Acta Biomaterialia 8 (2012) 302–312

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Effect of self-assembled nanofibrous silk/polycaprolactone layer on the osteoconductivity and mechanical properties of biphasic calcium phosphate scaffolds

S.I. Roohani-Esfahani [a], Z.F. Lu [a], J.J. Li [a], R. Ellis-Behnke [b,c,d], D.L. Kaplan [e], H. Zreiqat [a,*]

[a] Biomaterials and Tissue Engineering Research Unit, School of Aerospace, Mechanical and Mechatronic Engineering, The University of Sydney, Sydney 2006, Australia
[b] Department of Ophthalmology, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, 69167 Mannheim, Germany
[c] Nanomedicine Translational Think Tank, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, 69167 Mannheim, Germany
[d] Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[e] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA

ARTICLE INFO

Article history:
Received 9 June 2011
Received in revised form 5 October 2011
Accepted 6 October 2011
Available online 13 October 2011

Keywords:
Silk
Biomimetic materials
Scaffold
Bone
Osteoblasts

ABSTRACT

We here present the first successful report on combining nanostructured silk and poly(ε-caprolactone) (PCL) with a ceramic scaffold to produce a composite scaffold that is highly porous (porosity ~85%, pore size ~500 μm, ~100% interconnectivity), strong and non-brittle with a surface that resembles extracellular matrix (ECM). The ECM-like surface was developed by self-assembly of nanofibrous structured silk (20–80 nm diameter, similar to native collagen found in ECM) over a thin PCL layer which is coated on biphasic calcium phosphate (BCP) scaffolds. The effects of different concentrations of silk solution on the mechanical and physical properties of the scaffolds were also comprehensively examined. Our results showed that using silk only (irrespective of concentration) for the modification of ceramic scaffolds could drastically reduce the compressive strength of the modified scaffolds in aqueous media, and the modification made a limited contribution to improving scaffold toughness. Using PCL/nanostructured silk the compressive strength and modulus of the modified scaffolds reached 0.42 MPa (compared with 0.07 MPa for BCP) and ~25 MPa (compared with 5 MPa for BCP), respectively. The failure strain of the modified scaffold increased more than 6% compared with a BCP scaffold (failure strain of less than 1%), indicating a transformation from brittle to elastic behavior. The cytocompatibility of ECM-like composite scaffolds was investigated by studying the attachment, morphology, proliferation and bone-related gene expression of primary human bone-derived cells. Cells cultured on the developed scaffolds for 7 days had significant up-regulation of cell proliferation (~1.6-fold higher, $P < 0.001$) and osteogenic gene expression levels (collagen type I, osteocalcin and bone sialoprotein) compared with the other groups tested.

1. Introduction

While commercially available ceramic scaffolds such as β-tricalcium phosphate (β-TCP), hydroxyapatite (HA), biphasic calcium phosphates (BCP), bioactive glasses (BG) and calcium silicates (CS) are bioactive [1–5], their use in load-bearing applications is limited by the inherent brittleness of ceramics. The weakness of ceramic scaffolds is particularly evident at the optimal porosity (>80%), interconnectivity (~100%) and pore size (200–900 μm) required for bone regeneration [6,7]. During routine fabrication ceramic scaffolds are exposed to high temperatures for long periods of time in order to increase their mechanical integrity, which can compromise their bioactivity. Moreover, the lack of fibrillar proteins in these scaffolds results in poor surface reactivity and interaction with biological entities [7,8]. Silk fibroin, a naturally occurring structural protein with excellent mechanical properties, biocompatibility and biodegradability [9–14], has been widely used in tissue engineering applications. Recent studies reported the efficacy of silk in bone tissue regeneration, both as a single material or in combination with other constituents [15–19]. Silk fibroin scaffolds can be prepared using various methods [19–25]. Although these scaffolds excel amongst polymer-based scaffolds for bone regeneration, their mechanical strength is significantly less than that of ceramic scaffolds with similar physical characteristics, due to partial degradation of the protein structure. Combining silk with ceramics (in particle or scaffold form) remains a major challenge in the field due to the poor adhesion of silk to ceramic materials, which limits its application in drug delivery devices [26–30]. The use of silk to address the brittleness of ceramic scaffolds also requires further investigation. Polycaprolactone (PCL), a semi-crystalline linear ali-

* Corresponding author. Tel.: +61 2 93512392; fax: +61 2 93517060.
E-mail address: hala.zreiqat@sydney.edu.au (H. Zreiqat).

1742-7061/$ - see front matter © 2011 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2011.10.009

phatic polyester (melting point ~60 °C, glass transition temperature −60 °C), is approved by the US Food and Drug Administration and is one of the most popular polymers used for tissue engineering purposes. PCL is known for its biocompatibility, slow degradation and high fracture energy (compared with other biomedical polymers) [31]. This report presents a novel route for combining ceramic scaffolds with silk, tackling the current drawbacks by introducing a dual nanofibrous structured silk–PCL layer onto the surface of a BCP ceramic scaffold. Moreover, for the first time the ability of silk to improve the toughness of ceramic scaffolds is investigated. Primary human osteoblasts (HOB) were seeded on the modified scaffolds and osteogenic gene expression was analyzed as an indicator of osteoconductivity. The scaffolds were tested for both early (Runx2 and collagen type I) and late (bone sialoprotein (BSP) and osteocalcin) markers of osteogenic differentiation. These genes are known to be induced during different stages of the osteogenic differentiation pathway [32,33].

