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Preparation and characterization of aligned porous PCL/zein scaffolds as drug delivery systems via improved unidirectional freeze-drying method

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ABSTRACT

A novel type of drug-delivery scaffold based on poly(ε-caprolactone) (PCL) and zein blends was prepared by improved unidirectional freeze-drying. Scaffolds with tube-like pore structure and high porosity, up to 89%, were obtained by adjusting the concentration of the PCL and zein solutions. Characters of the prepared scaffolds, such as microstructural, porosity, and compressive strength, were evaluated. The hydrophilicity and the degradability of the composite films were investigated in contact with phosphate buffer saline (PBS). It was found that the presence of zein accelerates the degradation rate of the scaffolds in the period time of investigation (28 days). The results showed an acceptable way for controlling the in vitro degradation behavior of PCL composite scaffolds by adapting the concentration of zein. In vitro protein release and degradation results revealed that the absolute weight loss of the PCL/zein scaffolds exhibited an increasing trend by increasing the amount of zein concentration in the scaffolds. The drug delivery capability of the scaffolds was tested using tetracycline hydrochloride (TCH). Sustained release of the drug was obtained, and it was found that the proportion of zein in the scaffold had a great impact on the drug release kinetics. The results demonstrated the potential of the PCL/zein biocomposite scaffolds as a suitable candidate in tissue engineering strategies for bone defect treatment.

1. Introduction

One important group of tissue engineering approaches involves the implantation of a highly porous biomaterial scaffold populated with appropriate cells, providing a three-dimensional environment in which new tissue can grow [1–4]. After tissue restoration, the ideal biomaterial will be resorbed and removed by the body itself or integrated within the new tissue without any requirement for further surgery [5–7]. In this context, the scaffold, which is an engineered material with high porosity, should perform as 3D template for cell adhesion, proliferation, migration and ultimately the formation of new tissue [2]. One of the scientific challenges for tissue engineers is associated with the required properties of the scaffold, which include controlled degradation behavior, time-dependent mechanical properties, and biological activity [8]. Zein, a class of the main storage proteins, is a prolamine rich as well as alcohol-soluble protein and its amount is 2.5–10% (dry basis) in corn [7]. Zein has received attention in recent years regarding its pharmaceutical application, where it has been successfully used in tableting, film formation [9], active food packaging materials [7,9], encapsulation of essential oils, aromas and flavors [10], and controlled release of active additives or drugs [11–16]. The disadvantages of zein are related to its low mechanical properties [17], especially considering that pure zein films are too fragile and rigid to be used for any load-bearing application especially bone tissue engineering [8,18]. Zein is also excessively sensitive to moisture and pure zein films take up water [19]. In order to overcome these drawbacks, some polymers having superior mechanical properties and resistance to absorption of moisture are being considered to improve the flexibility and toughness of zein for tissue engineering application. Such polymers include chitosan [14], alginate [20], poly(vinylpyrrolidone) (PVP) [21], poly(lactic acid) (PLA) [22] and poly(lactic-co-glycolic acid) (PLGA) [13]. Among the variety of polymeric materials which have been used for fabrication of bone tissue engineering scaffolds, poly(ε-caprolactone) (PCL) has been widely considered due to its biocompatibility, suitable mechanical properties, easy-processing ability, and non-toxic degradation products [11,12,20–25]. However, some drawbacks of PCL scaffolds such as slow degradation rate, hydrophobicity, and acidic degradation products have limited their wider applications [26]. Blending synthetic and natural polymers constitutes a very efficient way to overcome the aforementioned limitations and to

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enhance the control over scaffold degradation and hydrophilicity [12, 27].

Salerno et al. [28] developed PCL/zein composite scaffolds via the supercritical CO_2 foaming technology. The porous scaffolds were prepared from PCL-modified zein which was obtained by mixing zein with poly(ethylene glycol) (PEG). They prepared blends of PCL/zein which were modified by PEG for improving the hydrophilic properties and the cell adhesion and proliferation of the scaffolds. Wu et al. [17] reported that the toughness of zein can be improved by using PCL and hexamethylene diisocyanate (HDI) as a prepolymer, which was used to modify zein-based polymers. Their results showed that amino acids in zein reacted with the prepolymer, and urea–urethane links were formed. Porous PCL/zein scaffolds have been fabricated by solvent casting–particulate leaching method [29]. Moreover, Gong et al. [30] utilized zein for fabricating tissue engineering scaffolds by the salt-leaching method. Recently, Cooper's group reported a simple and effective way to produce porous materials with oriented structure by unidirectional freeze-drying method [31,32]. They controlled the growth speed and orientation of the ice crystals and obtained unidirectional porous scaffolds after the freeze-drying process. The pore size of the scaffolds could be adjusted by varying the concentration of the solution.

Based on the developments discussed above, this work reports the fabrication of a novel porous PCL/zein scaffold with microtubule orientation structure via improved unidirectional freeze-drying method. We used a blend of zein and PCL to prepare tissue scaffolds. It was expected that blending PCL with zein would enable control over hydrophilicity and degradation behavior as well as influencing the drug delivery of the scaffolds. Our hypothesis was that pore size and porosity could be adjusted by altering the relative concentration of PCL and zein solutions. The pore structure, hydrophilicity and mechanical properties of the scaffolds were determined. In vitro degradation behavior and protein release of the scaffolds in phosphate buffered saline (PBS) were monitored. In addition, the ability of the 3D scaffolds as a controlled release device for tetracycline hydrochloride (TCH) as a model drug was evaluated.

