Materials Science and Engineering C 70 (2017) 1149–1155

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

# Uniformly-dispersed nanohydroxapatite-reinforced poly(ε-caprolactone) composite films for tendon tissue engineering application

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ARTICLE INFO

*Article history:*
Received 15 January 2016
Received in revised form 26 February 2016
Accepted 14 March 2016
Available online 18 March 2016

*Keywords:*
Poly(ε-caprolactone)
Hydroxyapatite
Tendon tissue engineering
Tendon-to-bone interface

ABSTRACT

Regeneration of injuries at tendon-to-bone interface (TBI) remains a challenging issue due to the complex tissue composition involving both soft tendon tissues and relatively hard bone tissues. Tissue engineering using polymeric/ceramic composites has been of great interest to generate scaffolds for tissue's healing at TBI. Herein, we presented a novel method to blend polymers and bioceramics for tendon tissue engineering application. A homogeneous composite comprising of nanohydroxyapatite (nHA) particles in poly(ε-caprolactone) (PCL) matrix was obtained using a combination of solvent and mechanical blending process. X-ray diffraction analysis showed that the as-fabricated PCL/nHA composite film retained phase-pure apatite and semi-crystalline properties of PCL. Infrared spectroscopy spectra confirmed that the PCL/nHA composite film exhibited the characteristics functional groups of PCL and nHA, without alteration to the chemical properties of the composite. The incorporation of nHA resulted in PCL/nHA composite film with improved mechanical properties such as Young's Modulus and ultimate tensile stress, which were comparable to that of the native human rotator tendon. Seeding with human tenocytes, cells attached on the PCL/nHA composite film, and after 14 days of culturing, these cells could acquire elongated morphology without induced cytotoxicity. PCL/nHA composite film could also result in increased cell metabolism with prolonged culturing, which was comparable to that of the PCL group and higher than that of the nHA group. All these results demonstrated that the developed technique of combining solvent and mechanical blending could be applied to fabricate composite films with potential for tendon tissue engineering applications.



## 1. Introduction

Tendon-to-bone interface (TBI) serves as the force transmission from relatively soft tendon tissue to harder bone [1]. However, injuries occur commonly at this specific portion [2], and because of its complex composition, the regeneration of TBI remains a significant challenging issue clinically. Tissue engineering can be a promising strategy in solving this problem, and one of which is using polymeric/ceramic composites to engineer biomimetic scaffolds for healing tissue at TBI [3]. Previously, studies have been conducted to generate hybrid scaffolds composing of ceramics (e.g. hydroxyapatite (HA) and tricalcium phosphate) in polymeric matrices such as poly-*L*-lactic acid (PLLA), polyglycolic acid (PGA) and polylactic-*co*-glycolic acid (PLGA) [4]. These hybrid scaffolds could mimic the composition of tissue matrix at TBI, with improved osteoconductive nature for faster fixation. Furthermore, the hybrid scaffolds had obtained compromised mechanical properties, which was in between that of the relatively soft tendon tissue and harder bone.

Different techniques have been developed to obtain polymeric/ceramic composites including mechanical [5,6] and solution blending [7,8]. Mechanical blending has been intensively investigated to mix polymer and ceramic in an extruder. However, this method allowed particle aggregation and phase separation between the polymers and ceramic particles to occur easily due to poor interaction [6,9]. Such problems were met even when low amounts of ceramic particles were added [6], and made the technique difficult to incorporate higher amounts of ceramic particles. The other commonly used method for hybrid scaffold fabrication was solvent casting, which involved casting ceramic particle-infused polymer solutions in a mould [7,8]. Although this method could allow for suspension with homogeneous distribution of ceramic particles, the particles tend to deposit at the bottom during the process of solvent evaporation. Meanwhile, electrospinning has been applied to create HA/poly(ε-caprolactone) (PCL) composite fibers [9,10]. However, this method was reported to create beads-like fibers with an inhomogeneous distribution of ceramic particles, leading to deteriorative mechanical properties [11]. A newly reported method was cryo-milling that involved the grinding of ceramic particles by repeated collision with milling balls at high-speed rotations and ultra-low temperatures (e.g. −179 °C) [12]. However, due to the repeated process of high impact grinding, this method caused unwanted damage to the

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http://dx.doi.org/10.1016/j.msec.2016.03.051
0928-4931/

surface and structure of the ceramic particles. Moreover, not all polymers or ceramic particles are suitable to undergo very low temperature process [13].

Current blending strategies cannot result in an ideal polymeric/ceramic composite for tendon tissue engineering applications. Herein, we developed a novel method via a combined solvent and mechanical blending process for the generation of spatially homogeneous polymeric/ceramic composite. PCL was selected as the polymeric matrix because of its unique biocompatibility, bioresorbablity and mechanotransductivity for applications in regenerative medicine [14–16]. HA, the main mineral component of bone was employed as the ceramic composition of polymeric matrix due to its high biocompatibility and bioactivity [17,18]. The PCL/HA composite could mimic the composition of TBI tissue matrix, with the potential to be applied for tendon regeneration purposes [6,19,20].

## 2. Materials and methods

### 2.1. Materials

PCL ($M_w$ = 80.000), dichloromethane (DCM), methanol, fluorescein-diacetate (FDA), propidium iodide (PI), glass petri dishes and falcon tubes were purchased from Sigma-Aldrich (Singapore). Calcium hydroxide (95%) and orthophosphoric acid (85%) were purchased from Merck (Singapore). Ammonia (25%) was purchased from VWR (Singapore). Phosphate buffer saline (PBS) and alamarBlue® medium were purchased from Thermo Fisher Scientific (Singapore). Human tenocytes (CSC-C1584, USA) and growth medium (HTGM-500) were purchased from Creative Bioarray (USA).

