INTRODUCTION: The transplantation of an in vitro tissue-engineered cartilaginous implant is currently under consideration because of the loss of cells that occurs in the cell transplantation technique. The alginate-recovered-chondrocyte method (ARC method) was developed as a two-step scaffold-free culture system for the production of cartilaginous tissue in vitro [1]. The addition of the growth factor, osteogenic protein-1 (OP-1), to this system promoted the formation of cartilaginous tissue [2]. Furthermore, the transfer of the OP-1 gene to chondrocytes and intervertebral disc cells has proven to be effective in inducing the enhancement of proteoglycan (PG) metabolism [3, 4].

Particle-mediated gene transfer using gold particles, otherwise known as gene-gun-mediated gene transfer, has several advantages over viral-mediated methods, including increased safety, patient acceptance and simplicity of methodology, for future clinical trials. In addition, the gene gun system has made it possible to transfer genes directly to the target tissue in an extremely localized area. For example, the direct in situ gene transfection of a marker gene, β-galactosidase, to the surface of tissue-engineered cartilage tissue was possible using this system [5].

The purpose of this study was (i) to examine the effects of gene gun-mediated OP-1 gene transfection to tissue-engineered cartilage produced by the ARC method, and (ii) to evaluate the effectiveness of OP-1 gene transfection to induce changes in PG metabolism.

MATERIALS AND METHODS:
Cell culture and engineering of the ARC Tissue [2]: Bovine articular chondrocytes from metacarpophalangeal joints of 14-18 month old animals were isolated by sequential enzymatic digestion. The cells were encapsulated in 1.2% alginate beads at 4 million cells/ml and cultured in DMEM/F12 medium containing 20% FBS and supplements. After 10 days in culture, the cells with their cell-associated matrix (CM) were recovered by dissolving the alginate and seeded onto a culture insert (10 mm diameter).

Gene-gun-mediated gene transfer to ARC tissue: The human OP-1 gene expression vector with CMV promoter, pW24, and its vacant control vector were a generous gift from Dr John C. Lee (University of Texas Health Science Center at San Antonio). The gWiz β-gal with CMV promoter (Gene Therapy Systems) served as a reporter gene. The vectors were precipitated on gold particles (1 μm diameter) at a concentration of 2 μg DNA/mg gold. The tubing coated with the gold particles was cut into 0.5-inch segments and used as the “bullets” for the gene gun.

After two weeks in culture on the insert, gene transfection to the de novo formed ARC tissue was carried out using the Helius™ Gene Gun System (Bio Rad) with one shot (1 μg plasmid DNA) per each tissue. At the time of gene transfection, a pulse of high-pressure helium gas (150 psi) was released through the gold-coated tubing, accelerating the DNA-coated gold particles to penetrate target cells.

Assessment of transfection efficiency: Three days after the gene transfection, the expression of β-galactosidase was assessed by X-gal staining using an in situ β-galactosidase staining kit (Specialty Media). OP-1 protein in the culture media or in the tissue was measured with immunoblotting and sandwich ELISA using an anti-human OP-1 monoclonal antibody (R&D Systems) on day-3 after transfection.

Measurement of DNA, PG and collagen contents of the tissue constructs: At various time points (day-0, -21, and -42 after transfection), the tissues were collected and treated with papain at 60°C. The papain digests were analyzed for DNA and PG contents using the Hoechst 33258 dye and the DMMB methods, respectively [5]. The content of hydroxyproline, as a measure of collagen, was also measured using reverse phase HPLC after hydrolysis with 6M hydrochloric acid and derivatization with phenylisothiocyanate.

Statistical Analysis: The data are expressed as the mean ± standard deviation. Significant differences among the groups were assessed by one-way ANOVA and Fisher’s PLSD post hoc test.

RESULTS: When the engineered cartilage was transplanted with the gWiz β-gal vector using the gene gun transfer method, β-galactosidase positive cells were observed only in the surface layer of the ARC tissue (depth, approximately 40 μm, Figure 1, A). On day-3 after transfection of the OP-1 gene, human OP-1 protein was detected only in OP-1 gene transfected ARC tissue in both the culture media and tissue homogenate (media: 32.9 pg/tissue/24h; homogenate: 14.7 pg/tissue/24h). The level of OP-1 decreased with time and was not detectable on day-7. The thickness, wet weight, dry weight and DNA content of ARC tissue did not show any significant differences between control and OP-1 gene transfected groups at each time point. However, PG accumulation in the OP-1-gene transfected ARC tissue was significantly higher than that in the control group (Figure 2, A. +36%, p<0.01). The intensity of Safranin-O staining of the tissue was higher in the OP-1 group (Figure 1, B & C). Collagen accumulation in the OP-1 group tended to be higher than that of the control group, but the difference was not statistically significant (Figure 2, B).

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