Materials Letters 117 (2014) 237–240

Contents lists available at ScienceDirect

Materials Letters

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

A novel integrated biphasic silk fibroin scaffold for intervertebral disc tissue engineering

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ARTICLE INFO

Article history:
Received 22 August 2013
Accepted 6 December 2013
Available online 16 December 2013

Keywords:
Tissue engineering
Intervertebral disc
Annulus fibrosus
Nucleus pulposus
Silk fibroin
Biphasic scaffold

ABSTRACT

The intervertebral disc (IVD) tissue engineering construct provides a promising approach for the treatment of symptomatic degenerative disc diseases. In this study, a novel 3D silk fibroin biphasic scaffold for integrated IVD tissue engineering was fabricated by sequentially using the paraffin sphere-leaching method for annulus fibrosus (AF) phase and the phase separation method for nucleus pulposus (NP) phase. Both phases were perfectly integrated and possessed a highly interconnected porous structure with pore size of 220 ± 23.1 μm for AF and 90 ± 17.8 μm for NP phase. Furthermore, the scaffolds were found to have a high porosity of 91% and 93% for AF and NP phases, respectively. In addition, this silk biphasic scaffold had a relative high compressive modulus under wet conditions (150.7 ± 6.8 kPa). Rabbit AF and NP cells could attach, grow and further penetrate into the scaffold and distribute uniformly. This silk fibroin biphasic IVD scaffold emerges as a potential candidate for IVD tissue engineering.

1. Introduction

Degeneration of the intervertebral disc (IVD) is considered a major source of lower back pain and limited mobility [1]. IVD degeneration is characterized by progressive microstructural disorder of the outer AF and dehydration of the inner NP extracellular matrix [2]. Currently, therapeutic methods in clinic for IVD diseases are spinal discectomy and fusion. However, these surgical procedures are mainly focused on relieving symptoms of pain and repairing neurological deficits at the expense of the biological functions and mechanical properties of the IVD [3].

Tissue engineering technology provides a promising alternative to restore physiological functionality of the damaged IVD [4]. Successfully regenerated IVD tissues should include both AF and NP tissues because the degeneration process involves both AF and NP of IVD. In previous studies, several biphasic composite scaffolds for entire IVD tissue engineering including PGA/PLA and alginate scaffold [5], decellularized scaffold [6] have been studied. Silk fibroin as biopolymer was wildly used to prepare tissue engineering scaffolds due to its unique biocompatible, strong mechanical properties, excellent biodegradability and its cost effective production [7]. Chang et al. [8,9] developed a porous silk scaffold using salt as porogen for AF tissue engineering. However, their results showed the lack of high interconnectivity of porous which resulted in limited cell penetration into scaffold. The aim of this study was to fabricate and evaluate a novel AF–NP porous silk fibroin biphasic scaffold made by the paraffin sphere-leaching method for outer AF phase and the phase separation method for inner NP phase. The AF and NP phases were found to integrate perfectly and possess high interconnected porous. We formed a hypothesis that this silk biphasic scaffold seeded with AF and NP cells could form an integrated IVD composite which could provide the potential for the regeneration of IVD.

2. Materials and methods

Fabrication of the silk fibroin biphasic scaffold: After being boiled in a 0.02 M Na_2CO_3 solution, the dried silk fibroin fibers from Bombyx mori cocoon were dissolved in a 9.3 M LiBr solution followed by dialysis against distilled water, and subsequently concentrated in 15% PEO aqueous solution. The obtained 20% (w/v) silk fibroin solution was added into the paraffin spheres assembly in Teflon molds with a stainless rod at the center of the mold (Fig. 1(A)–(C)). The silk fibroin solution was filled in the interspaces between the paraffin spheres under vacuum condition. The aim of above

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http://dx.doi.org/10.1016/j.matlet.2013.12.029

procedure is to prepare the AF phase. Next, the stainless rod was pulled out leaving a space at the center of the mold (Fig. 1(D)). Then, 7% of the SF solution containing 15% ethanol was immediately injected into the space (Fig. 1(E)). The molds were frozen for 12 h at −20 °C and then lyophilized for 48 h. The dried silk fibroin composite samples were taken out from the mold (Fig. 1(F)) and then placed into a Soxhlet's extractor to leach out paraffin spheres by boiling hexane (Fig. 1(G)). The obtained porous silk fibroin composite scaffold was dried at room temperature and sterilized by autoclaving for further use.

Characterization and evaluation of scaffolds: The scaffold specimens were cut to a thickness of 1 mm using a scalpel blade. The cross-section microstructure of the scaffold was examined under a stereo microscope (Leica, S8APO, Germany) and a scanning electron microscope (SEM, Hitachi, Janpan) (Fig. 2). The average pore size was determined by measuring 50 random pores from SEM images of the biphasic scaffolds with the Image J software (Wayne Rasband, National Institute of Health, USA). The porosity of the biphasic scaffolds was measured by the liquid displacement method [10]. The compressive modulus was obtained from the slope of a stress–strain curve which was recorded by using the dynamic mechanical performance test system (EnduraTE-CELF3200) at a compressive speed of 1 mm/min. AF cells and NP cells were isolated from the IVDs of 4-week-old New Zealand White rabbits. 20 ml of passage 2 NP cell suspension (1×10^7 cells/ml) was once seeded into the regions of the NP scaffold followed by the addition of a certain volume of culture medium to make the scaffold saturation. Then the 20 µl of AF cell suspension

