Biodegradable Poly (Ester) Micro-Particle Co-Incorporating Growth Factor and Dexametason in Scaffolds for Neocartilage Formation

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(Received: October 18th, 2010; Accepted: October 25th, 2010)

Abstract: Chondrogenic differentiation is a complex process by stimulation of several kinds of factors. We have evaluated the dexamethasone (DEX) and transforming growth factor-β3 (TGF-β3) loaded/coated on small sized micro-particle integrated porous scaffolds embedded with rabbit bone marrow stromal cells (rBMSC). DEX and TGF-β3 loaded/coated micro-particles integrated in the porous scaffolds significantly increased the chondrogenesis of BMSC comparing to the other constructs. To determine the release profiles of growth factor and DEX from micro-particles embedded in porous scaffolds, we evaluated the growth factor and DEX release profiles from scaffolds and confocal laser microscopic views of scaffolds integrated with TGF-β3-coated micro-particles containing DEX. RT-PCR results for the resultant cartilage tissue revealed that porous scaffolds with TGF-β3 3-coated micro-particles containing DEX was suitable scaffolds in that collagen Type II and COMP gene expression was actively released from transplanted rBMSCs. In addition, the fact that the porous scaffold with TGF-β3 3-coated micro-particles containing DEX showed faster formation of new cartilage than those shown by other scaffolds certified the results of histology and immunochemical histology. These results suggest that the porous scaffold including TGF-β3 3-coated micro-particles containing DEX is a promising candidate scaffold for cell-based tissue engineering.

Key words: TGF-β3, dexamethasone, porous scaffolds, BMSC, cartilage formation

1. Introduction

The scaffold-based tissue engineering approach has become an important method of cartilage repair and regeneration. It is based on the use of polymeric scaffolds for mechanical support and tissue guidance, and in some cases, as carriers for growth factors or specific drugs to accelerate tissue healing when used in vivo.1, 2 The polymeric materials used for tissue engineering, either synthetic or natural, must be able to perform in the presence of appropriate host responses, and must be biodegradable so that the materials can be broken down and absorbed by a biological system.3 Furthermore, the three-dimensional (3-D) scaffolds derived from the biomaterials for tissue engineering require 3-D structures that serve as support for initial cell attachment and subsequent tissue formation suitable to fulfill their in vivo functions.

Our laboratory has developed a poly(lactic-co-glycolic acid) (PLGA) microsphere-based approach to create scaffolds with a porous interconnected structure for cartilage tissue engineering.4 This technique is based on surface modification of PLGA microspheres using heparin nanoparticles. In this method, a novel 3-D microsphere scaffold was fabricated by biocompatible surface modification.

In addition, the presence of signaling molecules such as growth factors (GFs) within extracellular matrix (ECM)-mimicking scaffolds is also critical for tissue repair, guidance, and development.5-7 This suggests a possible solution that could be used to reproducibly reduce the time needed to expand the chondrogenic differentiation, even when isolated from elderly individuals.

Biodegradable PLGA-based microspheres have already demonstrated their potential use as specific drugs in tissue equivalents.8-10 Specifically, glucocorticoids have been demonstrated to promote the differentiation of chondrocytes and maintain the integrity of the cartilaginous matrix in isolated primary cell populations, as well as in mesenchymal stem cells.11-14 Some researchers have been focused on dual drug and proteins or genes loaded/coated in and on the microspheres for both cell delivery and cell differentiation simultaneously.15-17
However, this methods showed some limitation for safe cell delivery due to their small space encapsulating the cells.

In this study, dexamethasone, either alone or in combination with TGF-β3, is well-investigated among the chondrogenic inducers of rabbit mesenchymal stem cells in vitro and in vivo. Porous tissue-engineering scaffolds have been developed to serve as vehicles for the delivery of drugs or bioactive factors that can direct cellular responses within or around the scaffolds. However, the factors controlling drug and protein release within microsphere-integrated scaffolds have not yet been fully elucidated. In the case of small-sized microsphere-loaded in porous PEG-PCL scaffolds, the chemophysical properties of the template (e.g. hydrophilicity, composition) may also affect the release kinetics and should be properly taken into account. Furthermore, the knowledge of protein concentration gradients realized within the scaffold is crucial for the effective direction of neo-tissue formation. Therefore, a rational design of tissue engineering constructs based on the combined use of microspheres and scaffolds requires the evaluation of TGF-β3 and DEX release profiles directly within the polymeric template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template. The attachment, proliferation, and differentiation of rMSCs were evaluated according to the release profile of TGF-template.

