INTRODUCTION:
Tissue engineering is a promising technique which has the potential to create tissues and organs de novo [1]. The prevalence and health care cost of low back pain associated with intervertebral disc (IVD) degeneration has prompted researches for engineering disc tissue to treat degenerated disc. Traditional treatment for patients with degenerative disc disease has focused on fusion of the painful motion segment. As an alternative to spinal fusion, tissue engineering of disc tissues have been reported in the literatures [2,3]. Various growth factors were known to stimulate matrix synthesis of IVD cells. Among those, transforming growth factor-ß1 (TGF-ß1), bone morphogenetic protein-2 (BMP-2) best candidates for regenerating IVD [4, 5]. Tissue engineering depends on optimal scaffold and biologic stimuli for cell amplification and retaining tissue specific phenotypes. In this regard, non toxic scaffold and certain biologic stimuli are essential for ultimate tissue engineering. Accordingly, the objective of this study was to examine the synthetic activity of IVD cells seeded on atelocollagen type I scaffold under the stimulation with TGF-ß1 and BMP-2.

MATERIALS AND METHODS:
Cell isolation: Lumbar IVD were harvested from New Zealand white rabbits, and the nucleus pulposus was separated from surrounding annulus fibrosus, and minced under aseptic conditions. Nucleus pulposus cells were isolated from tissue by sequential enzymatic digestion. Cells were passaged when confluence was reached. The medium was changed at three-day intervals. After 3 weeks in culture, cells were removed from flask with 0.05% trypsin and seeded into implants.

Production of atelocollagen scaffold: 56 µl of 1% type I atelocollagen (RBC I, Regenmed, Seoul, Korea) dispersion was poured into a 96 multiwell plate (diameter 5 mm), frozen at −70 °C, and then lyophilized at −50 °C. The fabricated porous collagen matrices were crosslinked in 50 mM of 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide (EDC, Sigma Chemical Co., St. Louis, MO, USA) solution (H2O-ethanol=5:95) for 24 hrs. The matrices obtained were washed in D.W using a sonicator, and then re-lyophilized at −50 °C. These matrices and methods were kindly supported to Ph.D. Park.

Cell Culture in Atelocollagen Type I Scaffold: Cell suspensions were imibed by surface tension into a scaffold consisting of atelocollagen type I. 4.4 x 10^5 cells per 96-well plate in 30ul DMEM/F12 containing 10% FBS, ascorbic acid(25ug/ml) and 1% antibiotics were seeded in each matrix. After incubation in 37 °C, 5% CO2 atmosphere for 4 hours, added the each culture media-5% FBS including TGF-ß1 of 10ng/ml, BMP-2A of 100ng/ml and the mixture both factors in the ratio of 1:1. Mixed medium was changed every 2 days for a week. All analyses were performed in triplicate, and each analytical condition was repeated three times using different cell preparations.

DNA Synthesis and newly synthesized Proteoglycan: DNA synthesis was measured by [3H] thymidine incorporation. Newly synthesized proteoglycan was measured by incorporation of [35S] sulfate in chromatography on Sephadex G25 in PD-10 columns. Scintillation count for [35S] sulfate was normalized by DNA synthesis. Reverse transcription-polymerase chain reaction for mRNA expression of collagen type I, II, aggrecan, osteocalcin: Total RNA was isolated by the RNeasy mini kit. cDNA was synthesized from 1ug of total RNA with the oligo(dT) priming method in a 50ul reaction mixture. Amplification reactions specific for the following cDNAs were performed: β-actin, collagen type I, collagen type II, aggrecan, and osteocalcin. PCR products were analyzed by electrophoresis in 2% agarose gels, and detected by staining with ethidium bromide. The intensity of the PCR products was quantified by TINA 2.0e.

RESULTS:
IVD cultures in atelocollagen type I with TGF-ß1 and BMP-2 demonstrated maximal synthetic response in proteoglycan synthesis 250% increase compared to control (Figure 1). Other cultures with TGF-ß1 or BMP-2 only demonstrated similar response to control. IVD cultures in atelocollagen type I scaffold with BMP-2 exhibited upregulation of type I collagen mRNA expression while those with TGF-ß1 showed upregulation of type II collagen and aggrecan mRNA expression. In any combination of growth factor, IVD cultures in atelocollagen type I did not exhibit upregulation of osteocalcin mRNA. Furthermore there is no synergistic effect of TGF-ß1 and BMP-2 in matrix synthesis and mRNA expression of matrix components.

DISCUSSION:
Nucleus pulposus cells from rabbit were viable in atelocollagen type I scaffold and biologically responsive to TGF-ß1 and BMP-2 in term of proteoglycan synthesis and expression of chondrogenic phenotypes i.e., collagen type II and aggrecan mRNA. Neither TGF-ß1 nor BMP-2 upregulated osteogenic phenotype i.e., osteocalcin mRNA in nucleus pulposus cells in atelocollagen type I scaffold with anabolic growth factors provide a mechanism for tissue engineering of intervertebral disc.

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ACKNOWLEDGMENT
This study was supported in part by Brain Korea 21 Project.