CONTROLLED RELEASE OF TRANSFORMING GROWTH FACTOR-BETA1 ENHANCES CHONDROGENESIS OF COLLAGEN/CHITOSAN/GAG SCAFFOLD

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INTRODUCTION: Transforming growth factor-β1 (TGF) has been reported to influence the proliferation of chondrocytes and also stimulate proteoglycan synthesis. However, this chondrocyte’s response to TGF can be achieved through prolonged exposure to TGF. Therefore, a focal application of controlled releasing system remains attractive for enhancing chondrogenesis of tissue engineered constructs. Porous collagen based scaffold has a great potential in cartilage tissue engineering. Chondroitin sulfate (CS), a component of glycosaminoglycans (GAG) present in articular cartilage, and chitosan similar with various GAG are also used as biomaterials available to stimulate chondrogenesis. In this study, in order to develop a novel tissue engineered cartilage, TGF loaded chitosan microspheres were incorporated into the porous collagen/chitosan/CS scaffolds. In addition, we evaluated whether TGF loaded chitosan microspheres based delivery systems modulate controlled release of TGF in a manner that favorably influence chondrocyte’s chondrogenesis.

METHODS: Microsphere preparation: Coarse emulsions were prepared by homogenizing mixtures of the oil phase and the aqueous phase containing chitosan (MW 100,000, Synyoung Chitosan Co., Korea) and TGF (Sigma Chemicals Co.) at 13,500 rpm for 10 min. The coarse emulsions were crosslinked by 10% tripolyphosphate and then freeze-dried. Surface and size of microspheres were examined using a scanning electron microscope (SEM). In vitro TGF release tests were performed using an enzyme linked immunosorbent assay. Scaffold fabrication: Blended solutions of collagens (5 mg/ml) and chitosan (10 mg/ml) were mixed in the ratio of 5:5 (collagen:chitosan, v:v). This mixture was lyophilized and then cross-linked using 33 mM of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Fluka) in the presence of 1% (w/v) CS (Sigma Chemicals Co.). The TGF loaded microspheres were incorporated into the fabricated collagen/chitosan/CS scaffold (10,000 TGF/scaffold). Two types of scaffolds were prepared: i.e. TGF-microsphere incorporated (Scaffold-TGF) and non-incorporated scaffold (Scaffold). Cross-sectional views of these scaffolds were observed using a SEM. In vitro cell culture: Rabbit chondrocytes (PI) were seeded in each scaffolds (7 mm x 3 mm) at a cell density 3x10⁶ cells/scaffold using a syringe under weak negative pressure. Cell seeded scaffolds were cultured for 1, 3, 7, 14 d and evaluated by cell attachment, proliferation, and GAG synthesis. Attached cell number was assayed by MTS assay, proliferation rate by 3H-thymidine uptake, and synthesized GAG content by 35Sulfate incorporation. Histological assessment was performed using a Safranin-O staining for proteoglycan and an immunohistochemistry for collagen type II. Statistical analysis: Statistically significant differences were determined by Student’s t test.

RESULTS: The fabricated scaffold was three-dimensionally porous with 195.7 ± 44.4 μm pore size and its interconnected network was observed (Fig. 1). TGF loaded microspheres and their incorporation into scaffold were observed in Fig 1. Controlled release of TGF from chitosan microspheres has been observed for 7 days (Fig. 2). The number of attached cells was higher in microsphere-incorporated scaffold. Through the culture period, TGF microsphere-incorporated scaffold increased the proliferation rate as well as the GAGs synthesis of cells compared to non-incorporated one (Fig. 3). The newly synthesized extracellular matrix around the chondrocytes was more prominent in TGF incorporated scaffold than non-incorporated one (Fig. 4).

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REFERENCES:

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