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In vitro and *in vivo* test of PEG/PCL-based hydrogel scaffold for cell delivery application

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Abstract

Biodegradable elastic hydrogel scaffolds based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(ε-caprolactone) (PCL) were fabricated and investigated as a delivery vehicle of rabbit chondrocytes for the formation of neocartilage. The diacrylated forms of PEG and PCL were used as building blocks to prepare a series of hydrogel scaffolds with different block compositions and, thus, different physico-chemical properties. The porous hydrogel scaffolds were prepared by using the salt leaching method that is generally used for the creation of porous scaffolds, and their *in vitro* cell interactions were examined using chondrocytes. The hydrogel scaffold with a relatively high PEG content showed better cell growth for chondrocytes, while the scaffold with a relatively low PEG content showed lower chondrogenic differentiation. It was observed that different kinds of scaffolds and rabbit chondrocytes were shown to have different swelling ratios in the scaffold for effective cell growth and tissue regeneration. RT–PCR results for the resultant cartilage tissue revealed that a PEG–PCL ratio of 14 to 6 scaffold was optimal for cartilage tissue formation in terms of collagen Type II, aggrecan, SOX9, and COMP gene expression. In addition, the hydrogel scaffold with a PEG–PCL ratio of 14 to 6 showed faster formation of new cartilage than those shown by other scaffolds.

Keywords: Poly(ethylene glycol) (PEG); Poly(ε-caprolactone) (PCL); Hydrogel scaffolds; Chondrocytes; Cartilage formation

1. Introduction

Porous biodegradable polymeric scaffolds have been widely used for regeneration of cell-based artificial organs. Specially in cartilage engineering, scaffold served as the matrices of tissue formation plays a pivotal role, and has to fulfill a few basic requirements, that is, high porosity and proper pore size, required surface properties permitting cell adhesion, differentiation and proliferation, desirable mechanical integrity to maintain the predesigned tissue structure, non-cytotoxicity and osteoconductivity [1–3].

Numerous investigations, including scaffold fabrication [4], surface modification [5–7], and a bioreactor system [8], have been actively conducted for the development of the scaffolds, which can provide a desirable environment for cell growth. In particular, a great deal of research effort has been expended recently for repairing articular cartilage lesions by combining biodegradable scaffolds with chondrocytes [5–9]. In these investigations, a small population of articular chondrocytes was extracted from each patient, cultivated at a large scale, and seeded within porous biodegradable polymeric scaffolds. The resultant cell/scaffold construct was implanted back into the defect site for the regeneration of articular cartilage. In this plan for cartilage tissue engineering, the scaffold plays a pivotal role in dictating cell adhesion, proliferation, and differentiation for expressing desirable phenotypes. The scaffold must have an open, porous structure for sufficient cell seeding

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and for facilitating mass transfer of oxygen and nutrients. These scaffolds were fabricated by incorporating ammonium bicarbonate salt particles as a gas foaming salt porogen into a gel-like PLGA phase precipitated in an organic solvent [10–12]. Upon contacting this salt-PLGA mixture with a citric acid solution, macroporous scaffolds with highly interconnected pore structures could be obtained [13–15]. These scaffolds exhibited very high cell seeding density, as well as a homogeneous cellular distribution.

The aim of this study was to fabricate biodegradable elastic hydrogel scaffolds, of which properties such as biodegradability, swelling property, elasticity, and hydrophilicity can be easily modulated by simply changing the synthetic parameters, which include block composition, ratio, and other factors. The diacrylated forms of hydrophilic poly(ethylene glycol) (PEG) and biodegradable poly(ε-caprolactone) (PCL) were used as building blocks for the synthesis of a series of hydrogels [16–19]. Our previous study showed that PEG/PCL-based hydrogels could be useful for applications in drug delivery and tissue engineering due to several promising properties, such as elasticity, degradability, and other tailor-made properties [20]. This study investigated a porous hydrogel scaffold mixed with differentiation materials as a three-dimensional (3-D) culture for the macro-encapsulation of chondrocytes in conditioned media. The hydrogel scaffold can help to penetrate the inner state of the scaffold [21–23], which prevents the dedifferentiation of chondrocytes when they are implanted into the body [24–26]. Moreover, essential factors that are helpful for enhancing chondrogenic differentiation are also easily loaded into the inner state of the scaffold when they are mixed with chondrocytes. In this study, we hypothesized that the PEG/PCL-based hydrogel scaffold can offer a suitable environment for the retention of the chondrocytic phenotype, and can allow the synthesis of mechanically functional cartilage of the extracellular matrix (ECM) for cell therapy.