### 2.2. Synthesis of nanohydroxyapatite (nHA)

Phase-pure HA (Ca/P molar ratio: 1.67) was synthesized via a wet precipitation method at room temperature as previously described [21]. 0.3 M aqueous phosphoric acid was added dropwise into 0.5 M aqueous calcium hydroxide under continuous stirring. The pH of the mixture was ensured to be above 10.5 by the addition of aqueous ammonia. The mixture was stirred for an additional 18 h after the aqueous phosphoric acid was completely added. The precipitate was left to age for 2 weeks, autoclaved at 124 °C, and centrifuged at 3000 rpm to remove any excess water from the apatite precipitates. The precipitates were later oven-dried at 80 °C overnight and ground into fine powders.

### 2.3. Fabrication of PCL/nHA composite films

#### 2.3.1. Mechanical blending

Fig. 1 illustrates the blending methods that were used for fabricating PCL/nHA composite films. Technique #1 involved mechanical blending of PCL pellets and as-synthesized nHA powders using a 2-roll milling machine. Briefly, PCL pellets were first added into the 2-roll milling machine at an elevated temperature of 90 °C and a rotating speed of 1.8 rpm. After complete melting of PCL, a range of nHA powders (10, 30 and 50 wt.%) was added into the 2-roll milling machine, to blend with PCL mechanically for 2 h. The resultant PCL/nHA composites were then collected, cut into pallets (~5 g per pallet) and allowed to cool down to 24 °C into solid masses. The solid masses were subsequently fabricated into films using a hydraulic press machine operating at 80 °C and 300 MPa for 2 h [22,23].

#### 2.3.2. Combination of solvent and mechanical blending

Technique #2 combined mechanical blending and solvent blending. Briefly, PCL pellets were dissolved in DCM and a range of as-synthesized nHA powders (10, 30 and 50 wt.%) was dispersed in methanol, respectively. The ratio of DCM (to dissolve PCL) and methanol (to disperse nHA) was at 7:3 (v/v) according to previous report [24]. Subsequently, PCL solution was mixed with the nHA suspension using an orbital shaker (MRC TOS-4030PD, Israel) to form PCL/nHA composites. To remove the solvents, PCL/nHA composites were casted onto a petri dish in a fume hood at 24 °C for 3 days. The resultant PCL/nHA sheets were then subjected to mechanical blending using a 2-roll milling machine, followed by hydraulic pressing into films as described in Section 2.3.1.

#### 2.3.3. Modified combination of solvent and mechanical blending

Technique #3 involved an additional step of sieving. Briefly, the as-synthesized nHA powders were passed through a test sieve (mesh size: 75 μm; W.S. Tyler, USA) to eliminate powders of particle sizes larger than 75 μm. PCL pellets and a range of sieved nHA powders (10, 30 and 50 wt.%) were then blended using Technique #2 as described in Section 2.3.2. The resultant PCL/nHA composites were fabricated into films using hydraulic press as described in Section 2.3.1.

### 2.4. Measurement of mechanical properties

The mechanical properties of PCL/nHA composite films were studied using a tensile testing machine (Model 3345, Instron, USA). The film samples were cut into rectangular shapes of width 3 mm and length 30 mm. The thickness of each film sample was measured using a digital micrometer (APB-1D, Mitutoyo Corporation, Japan) at three random

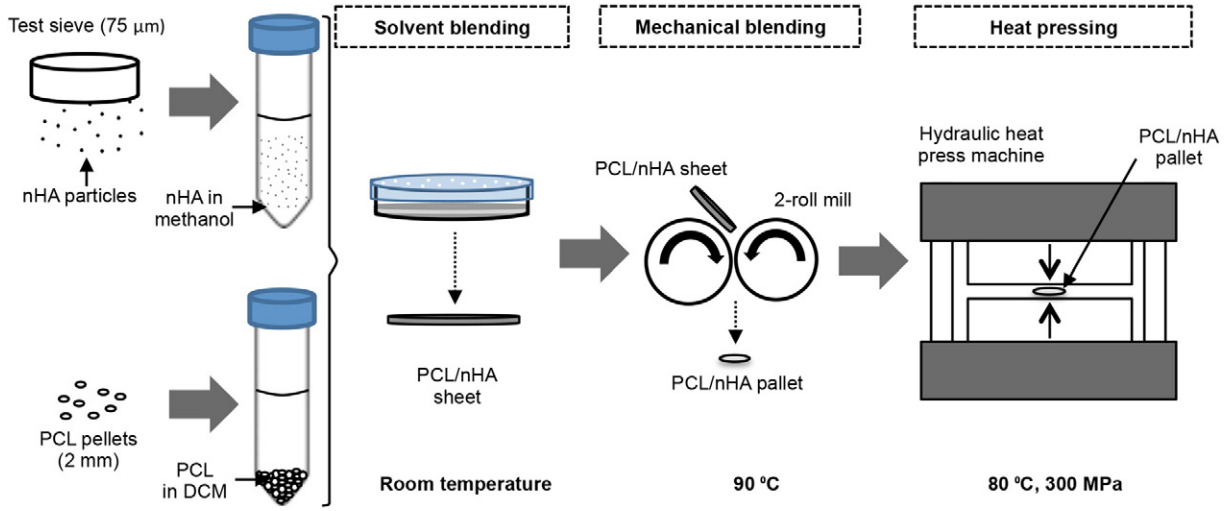


**Fig. 1.** Schematic diagram illustrating the fabrication processes of PCL/nHA composite film.