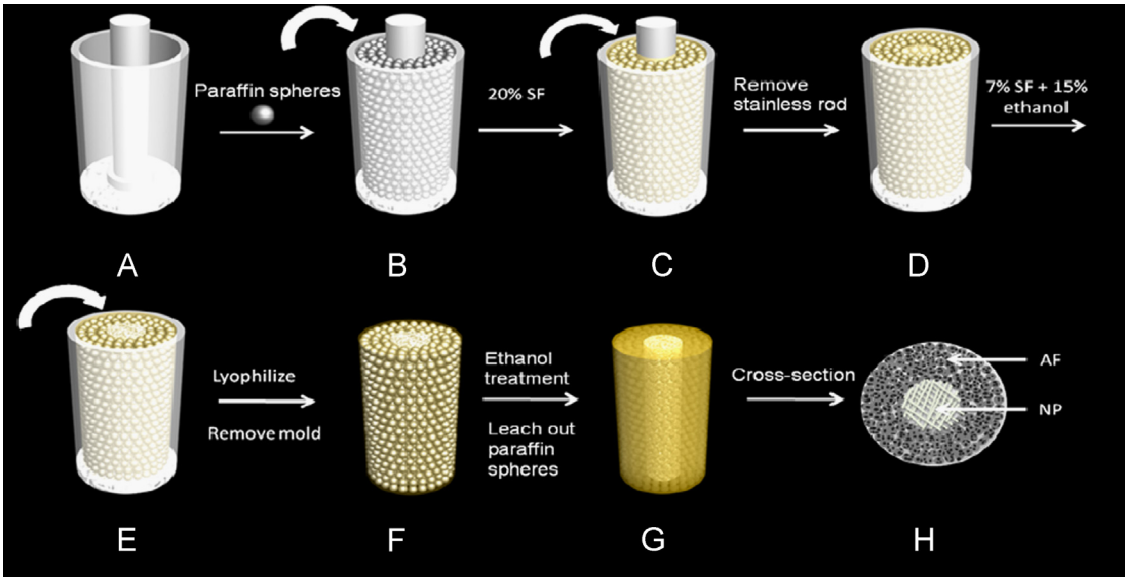

Fig. 1. Schematic diagram of the fabrication process for silk fibroin biphasic scaffolds.

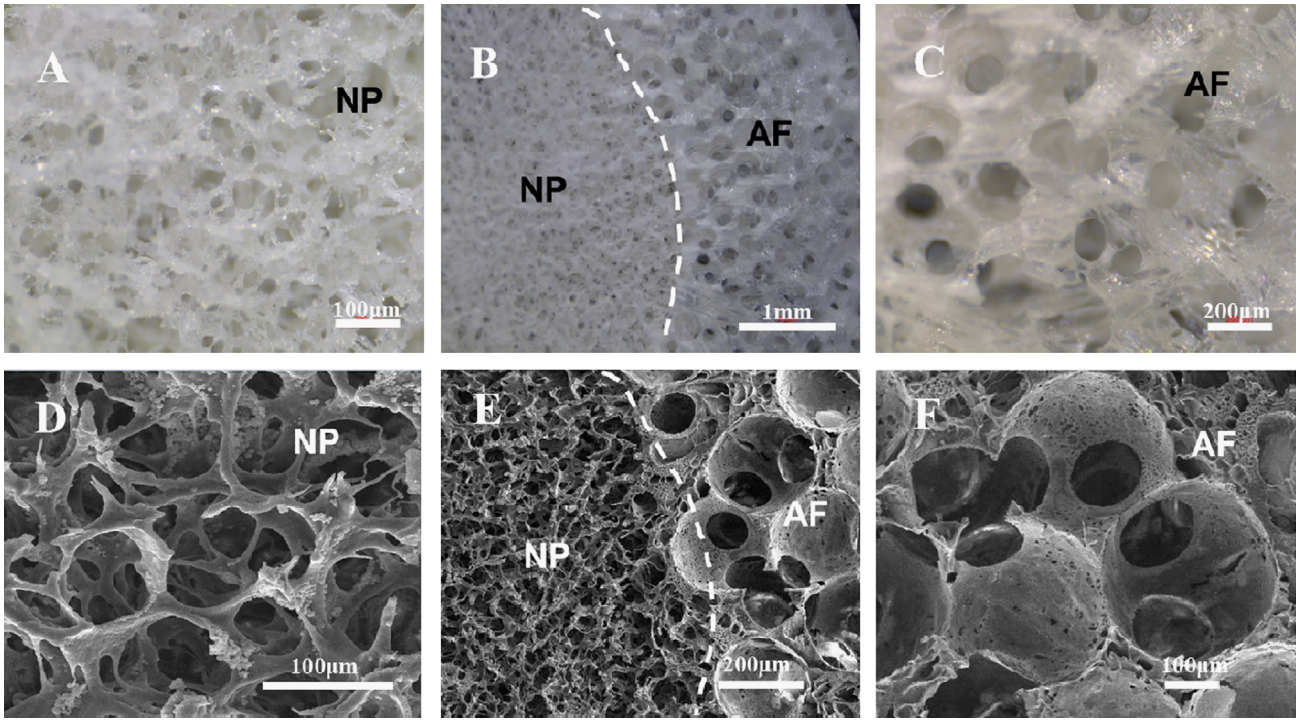

Fig. 2. Ultrastructure images of the scaffolds: stereo microscope of scaffold: (A) NP phase, (B) junction region between NP and AF phases, and (C) AF phase; SEM images of scaffolds: (D) NP phase (E) junction region, and (F) AF phase. (Dotted lines indicate the different porous structures of AF and NP phases).