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Fabrication and characterization of poly(γ-glutamic acid)-graft-chondroitin sulfate/polycaprolactone porous scaffolds for cartilage tissue engineering

Kuo-Yung Chang [a], Li-Wei Cheng [a], Guan-Huei Ho [b], Yun-Peng Huang [b], Yu-Der Lee [a,*]

[a] Department of Chemical Engineering, National Tsing Hua University 101, Section 2, Kuang Fu Road, Hsin Chu 300, Taiwan, ROC
[b] Vedan Enterprise Corporation, 65, Hsin-An Road, Shalu, Taichung 433, Taiwan, ROC

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Abstract

The development of blended biomacromolecule and polyester scaffolds can potentially be used in many tissue engineering applications. This study was to develop a poly(γ-glutamic acid)-graft-chondroitin sulfate-blend-poly(ε-caprolactone) (γ-PGA-g-CS/PCL) composite biomaterial as a scaffold for cartilage tissue engineering. Chondroitin sulfate (CS) was grafted to γ-PGA, forming a γ-PGA-g-CS copolymer with 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) system. The γ-PGA-g-CS copolymers were then blended with PCL to yield a porous γ-PGA-g-CS/PCL scaffold by salt leaching. These blended scaffolds were characterized by ^{1}H NMR, ESCA, water-binding capacity, mechanical test, degradation rate and CS assay. The results showed that with γ-PGA-g-CS as a component, the water-binding capacity and the degradation rate of the scaffolds would substantially increase. During a 4 week period of culture, the mechanical stability of γ-PGA-g-CS/PCL scaffolds was raised gradually and chondrocytes were induced to function normally in vitro. Furthermore, a larger amount of secreted GAGs was present in the γ-PGA-g-CS/PCL matrices than in the control (PCL), as revealed by Alcian blue staining of the histochemical sections. Thus, γ-PGA-g-CS/PCL matrices exhibit excellent biodegradation and biocompatibility for chondrocytes and have potential in tissue engineering as temporary substitutes for articular cartilage regeneration.
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Keywords: Articular cartilage; Poly(γ-glutamic acid); Chondroitin sulfate; Poly(ε-caprolactone)

1. Introduction

Articular cartilage is a particular tissue that lacks a blood supply to support repair and remodeling. It has limited capacity to restore defects that are caused by joint injury and aging. Cartilage tissue engineering offers an alternative method for treating arthritis: it involves culturing the chondrocytes in vitro in a scaffold and then implanting the resulting tissue-engineered construct back into the patient. A biodegradable scaffold could be adopted as a template to transfer the chondrocytes to a cartilage defect and to offer temporary mechanical support and bioactive substances to the cells during the period of proper tissue regeneration.

Poly(γ-glutamic acid, γ-PGA), a biodegradable and non-toxic biomaterial, is produced by microbial fermentation [1,2]. It is made of D- and L-glutamic acid units that are connected by amide linkages between the α-amino and γ-carboxylic groups [3]. Its physical factors are greatly affected by humidity and have high hydrophilic property and excellent water-binding capacity. During the past decade, γ-PGA has been used as a material with fine swelling ability and biocompatibility, which makes it suitable for use in such clinical fields as bioglue [4–6], drug delivery systems [7–10] and tissue engineering [11–13]. Chondroitin

* Corresponding author. Tel.: +886 3 5713204; fax: +886 3 5715408.
E-mail address: ydlee@che.nthu.edu.tw (Y.-D. Lee).

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sulfate (CS) is one of the glycosaminoglycans (GAGs) that exist in cartilage and skin. It associates with collagen in connective tissue and tendons in animals [14]. CS proteoglycan involves in intracellular signaling, cell recognition and the connection between ECM components and cell-surface glycoproteins [15]. However, the high solubility of CS in water is disadvantageous. To overcome this shortcoming, chemical cross-linking methods are utilized to reduce the solubility of this natural polysaccharide in water [16].

