Human Intervertebral Disc Engineering with Atelocollagen and Scaffold and by Adenovirus-mediated Gene Transfer

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Introduction: Intervertebral disc (IVD) degeneration is a chronic process that the nucleus pulposus (NP) have been changed on account of multiple factors (malnutrition, mechanical stress and apoptosis, among others) leads to subsequent loss of proteoglycan (PG)-rich matrix. [1] Rather than traditional treatment, by the technique of tissue engineering, composite IVD implants are fabricated as novel materials for disc replacement and gene therapy is accounted to be a potential application in treatment of IVD disorders. Among artificial scaffolds which have bionic affinity, atelocollagen has an advantage in safety over others. [2] While adenovirus-mediated transfer of a therapeutic gene has been determined. [1] On the other hand, growth factors were known to stimulate matrix synthesis of IVD cells, among of them, transforming growth factor – beta1 (TGF-beta1) and bone morphogenetic protein-2 (BMP2) are the best applicant for IVD degeneration. IVD engineering by BMP-2 and TGF-beta1 using atelocollagen using rabbit IVD cell was attempted, nevertheless, human IVD cells and gene transfer approach was not tried before. Hence, In this study, we elucidated the effect of adenoviral TGF-beta1 and BMP-2 gene transfer to human IVD cells cultured in atelocollagen scaffolds.

Materials and Methods: Human NP Cell Isolation and Culture: Human disc tissues were collected during surgery with lumbar spinal stenosis. The NP (nucleus pulposus) cells were isolated from disc tissue by sequential enzymatic digestion and cultured. Cells were passaged when confluence was reached. Primary culture were sustained for 3 weeks in culture media (DMEM-F12 containing 10% FBS, 1% antibiotics, ascorbic acid ) in 5% CO2 at 37c incubator and media were changed twice a week.

Production of Atelocollagen scaffold: 56 μl of 1% type I Atelocollagen (Dalamtissene, Seoul, Korea) dispersion was poured into a 96 well plate (diameter 5 mm), frozen at ~70 c, and then lyophilized at ~50 c. The fabricated porous collagen matrices were cross-linked in 50 mM of 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (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 ~50c.

Cell transfection by Adenovirus vector: NP cells were plated in the 60mm culture dish at the density of approximately 7 x 105 cells per dish for adenovirus vector transfection. After being plated for over night, cells were treated with 150 multiplicity of infection (MOI) by Ad/TGF-beta1, Ad/BMP2 and mixture of Ad/TGF-beta1 and Ad/BMP2, and then all were cultured in 5% CO2 at 37c with humidity for 1 hour under gentle agitation. Subsequently, culture media were added to each dish, and the cells were further incubated in 5% CO2 at 37c with humidity for over night.

Transfected cell culture in Atelocollagen type I scaffold: Cell suspensions were imbibed by surface tension into a scaffold 1x105 cells per 96-well plate in 30ul culture media were seeded in the scaffolds. After incubation in 37c, 5% CO2 atmosphere for 4 hours, all the scaffolds were removed to the 48-well plate, and added culture media. The culture media was changed every 2 days.

Alamar blue assay for cell cytotoxicity assay: At first, prepared appropriate dilution of test agent in growth media and then add 250μl of each dilution of test agent to the wells. The cells were incubated for 2days, and also added 25μl of the indicator to each well. After for 3 hours incubation, read the panels at 570nm and 600nm.

Reverse transcription-polymerase chain reaction for mRNA expression of collagen type I, collagen type II, aggrecan and Msx2: 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 : beta-actin, collagen type I, collagen type II, aggrecan, osteocalcin and Msx2. 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.

Scanning electron microscopy(SEM): Acellar and cell scaffolds were observed at 3 and 5 days .Specimens for SEM were washed twice with sterile PBS and then fixed in 4% paraformaldehyde (w/v) for 2 days. The specimens were examined using a Hitachi 3500 scanning electron microscope (Hitachi, Tokyo, Japan) in secondary electron mode at 15.0kV.

Results: Results of Alamar blue test to cytotoxicity showed in human NP cell which transfected by Adenovirus was not cytotoxic. IVD cultures with Ad/BMP-2 in atelocollagen matrix exhibited upregulation of type I collagen mRNA expression while those with Ad/TGF-beta1 showed upregulation of type II collagen and mRNA expression. IVD cultures in atelocollagen matrices did not exhibit upregulation of Msx2 mRNA and aggrecan expression did not considerable changed. Furthermore there is no synergistic effect of Ad/TGF-beta1 and Ad/BMP-2 in matrix synthesis and mRNA expression of matrix components.

Discussion: Nucleus pulposus cells from human were viable in atelocollagen matrices and biologically responsive to Ad/TGF-beta1 and Ad/BMP-2 expression of chondrogenic phenotypes i.e., collagen type II and mRNA. Neither Ad/TGF-beta1 nor Ad/BMP-2 upregulated Msx2 phenotype i.e., and mRNA expression in human NP cells in atelocollagen. Taken together, nucleus pulposus cells in atelocollagen scaffolds with anabolic growth factor gene transfer provide a mechanism for tissue engineering of IVD.


Acknowledgements: This study was supported in part by Brain Korea 21 project and by grant No. R01-2006-000-10933-0 from the Basic Research Program of the Korea Science & Engineering Foundation.