2. Materials and methods

2.1. Materials

Polycaprolactone diol (PCL diol, Mn=1250), benzene (anhydrous grade), acryloyl chloride, triethylamine, dimethyl sulfoxide (DMSO, anhydrous grade), and PEG diacrylate (PEG–DA, Mn=700) were purchased from Sigma-Aldrich. 2,2′-Azobisisobutyronitrile (AIBN) was obtained from JUNSEI Chemicals (Japan) and used after purification by recrystallization in methanol. Sodium chloride powder (size distribution: 180–400 μm, 99%) was purchased from Samchun Pure Chemical. The other chemicals were of reagent grade, and were used as received. Dulbecco's modified Eagle's medium (D-MEM low and high), fetal bovine serum (FBS) and pen-streptomicin were from GIBCO BRL, Life Technologies (Grand Island,NY); 3–(4,5–Dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide (MTT), Trypsin–EDTA and dexamethasone (Dex) were from Sigma Chemical Co. (St. Louis, MO, USA); recombinant transforming growth factor-β3 was from R&D Systems (Minneapolis, MN); ITS-plus Premix was from BD Biosciences (Bedford, MA); and anti-collagen Type I and Type II were from Chemicon International Inc. (Temecula, CA, USA).

2.2. Diacrylation of PCL

A predetermined amount of PCL diol (5 g, 4 mmol) was dissolved in anhydrous benzene. Triethylamine (1.01 g, 10 mmol) and acryloyl chloride (0.91 g, 10 mmol) were added to the solution, and the mixture was stirred for 3 h at 80 °C. Triethylamine hydrochloride formed as a reaction by-product in the reaction solution and was filtered off. The filtrate was precipitated in an excessive amount of cold *n*-hexane. The resulting product was collected and dried in a vacuum oven for 24 h (Yield ~87%).

2.3. Preparation of PEG–PCL hydrogel scaffolds

The predetermined amounts of PCL–DA and PEG–DA were dissolved in 5 ml of DMSO and placed into 15 ml of polypropylene conical tubes containing 7 g of sodium chloride salt particulates (size distribution: 180–400 μm, 99%). The feed ratio between PCL–DA and PEG–DA was varied from 7:3 to 3:7, but the total polymer concentration was fixed to 20 wt.%. After the addition of a small amount of AIBN, the reaction solution was placed in a convection oven maintained at 70 °C for 12 h. The resultant hydrogel was removed from the tube and cut into discs with thicknesses of 3 mm. The hydrogel samples were immersed in distilled water to dissolve the salt, and were then immersed in ethyl alcohol to remove any residual chemicals. Finally, the scaffolds were washed with distilled water several times and freeze-dried for 2–3 days.

2.4. Characterization

The reaction for diacrylation of PCL diol was confirmed by ^{1}H–NMR (JNM-AL400 spectrometer, JEOL Ltd, Akishima, Japan) and Fourier transform-infrared (Nicolet, USA) measurements. To measure the swelling ratio, the disk-typed hydrogel samples were immersed in distilled water. After reaching the equilibrium swelling state, the excessive water on the surface was removed by tapping with filter paper. The weight swelling ratio (Sr) was calculated from the equation, Sr=Ws/Wd, in which Ws and Wd are the weights of swollen and dried scaffolds, respectively. The contact angle of the hydrogels was measured (DSA100, KRÜSS). The PEG–PCL hydrogels with the same chemical compositions but without porous structure were prepared in the film type and used for contact angle measurements because the porous hydrogels could not be measured due to their tendency to absorb water instantaneously through pores on the surface. The morphologies of the scaffolds were measured by a scanning electronic microscope (S-2460N, Hitachi, Tokyo, Japan). Cross-sections of PEG–PCL scaffolds were mounted onto aluminum studs and sputter-coated with gold.

2.5. Chondrocyte isolation and cell culture

Chondrocytes were isolated from White New Zealand rabbit knee articular cartilage by collagenase digestion [27]. In brief, female rabbits weighing 250 g were sacrificed by an overdose of Nembutal. The non-fibrillated articular cartilage of the knee was removed by sterile dissection. The cartilage was finely minced, suspended in calcium-and magnesium-free phosphate-buffered