Biodegradable polyesters are widely employed in tissue engineering because they typically have good strength and an adjustable degradation speed [17–19]. Poly(ε-caprolactone) (PCL) prepared from ε-caprolactone has received FDA approval for several clinical applications in humans. They are applied in medical implants with a temporary mechanical or therapeutic function [20–24]. However, the poor hydrophilicity of this polymer is the main limitation to its use as a scaffold [25] as well as the fact that it has no cell recognition site and, therefore, poor cell affinity. Furthermore, PCL does not have any active functional groups on its backbone except the hydroxyl groups at the chain ends. Therefore, numerous researchers recently have investigated the blending methods for combining hydrophilic natural biomacromolecules and hydrophobic polyesters, such as chitosan/PCL [25–27], starch/PCL [28], collagen/PCL [29], gelatin/PCL [30], gelatin/PLLA [31], chitosan/PLA [32,33] and hyaluronic acid/PLGA [34]. In this study, γ-PGA-g-CS/PCL matrices were designed to serve as cell carriers for the construction of tissue-engineered cartilage. PCL is critical to provide a physical support to the scaffold while the γ-PGA-g-CS copolymer serves as a substrate that regulates cellular growth and function.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL, $M_w = 80{,}000$) was purchased from Acros. Chondroitin sulfate (CS, $M_w = 50{,}000$) was purchased from Calbiochem. γ-PGA ($M_w = 470{,}000$, $M_w/M_n = 1.82$, GPC analysis) was kindly supplied by the Vedan Enterprise Corp. (Taiwan) and was used without further purification. 4-Dimethylaminopyridine (DMAP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 1,6-hexanediamine were purchased from Sigma–Aldrich.

2.2. Preparation of γ-PGA-g-CS copolymer

Chemical grafting of the γ-PGA-g-CS copolymer was prepared with EDC system [13]. Briefly, γ-PGA (0.5 g) and DMAP (0.047 g) were dissolved in 18 ml of DMSO (TEDIA) by ultrasonication. The solutions were then mixed with 1 ml of CS solution (0.89 g, in deionized water). The CS solution was mixed dropwise to the γ-PGA/DMAP solution and an EDC solution (0.55 g, in 2 ml of DMSO) was then added at room temperature. After 3 h, the mixed solution was then poured into excess amount of acetone to produce the precipitates. The precipitates were then dissolved in phosphate-buffered saline (PBS) and dialyzed with a membrane (spectrum, MWCO: 100,000) for two days to remove the ungrafted CS. After it had been lyophilized until dryness, the sample was dissolved in 10 ml of deionized water solution and excess 1,6-hexanediamine (0.495 g) was then added at room temperature for 24 h to modify the polymer. The solution was further dialyzed with a membrane (spectrum, MWCO: 3500) for two days to remove the unreacted 1,6-hexanediamine. Finally, the product was lyophilized until dry. The preparation methods of γ-PGA-g-CS copolymer are illustrated in Scheme 1.

2.3. Fabrication of γ-PGA -g-CS/PCL scaffolds

To prepare a 3D porous scaffold by blended materials, a suitable method and concentration are important [31,34]. Table 1 shows the composition and the γ-PGA-g-CS content of various scaffolds. First, γ-PGA-g-CS was dissolved in a co-solvent (deionized water:DMSO = 1:1) and it was then added dropwise to an equivalent volume of chloroform with fast stirring. After PCL had been added and dissolved, the solution was then mixed with 90 wt.% sodium chloride (250–420 μm). The mixtures were poured into a Teflon mold and were then rapidly frozen at −20 °C. They were lyophilized to dryness, before being washed in a solution (deionized water:methanol = 1:2) to remove all of the sodium chloride. Finally, the resulting scaffolds were lyophilized until dry again.

2.4. Characterizations

The γ-PGA-g-CS copolymers were characterized via NMR spectra recorded on UNIYTINOVA-500 NMR (VARIAN) in co-solvent (D_2O:DMSO-d_6 = 1:1) with tetramethylsilane (TMS) as an internal standard. Complex scaffolds are generally characterized using electron spectroscopy to analyze the chemical composition (ESCA, Physical Electronics, Auger 670 PHI Xi/ESCA PHI 1600). Functional groups of the scaffolds were identified by the high-resolution peak analysis of the carbon 1s (C 1s) envelopes. Additionally, the internal structures of the produced scaffolds were examined by a scanning electron microscope (SEM) (JSM-5600, JEOL). The scaffolds were freeze-dried and then coated with an ultrathin layer of gold for examination.

2.5. Quantitative analysis

The chondroitin sulfate content of the γ-PGA-g-CS copolymers and γ-PGA-g-CS/PCL scaffolds were determined by 1,9-dimethylmethylene blue (DMB, Sigma) staining [35]. Briefly, the matrix was immersed in a 40 μl of degradable buffer for 24 h at room temperature and then

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