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 (99%) was purchased from Samchun Pure Chemical. The other chemicals were of reagent grade, and were used as received. Alpha-minimum essential medium (α-MEM), fetal bovine serum (FBS), and pen-streptomycin were obtained from GIBCO BRL, Life Technologies (Grand Island,NY); dexamethasone (DEX) were from Sigma Chemical Co. (St. Louis, MO, USA); recombinant transforming growth factor-β3 was from R&D Systems (Minneapolis, MN); and anti-collagen Type I and Type II were from Chemicon International Inc. (Temecula, CA, USA).

2.2 Conjugation of TGF-β3 to Cy5.5 and Dexamethasone to FITC

Cy5.5 labeling was carried out by following the manufacturer’s instruction. TGF-β3 (1.44 mg, 58 nmol) was washed with 3 mL sterile 0.15 M NaCl solution by using Centricon (MWCO 10,000). Finally, TGF-β3 was harvested in 1 mL 0.1 M sterile NaHCO3 buffer. Stock solution of Cy5.5-NHS (GE Healthcare) was prepared in 1 mL anhydrous dimethyl sulfoxide (1 mg/mL) and 0.9 mg (290 nmol) Cy5.5-NHS was added into TGF-β3 solution. Reaction was performed for 30 min at -4°C. During reaction, solution was briefly agitated in every 10 min. By using Centricon (MWCO 10,000), labeled TGF-β3 solution was repeatedly washed with 2 mL of 0.15 M NaCl solution to eliminate ionic interaction between Cy5.5 and TGF-β3 till no Cy5.5 in the filtrate was detected by UV spectrophotometer (675 nm). Finally, Cy5.5-labeled TGF-β3 was collected 1 mL sterile PBS (pH 7.4). By measuring UV absorbance at 280 and 675 nm, dye-to-protein (D/P) ratio could be calculated (0.26). The extent of modification was calculated as a ratio of the integral peak intensity at a wavelength of 216 nm (for protein) and 598 nm (for Cy5.5).

DEX (100 nmol) and dibutyltin dilaurate (5 μL) were dissolved in 2 mL anhydrous DMF. TRITC (120 nmol) dissolved in 1 mL anhydrous DMF was added into dexamethasone-containing solution and reaction was carried out for 4 hr at 60°C. After cooling at RT, the solution was repeatedly precipitated against 50 mL diethyl ether. Orange-colored precipitant was dried under vacuum with P2O5 for 2 days. Reverse phase-HPLC (Agilent 1100 series; 240 nm photodiode array detector; Waters Symmetry C18 3.9×150 mm column, 5 μm pore size; binary gradient of H2O and acetonitrile from 20% H2O to 60% H2O for 20 min) confirmed that the TRITC-labeled dexamethasone had more than 99% purity. Dexamethasone showed a sharp peak at 13.1 min, while TRITC presented a broad peak from 15.5 min to 18.5 min. TRITC-labeled dexamethasone had a sharp peak at 16.1 min as showed below. Because of partial solubility of TRITC and dibutyltin dilaurate in ethyl ester, triple precipitations removed most of impurities. In addition, due to the excess amount of TRITC, remaining dexamethasone was not observed in HPLC chromatogram, which means that almost all dexamethasones were converted to TRITC-labeled dexamethasone.

2.3 Preparation of Dexamethasone (DEX)-Loaded PLGA Microspheres

The small-sized microspheres were prepared using a water-in-oil-in-water solvent evaporation technique. In brief, 2 mg of dexamethasone were emulsified with 1 mL of methylene chloride containing 200 mg of PLGA by sonication for 30 s (sonifier: Bandelin electronic UW 70/HD 70, tip: MS 72/D, Berlin, Germany). After the addition of 3 mL of a 2% (w/v)