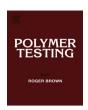
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Material properties

Effect of starch content on the biodegradation of polycaprolactone/starch composite for fabricating *in situ* pore-forming scaffolds



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ABSTRACT

Bone tissue engineering is an efficient approach to regenerating bone-related defects. The optimal scaffold used for bone tissue engineering must possess adequate porosity and suitable mechanical properties. This work described the development of a biodegradable polymeric composite based on polycaprolactone (PCL) and starch that can form a porous structure in situ. The scaffold exhibited the required mechanical properties at the initial stage of implantation by controlling in situ degradation and subsequent pore formation. PCL/starch (SPCL) scaffolds with 100/0, 70/30, and 50/50 ratios were developed. Degradation studies were performed in phosphate buffer saline (PBS) containing α -amylase or lipase at 37 °C for 4 weeks. Fourier-transform infrared spectroscopy was used to analyze chemical bonds and their changes after degradation. Differential scanning calorimetry was applied to determine the crystallinity and recrystallization of samples before and after degradation. Mass loss and starch release were observed during degradation, and the porosity of samples was measured by the ethanol replacement method. Morphology was further determined using scanning electron microscopy. Finally, variations in compressive strength and modulus during degradation and pore formation were also measured. The porosity of samples reached 45% after 1 month of degradation, and mechanical properties were still appropriate for human bone tissue. Reduction in mechanical property after mass loss, starch release and pore formation was controlled by the hydrogen bonding and recrystallization effect of PCL after degradation. Results suggested that SPCL composite had potential to form porous scaffold with adequate mechanical properties in situ and is promising for bone tissue engineering applications.

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1. Introduction

Scaffolds designed for a particular tissue require different characteristics, and porosity is an inevitable

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property for all scaffolds to enable cell immigration, storage and proliferation [1]. In bone tissue engineering, scaffolds must possess adequate porosity and pore size to increase the potential of bone formation *in vitro* and *in vivo* [2]; they must also provide sufficient temporary mechanical support that matches the mechanical properties of the host tissue

to withstand *in vivo* stresses and loading [3]. As porosity affects the mechanical characteristics of the constructs, one of the most competitive challenges in bone tissue engineering is the preparation of porous scaffolds; such scaffolds must exhibit adequate mechanical strength at initial stages to retain their sufficient structural integrity until the newly grown tissues become capable of supporting loads and stresses [4,5].

In situ pore formation was applied by Martins et al. [6] in 2008 to solve the challenges regarding the porosity of scaffolds. A scaffold capable of in situ pore formation does not produce pores after fabrication, but becomes porous through enzymatic and metabolic reactions after implantation in the body [6]. The preparation of such scaffolds requires a composite with at least two components, in which one component undergoes rapid degradation and functions as a porogen. In early works of Martins et al., blend of chitosan and starch was utilized as two natural polymers. In the present research, we focused on using a blend of PCL and starch to obtain the benefits of synthetic and natural polymers.

PCL exhibits enormous potential in biomedical applications, such as tissue engineering and drug delivery, because of its suitable mechanical properties, biocompatibility, bioresorbability and biodegradability [7-11]. The major limitation of this polymer is poor wettability and slow degradation (approximately years) in vivo because the close packed macromolecular arrays resist water uptake in the bulk [12,13]. Starch is totally biodegradable because of its highly hydrophilic nature, so starch-based blends have also been widely used in the biomedical and environmental fields [14]. Compared to PCL and starch, SPCL exhibits different mechanical properties and degradation pathways [15]. PCL degradation takes place in two different stages: a prolonged phase, in which ester groups are randomly hydrolyzed; and a subsequent phase, in which the formed oligomers and low-molecular-weight polymer chains diffuse through the bulk and cause weight loss. This phase begins with a highly crystalline polymer that consists of low-molecular-weight chains [16–18]. In SPCL composite, water may be efficiently absorbed into the bulk because of the hydrophilic nature of starch blended with PCL. Depending on starch amount and distribution, efficient water uptake can accelerate the high-molecular-weight PCL chain scission and, subsequently, speed up its prolonged degradation.

In this research, degradation and pore formation of PCL/starch composite in different aqueous environments were investigated. Three different composites with 0%, 30%, and 50% starch were selected to evaluate the effect of starch content on degradation, pore formation and consequent mechanical property evolution. Fourier-transform IR spectroscopy (FTIR) and differential scanning calorimetry [19] were used to determine the effect of degradation on chemical and molecular structures. Weight loss and starch release profile were measured within 4 weeks of degradation in phosphate buffered saline containing α -amylase or lipase at 37 °C. Porosity percentage, as well as pore structure and morphology, were observed using the ethanol replacement method and scanning electron microscopy (SEM), respectively. Finally, mechanical modulus

and strength were measured after degradation to determine the post-degradation properties of the composites.

2. Materials and methods

2.1. Sample preparation

PCL with an average molecular weight of 80.000 g/mol (Sigma—Aldrich) was dissolved in chloroform (Merck) at 60 °C to fabricate PCL/starch composite. Potato starch (Merck) was simultaneously dissolved in chloroform at 25 °C. The two solutions were mixed to form different PCL/starch composites with ratios of 100/0, 70/30, and 50/50. The mixed solution was prepared by gentle stirring at 60 rpm for 1 hour in a sealed reactor. The solutions were then cast into molds and maintained at 4 °C for 72 hours to provide sufficient time for residual solvent to leave the composite structure and reorient the polymeric chains to resist pore formation.

2.2. Degradation assay

The samples were immersed in PBS (pH 7.4) containing α -amylase or lipase at concentrations similar to human serum and then incubated at 37 °C. α -Amylase is an important enzyme that accelerates starch degradation in the human body, and lipase can speed up lipase degradation [20,21]. Degradation assay was performed for 4 weeks, followed by evaluation and analysis.[19]

2.2.1. Fourior-transform IR spectroscopy

FTIR with an IFS-48 device (constructed by Bruker Company) was used to characterize the chemical structure and changes in chemical bonds and component ratio of the samples after degradation. The samples were thoroughly ground with KBr, and pellets were prepared through compression in vacuum. Spectra were recorded from $4000~\rm cm^{-1}$ to $400~\rm cm^{-1}$.

2.2.2. Differential scanning calorimetry

A DSC instrument was used before and after degradation at a heating rate of $10~{\rm min}^{-1}$ at $10~{\rm ^{\circ}C}$ and $80~{\rm ^{\circ}C}$ to determine the effect of starch and its degradation on the crystallinity of PCL. An empty sample pan was used as reference. Crystallinity was calculated using the following equation:

$$X_c = \frac{\Delta H_m}{\Delta H_m^0} \times 100$$

where ΔH_m is the enthalpy of melting of each sample and ΔH_m^0 is the enthalpy of melting of 100% crystalline PCL, taken as equal to 136 J/g [19].

2.2.3. Mass loss

Mass loss may occur during immersion because of the degradation of composite components, and mass increase may occur because of water uptake and water trapping inside the composite. Water uptake, as well as subsequent hydrolysis and mass loss, of the composite were measured at predetermined times. To maintain the PBS medium

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