Carbohydrate Polymers 138 (2016) 172–179

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Preparation and characterization of bioactive composite scaffolds from polycaprolactone nanofibers-chitosan-oxidized starch for bone regeneration

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ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 19 October 2015
Accepted 23 November 2015
Available online 2 December 2015

Keywords:
Chitosan
Oxidized starch
Polycaprolactone
Calcium phosphate
Bone
Scaffold

ABSTRACT

The objective of this study was to fabricate and investigate the characteristics of a suitable scaffold for bone regeneration. Therefore, chitosan was combined with various amounts of oxidized starch through reductive alkylation process. Afterwards, chopped CaP-coated PCL nanofibers were added into the chitosan-starch composite scaffolds in order to obtain bioactivity and mimic bone extracellular matrix structure. Scanning electron microscopy confirmed that all scaffolds had well-interconnected porous structure. The mean pore size, porosity, and water uptake of the composite scaffolds increased by incorporation of higher amounts of starch, while this trend was opposite for compressive modulus and strength. Osteoblast-like cells (MG63) culturing on the scaffolds demonstrated that higher starch content could improve cell viability. Moreover, the cells spread and anchored well on the scaffolds, on which the surface was covered with a monolayer of cells.

1. Introduction

Large bone defects are believed to be one of the major issues caused by trauma and infection. Despite several efforts such as using bone grafts and implants, there are still limitations and drawbacks in bone regeneration. Over the past few decades, bone tissue engineering (BTE) has emerged as a promising alternative to classical therapies (Bose, Roy, & Bandyopadhyay, 2012; Shrivats, Dermott, & Hollinger, 2014). One of the most important aspects of BTE is designing a suitable scaffold that could modulate bone healing and mimic the extracellular matrix (ECM) role in bone tissue. Thus, ideal scaffolds should consist of biodegradable and biocompatible materials, which possess appropriate pore size, porosity, mechanical properties, and osteo-conductivity (Guarino et al., 2013). However, single-phase scaffolds do not provide proper structural and mechanical features required for bone regeneration. Recently, using composite materials has gained much attention to improve the biodegradability and bioactivity (Hutmacher, Schantz, Lam, Tan, & Lim, 2007). Meanwhile, the mechanical and biological properties of the composite scaffolds can be manipulated in order to design a suitable structure similar to the features of the bone tissue (Hutmacher et al., 2007; Lee & Shin, 2007).

Various natural and synthetic biodegradable polymers have been studied as scaffolds for BTE (Puppi, Chiellini, Piras, & Chiellini, 2010). In this regard, natural hydrogels have gained much popularity because of their similarity to the extracellular matrix (ECM) and higher regeneration rate (Peppas, Hilt, Khademhosseini, & Langer, 2006). Chitosan is a linear, amino polysaccharide derived from chitin through deacetylation process. It is widely used in various fields of biomedical engineering due to its biocompatibility, accessibility, biodegradability, and antibacterial activities (Croisier & Jérôme, 2013). Chitosan is barley used alone in BTE as it has poor mechanical properties, and high water sensitivity. Reports indicated that the functional properties of chitosan are generally improved by combining it with other natural biopolymers such as alginate (Baysal, Aroguz, Adiguzel, & Baysal, 2013), cellulose (Wu et al., 2004), and gelatin (Cheng et al., 2010). Among different kinds of biopolymers, starch is one of the most promising material in the field of biomedical engineering, since it is biocompatible, cost-effective, and accessible (Hanafi, Irani, & Zulkifli, 2013; Xie, Pollet, Halley, & Avérous, 2013). Bourtoom and Chinnan (2008) proposed that the water vapor permeability of rice starch-chitosan blend films is lower than the films prepared only by chitosan. Martins,

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http://dx.doi.org/10.1016/j.carbpol.2015.11.055
0144-8617/

Santos, Azevedo, Malafaya, & Reis (2008) found that the water uptake and biological activity of chitosan hydrogel improved when combining with starch, while its compression strength decreased.

As mentioned above, blending of chitosan with starch can improve some properties of chitosan-based hydrogels. However, there are still some limitations regarding their application as BTE scaffolds, including insufficient mechanical properties and bioactivity (Shakir, Jolly, Khan, Iram, & Khan, 2015). Over the past few years, the researchers have incorporated nano-hydroxyapatite into hydrogels to enhance such properties (Barbani et al., 2011; Chesnutt et al., 2009; Lai, Shalumon, & Chen, 2015;). However, the weak interfacial bonding and inhomogeneous distribution of ceramic fillers throughout the hydrogels were reported in such approaches. More recent studies suggested that hydrogel reinforcement with electrospun nanofibers improved its mechanical properties and biological activity (Hadisi, Nourmohammadi, & Mohammadi, 2015; Kai et al., 2012).