2. Materials and methods

2.1. Preparation of composite scaffolds and films

Poly(ε-caprolactone) (PCL) (Mw = 80,000, m.p. 60 °C) and zein (Z 3625, purity of 98%) were supplied by Sigma-Aldrich (St. Louis, Mo., U.S.A). Chloroform (99.8 vol.%), acetic acid glacial (100%) and ethanol (99.8 wt.%) were purchased from Merck Co. and used as received.

PCL pellets were weighed and dissolved in selected volumes of blend of chloroform–ethanol, chloroform–acetic acid glacial and acetic acid glacial. In order to use binary solvent, we dissolved zein in ethanol or acetic acid and PCL in chloroform. Then, zein solution was slowly added into the PCL solution followed by continuous stirring. The obtained solution was injected into a plastic tube with a length of 50 mm and a diameter of 10 mm. The wall of the tube was insulated with Styrofoam container and the bottom surface was placed on a metal substrate (see Fig. 1). This prepared set was placed into a 6 cm deep pool of liquid nitrogen to create a uniaxial thermal gradient [33]. By cooling the substrate (metal), the homogeneous solution started to freeze vertically from the bottom to the top and was then frozen completely. After solidification, these prepared sets were instantly transferred into a freeze-drying vessel under vacuum condition (Alpha1-2 plus, Christ, Germany, <20 Pa) and were freeze-dried for 72 h. The scaffolds were subsequently removed from the plastic tubes and preserved in a silica gel desiccator for further characterization. The effect of PCL and zein concentration on the morphology of the composite scaffolds was also investigated in three concentrations of 5%, 10%, and 15% w/v of PCL/zein. In order to prepare composite films, a solution with a desired concentration was blended in accordance with the above procedure. Afterwards, the solutions were spread over a flat surface such as a glass sheet and were dried at room temperature for 24 h.

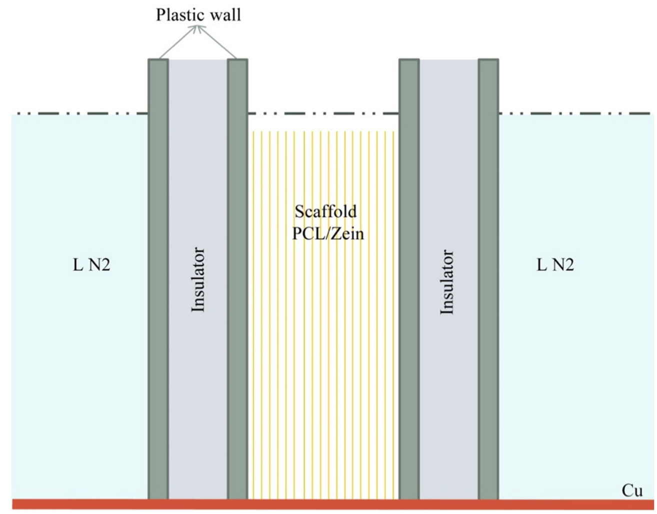

Fig. 1. Schematic representation of the isolated system used for preparation of composite scaffolds.

2.2. Characterization of PCL/zein composite scaffolds

2.2.1. Surface morphology

Macroscopic cross-sections of the scaffolds, which were cut using a razor blade, were observed and studied under light microscope (LEICA M50) attached to a camera (LEICA IC80 HD). Samples were then sputter coated with a thin layer of gold and studied under an accelerating voltage of 10 kV using a LEO 435VP scanning electron microscope (SEM). The pore size distribution of the scaffolds was quantitatively measured using SEM images by Image J software.

2.2.2. Structural analysis

Attenuated total reflection-FTIR (ATR-FTIR) spectra of different types of scaffolds were recorded on Tensor 27 FTIR spectrometer in transmittance mode in the mid infrared region (4000–400 cm^{-1}). The phase structural analysis of scaffolds was also examined by X-ray diffraction (XRD: Philips diffractometer, 40 kV, Cu Kα) analysis over the 2θ range from 5° to 70° using a time per step of 1 s and a step size of 0.02°.

2.2.3. Porosity

The porosity of the scaffolds was calculated according to the following formula:

$$\text{Porosity} = \frac{V_t - (V_{PCL} + V_{zein})}{V_t} \times 100 = \frac{V_t - \left(\frac{W_{PCl}}{\rho_{PCL}} + \frac{W_{zein}}{\rho_{zein}}\right)}{V_t} \times 100 \quad (1)$$

where V_t is the total volume of scaffold (cm^3), V_{PCL} and V_{zein} are the actual volumes taken by PCL and zein (cm^3), W_{PCL} and W_{zein} are the masses of PCL and zein (g), and ρ_{PCL} and ρ_{zein} are the densities of PCL (1.145 g/cm^3) [34] and zein (1.22 g/cm^3) [28,34], respectively.

2.2.4. Mechanical testing

The compressive strength of prepared composite scaffolds was measured using a Zwick/RoellZ050 mechanical tester equipped with a 50 N loading cell at across head speed of 0.5 mm/min. The cylindrical samples were 10 mm in diameter and 10 mm long. During compressive strength test, the load was applied until the strain reached 80%. The compressive strength was determined from the maximum value of the stress–strain