Enhancement of cell proliferation and matrix production in human annulus cells under bioreactor culture
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INTRODUCTION
Low back pain (LBP) is the leading resource of physical disability and result in high cost of healthcare among people under 45 years of age. It has been shown by a lot of studies that LBP is closely related to the disc degeneration, and an efficient pain relief can be achieved after treatment of disc degeneration. The therapeutic strategy based on tissue engineering approach has received considerable attention during the past decade. Due to existence of nutrition and excretion deficiencies, various bioreactors have been designed as the effective alternatives of the conventional (static) three dimensional culture systems for tissue engineering. We hypothesize that a bioreactor culture will improve cell proliferation and matrix production of disc cells, thus beneficial to construction of the intervertebral disc tissue.

METHODS
We obtained human intervertebral disc samples from six individuals (age 13-16 years) who had undergone discectomy for surgical management of scoliosis at University of Virginia Hospital and followed the approved guidelines set by the US National Institutes of Health Office of Human Subjects Research for use of surgical waste. Annulus fibrosis (AF) cells was isolated by a collagenase/trypsin digestion procedure and cultured within alginate beads in a 50 mL high-aspect-ratio vessels (Synthecon, Houston), either under a rotating wall vessel (RWW) bioreactor (BIO) or being maintained statically as controls (STA). The medium was changed every 3 days, and the cultures were maintained for up to 21 days. Cellular mRNA levels of target genes were determined by real-time RT-PCR. Histological studies were performed by using frozen sections of alginate beads. Cells were released by a depolymerization solution (55 mmol/L sodium citrate, 30 mmol/L Na2EDTA, and 0.15 mol/L sodium chloride, pH 6.8) and the cellular glycogenaminoglycan (GAG), hydroxyproline (OHP) and DNA were measured colorimetrically and normalized to total DNA. Data from 4 repeats of either gene expression or biochemical assays are expressed as mean ± SD. Statistical evaluation between two groups was carried out by Student’s t test in a two-tailed way, and p values of less than 0.05 and 0.01 were considered significant and very significant, respectively.

RESULTS
Alginate matrix and cell-produced GAGs are stain in light and heavy red, respectively, while cells themselves are in blue color by Safranin-O stain (Fig. 1, upper panel). It is noticed that the matrix and cells are both stained in blue by hematoxylin stain. After 3 week-culture, cluster of cells can be found in beads in a rotating but not static vessel (Fig. 1, lower panel).

Fig. 1 Histology of human annulus fibrosis cells under bioreactor culture. Upper panel: Safranin-O stain, 1 week; lower panel: Hematoxylin stain, 3 weeks. CC: cluster of cells; SC: single cell. Original magnification: X 400.

By real time RT-PCR, it is revealed that under bioreactor culture cellular mRNA levels of matrix proteins including aggrecan (AGG), type I and II collagens (COL I and COL II) are up-regulated significantly, while those of matrix degrading enzymes matrix metalloprotease 3 (MMP-3) and disintegrin and metalloprotease with thrombospondin type I repeats 5 (ADAMTS-5) are remarkably down-regulated (Fig. 2a), in comparison to static culture. For other genes investigated, transcription of IL-1β, hypoxia-inducing factors 1 and 2 (HIF-1 and 2), sex-determining region of Y chromosome box containing gene 9 (Sox 9) and glucose transporter 1 (Glu-1) is significantly down-regulated, while proliferating cell nucleus antigen (PCNA) is up-regulated. No significant differences in mRNA levels of IL-1R and CBP/p300-interacting transactivator with ED-rich tail 2 (Cited 2) between BIO and STA groups are found (Fig. 2b).

Fig. 2 Gene expression of human annulus fibrosis cells by real time RT-PCR under bioreactor culture.

Surprisingly, biochemical analysis shows that cellular contents of GAG and OHP (representing collagen) after 3-week-bioreactor culture are unchanged and decreased remarkably, respectively (Fig. 3).

Fig. 3 Colorimetric assays of cellular GAG and OHP in 3-week-bioreactor-cultured human annulus fibrosis cells.

DISCUSSION
The gene expression experiment shows a potential of bioreactor culture in stimulating matrix production in human AF cells, however, the biochemical experiment reveals unchanged GAG and decreased collagen contents. Under bioreactor culture, the culture medium around each bead keeps fresh all the time, and it is obvious that transportation of biomolecules from alginate bead to culture medium is greatly improved and thus results in a sharp decrease in their cellular levels. That might explain the discrepancy between data from the gene expression analysis and biochemical assays in the present experiments. It is concluded that bioreactor culture is beneficial to human annulus fibrous tissue engineering due to the ability of stimulation of synthesis of matrix proteins, inhibition of synthesis of matrix degrading enzymes and enhancement of cell proliferation.

The knowledge about regulation of gene expression in the intervertebral disc remains limited. The fact that expression of HIF and their target genes are varied with culture condition also indicated that HIF may probably play an important role in the regulation of matrix proteins in human AF tissue which deserves further investigation.