds (PCFSs)

Collagen extracted from Nile tilapia skin (4, 6, 8, and 10%) was dissolved in acetic acid (80%, v/v) by stirring the mixture at 300 rpm for 3 h at room temperature (26 ± 1 °C). Similarly, PCL (20%) was dissolved in the chloroform/methanol (3:1, v/v) solvent mixture by agitating the mixture with magnetic stirring (90-3, BAIXIAN, China) at 300 rpm for 2 h at room temperature. After the preparation of both polymeric solutions, all collagen solutions were mixed at a volume ratio of 80:20 with the help of a vortex (SA8, Stuart, England) and the mixture was kept for 48 h of incubation. After 48 h of incubation, collagen solutions were uniformly dispersed in the PCL solution whereby an immiscible blend (emulsion) of PCL/collagen was obtained, which was used for electrospinning.

PCFSs were fabricated by the electrospinning technique, the technical details of which have been discussed in previous literature [29]. In the electrospinning process, the polymer solution was placed into a 5-ml syringe with a needle of inner diameter 0.495 mm. A clamp connected a high voltage power supplier (DW-P403-1ACCC, DONGWEN, China) to the needle, and a piece of aluminum foil was placed at 150 mm directly below the needle to act as a grounded collector. The solution formed jets at the tip of the needle, and the jets formed fibrous scaffolds on the grounded collector by the combined force of gravity and an electrostatic charge. A high voltage of 15.0 kV was applied to the tip of the needle, and the feed rate of the solution was fixed at 0.3 ml/h. The electrospun scaffolds were collected on flat aluminum foil. The electrospinning process was carried out at room temperature, and humidity was maintained at 45% throughout the process.

2.4. Characterization of PCFSs

2.4.1. Morphological characterization

The morphology of PCFSs was studied under scanning electron microscope (JSM-6490 LV, JEOL, Japan) at an accelerating voltage of 20 kV, after sputter coating with gold. The diameters and the size of scaffolds were measured from the SEM images.

2.4.2. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectroscopic analysis of PCFSs was performed on spectrometer (Nicolet-67, Thermo Nicolet, USA) over the range 600–4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.4.3. X-ray diffraction (XRD)

The crystalline structure of PCFSs was analyzed by XRD on a wide-angle analyzer (D/MAX2500V, Rigaku, Japan) with a Cu target and K_α radiation at a scanning rate of 2° min^{-1}.

2.4.4. Differential scanning calorimetry (DSC)

The thermal behavior of PCFSs was characterized by DSC (Q2000, TA, USA) in the temperature range of 20–100 °C at a heating rate of 10 °C/min.

2.4.5. Mechanical testing

Mechanical properties of PCFSs were measured by a texture analyzer (TA-XT Plus, Stable Micro Systems, England), using test specimens of dimensions 10 mm breadth × 70 mm length with a thickness of 0.05 mm with the cross-head speed set at 10 mm/min. These tests were repeated three times and the tensile strength at the breaking point was recorded.