This study introduces a set of new bioactive nanocomposite scaffolds using chitosan, starch, and polycaprolactone (PCL) for BTE applications. Therefore, chitosan was combined with various amounts of oxidized starch through the reductive alkylation process. Afterwards, chopped calcium phosphate-coated PCL nanofibers were added into the fabricated chitosan-starch composite in order to achieve bioactivity and mimic bone ECM. Eventually, the morphology/structure, swelling, mechanical properties and biological response of MG 63 osteoblast-like cells cultivated on the surface of the scaffolds were investigated.

2. Materials and methods

2.1. Materials

Chitosan (medium molecular weight, deacetylation degree 85%), acetic acid, formic acid, and polycaprolactone (Mn = 80000 Da) were purchased from Sigma (St. Louis, USA). Sodium periodate ($NaIO_4$), calcium chloride ($CaCl_2$), Tris (Hydroxymethyl-amino-methane) ($(CH_2OH)_3CNH_2$), hydrochloric acid (HCl), sodium hydrogen phosphate (Na_2HPO_4), and sodium hydroxide (NaOH) were obtained from Merck (Germany). The soluble potato starch was supplied by BioBasic Inc. (Canada). All the chemicals used in this study were of analytical grade.

2.2. Electrospun PCL nanofibers preparation

In this study low toxic solvents of acetic acid and formic acid were chosen to produce elctrospun PCL nanofibers. The appropriate amount of PCL was dissolved in the mixture of formic acid and acetic acid with a ratio of 3:1 (v/v) in order to prepare 13 wt% solution. Afterwards, the resulting solution was filled in a 1 ml syringe with a 22 G blunted stainless steel needle. Then, electrospinning (Fanavaran Nano-Meghyas, Iran) was carried out at 11 kV with a constant flow rate of 0.4 ml h^{-1}. The rotating mandrel (50 m/min) was chosen as a collector and the needle tip distance from the collector was 10 cm.

2.3. Calcium phosphate (CaP) deposition on/in the electrospun PCL mats

The surface of the PCL mat was modified using a 2 M NaOH solution in a shaker incubator ($T = 37\,^{\circ}C$). After 3 h, the sample was removed from the NaOH solution, carefully washed with deionized water and dried at room temperature. For CaP deposition, the NaOH-treated sample was dipped in calcium and phosphate rich solutions, alternately (Taguchi, Kishida, & Akashi, 1999). Briefly, the fabricated mat was soaked into 10 ml of 0.5 M $CaCl_2$ (pH = 7.4) at 37 °C for 30 min, washed rapidly with deionized water, and immediately soaked into 10 ml of 0.3 M Na_2HPO_4 (pH = 8.5) at 37 °C for another 30 min. After repeating each cycle 3 more times, the mat was rinsed thoroughly with deionized water and then dried at room temperature.

2.4. Preparation of the oxidized starch

Oxidized starch was initially prepared through the procedure described by Hermanson (1996). Briefly, 1.25 ml of sodium iodate ($NaIO_4$) solution (10 mg/ml) and 2% (w/v) starch solution were mixed thoroughly in a light-protected glass vessel at room temperature. After 30 min, 1.125 ml of glycerin was added to the reaction vessel and stirred for another 10 min.

2.5. Preparation of composite scaffolds

Chitosan powder (1 g) was added to the 1% (v/v) acetic acid and stirred overnight to have 1% (w/v) clear solution. Subsequently, different amounts of oxidized starch and chitosan solutions were mixed thoroughly at room temperature for about 1 h. Meanwhile, 1% (w/v) of chopped CaP-coated PCL nanofibers were added into the solution. After stirring for 2 h, the mixture was poured into 24-well tissue culture polystyrene plates, frozen at −20 °C overnight and then freeze-dried. The code and composition of the fabricated scaffolds are presented in Table 1.

3. Characterization

3.1. Characterization of electrospun PCL mat

The morphologies of electrospun fibers before and after CaP deposition were observed using a Scanning electron microscopy (SEM, Lecia Cambridge S360). The diameter and distribution of the electrospun fibers were measured by Image J software (National institutes of Health, Bethesda, Maryland, USA). Approximately 100 random fibers of different SEM images were analyzed. The presence of calcium and phosphorus elements on the deposited layer was also evaluated by Electron dispersive spectroscopy (EDS). The changes in the chemical structure of electrospun PCL nanofibers after CaP deposition were studied by Fourier transform infrared spectrometer (FTIR; BRUKER IFS 48) in the range of 400–4000 cm^{-1}. Moreover, the phase compositions of electrospun PCL nanofibers were determined by X-ray diffractometry (XRD; X'Pert Pro MPD) using Cukα radiation. Metrohm pH meter 827 determined the changes in pH value during alternate soaking process.

3.2. Characterization of fabricated composite scaffolds

3.2.1. IR spectroscopy

The structural analysis of pure starch, oxidized starch, pure chitosan, and composite scaffolds were examined through Fourier transform infrared spectroscopy in the attenuated total reflectance mode (FTIR-ATR).

Table 1
The code and composition of each fabricated composite scaffolds.

Samples	Chitosan	Oxidized starch	CaP-coated PCL electrospun fibers (w/v %)
S1	97	3	1
S2	93	7	1
S3	90	10	1
S4	85	15	1