2. Materials and methods

2.1. Fabrication of BCP scaffolds

Calcium phosphate-deficient apatite powder was prepared by an aqueous precipitation reaction. Briefly, $Ca(NO_3)_2{\cdot}4H_2O$ (0.92 M) and $(NH_4)_2HPO_4$ (0.58 M) solutions were mixed gradually at room temperature and pH 11. The precipitated powder was thermally treated at 900 °C for 1 h, to produce BCP powder composed of approximately 40% HA and 60% β-TCP. The powder was crushed using a mortar and pestle and classified using stainless steel sieves to give particles of <75 μm size for scaffold fabrication. Fully reticulated polyurethane foam (The Foam Booth, Sydney, Australia) was used as a sacrificial template for scaffold replication via the polymer sponge method. The ceramic slurry was prepared by adding BCP powder to polyvinyl alcohol (PVA) solution to make a 30 wt.% suspension. Foam templates were cut to the appropriate dimensions and treated in NaOH solution for 30 min to improve surface hydrophilicity. After cleaning and drying the foams were immersed in the BCP slurry and compressed slightly to facilitate slurry penetration. Excess slurry was squeezed out and the foam was subsequently blown with compressed air to ensure a uniform ceramic coating on the foam surface. The weight of the polyurethane foams increased approximately five times after coating with BCP slurry. After drying at 37 °C for 48 h the BCP-coated foams were fired in air in an electric furnace using a four stage schedule: (i) heating from 25 °C to 600 °C at a heating rate of 1 °C min^{-1}, (ii) further heating from 600 °C to 1200 °C at 2 °C min^{-1}, (iii) holding the temperature at 1200 °C for 2 h and (iv) cooling to 25 °C at a cooling rate of 5 °C min^{-1}.

2.2. Modification of BCP scaffolds

Silk fibroin solution was prepared from cocoons of *Bombyx mori* silkworms using previously published procedures [9]. A 7.8 wt.% silk fibroin aqueous solution was used to modify the surface of the BCP scaffolds to produce the different study groups (Table 1). To prepare the BCP/PCL group PCL pellets (M_w = 80,000, Sigma-Aldrich, USA) were dissolved in chloroform (Sigma-Aldrich, USA) at a concentration of 7% (w/v). The BCP scaffolds were then immersed in the PCL solution for 1 min. The coated scaffolds were dried for 3 days in an oven at 37 °C and subsequently dried in a fume hood for another 24 h. To prepare the BCP/silk groups the BCP scaffolds were immersed in each concentration of silk solution (3, 5 and 7 wt.%) for 2 min and subsequently dried in an oven at 37 °C for 24 h. After drying the scaffolds were treated with 70% methanol for 1 h followed by drying at 37 °C for 1 week to remove residual methanol. To prepare the BCP/PCL–silk group optimal concentrations of silk and PCL was used for scaffold coating. BCP scaffolds were coated with PCL (5 wt.%) and dried using the procedure described above. Dried PCL-coated scaffolds were placed in 0.5 M NaOH solution at 50 °C for 1 h and subsequently rinsed at room temperature. Prior to coating with silk the scaffolds were exposed to methanol vapor for 2 h and then quickly dipped into the silk (3 wt.%) solution. PCL–silk-coated scaffolds were dried in an oven at 37 °C for 24 h and treated in methanol for 1 h, followed by drying at 37 °C for 1 week. Processing parameters for the PCL–silk-coated scaffolds should be carefully controlled for formation of nanofibrous structured silk on the PCL layer.

2.3. Physical and chemical properties of the scaffolds

The microstructure and fracture surface of the scaffolds were characterized by field emission scanning electron microscopy (FE-SEM) (Carl Zeiss, Germany). Internal structure, porosity and interconnectivity of the scaffolds were evaluated by micro-computerized tomography (SkyScan 1072, Belgium). A spatial resolution of 17 μm was selected for scanning. An optimized threshold was used to isolate the ceramic component from the background (void) for the evaluation of different morphological parameters. Thirty image slices were sampled at regular intervals for porosity analysis. Pore dimensions of the polyurethane foam before and after ceramic coating were determined via image analysis, to ensure that during scaffold fabrication the polyurethane template was replicated accurately with no change in pore shape or distribution. The coated silk had a submicron thickness and investigating the effect of processing parameters on its structural changes was almost impossible. Therefore, similar coating parameters were used to prepare silk, PCL and PCL–silk films as follows. To prepare silk films, 2 ml of silk solution was cast on polystyrene Petri dishes (diameter 3 cm) and subsequently dried 37 °C for 24 h. After drying the films were treated with 70% methanol for 1 h followed by drying at 37 °C for 1 week to remove residual methanol. To prepare PCL films PCL pellets were dissolved in chloroform at a concentration of 5 wt.%. The solution obtained was cast on glass Petri dishes and subsequently dried for 3 days in an oven at 37 °C and dried in a fume hood for another 24 h. To prepare PCL–silk films PCL films were placed in 0.5 M NaOH solution at 50 °C

Table 1
Designation, composition, weight, porosity and contact angles after coating the scaffolds.

Designation	Composition		Porosity (%)	Weight increase after coating (%)	Water contact angle (°)
	Scaffold substrate	Coating layer			
BCP	HA/β-TCP		89 ± 1		
BCP/PCL	HA/β-TCP	7 wt.% PCL	85 ± 2	19 ± 4	101 ± 5
BCP/PCL5	HA/β-TCP	5 wt.% PCL	87 ± 1	17 ± 3	104 ± 7
BCP/silk3	HA/β-TCP	3 wt.% silk	87 ± 1	15 ± 2	48 ± 3
BCP/silk5	HA/β-TCP	5 wt.% silk	85 ± 3	16 ± 2	51 ± 5
BCP/silk7	HA/β-TCP	7 wt.% silk	83 ± 1	18 ± 3	54 ± 6
BCP/PCL–silk	HA/β-TCP	3 wt.% silk + 5 wt.% PCL	85 ± 2	20 ± 1	52 ± 5