INTRODUCTION
Disc degeneration is characterized in part by loss of proteoglycans. Growth factors such as TGF-β1 have demonstrated strong anabolic effects in stimulating matrix synthesis. Increased in-vitro proteoglycan synthesis has been reported in canine disc tissue following treatment with exogenous TGF-β1. Increased proteoglycan synthesis has also been achieved in a rabbit model following adenovirus-mediated therapeutic gene transfer (encoding human TGF-β1). Before either approach to the treatment of human degenerative disc disease can be recommended, the relative efficacy of exogenous TGF-β1 vs. gene therapy for increasing proteoglycan synthesis should be addressed in-vitro. Therefore, the purpose of this study was to compare the proteoglycan synthesis rates of human intervertebral disc cells cultured in three-dimensional alginate beads exposed to either exogenous growth factors (TGF-β1) or transduced by adenovirus encoding for production of TGF-β1.

MATERIALS AND METHODS
All of the experimental protocols were approved by the human subjects Institutional Review Board at the University of Pittsburgh. Lumbar and cervical intervertebral disc tissue was obtained from twenty patients (age range: 18 to 67 years) during surgical disc procedures performed for idiopathic scoliosis, disc herniation, and spinal stenosis. Two different adenoviral constructs were prepared: Ad/TGF-β1 (encoding human TGF-β1) and Ad/luciferase (encoding firefly luciferase). The preparation of disc cells in alginate beads was performed as previously described. Cell viability was determined by trypan blue exclusion test. The cultures were organized into four groups. In Group 1 (control), disc cells were treated with normal saline and incorporated into alginate beads. Group 2 was similar to Group 1, except for administration of exogenous TGF-β1 at 2ng/ml of medium at the beginning of the experiment. In Group 3 (viral control), cells were transduced with Ad/luciferase at a multiplicity of infection (MOI) of 75 in monolayer culture, and then were incorporated into alginate beads. Group 4 was similar to Group 3, except that Ad/luciferase was replaced with Ad/TGF-β1 at an MOI of 75. All cultures were incubated for 1 day after transduction for stabilization. Afterwards, alginate beads were cultured in a serum-free condition for 1 day and 3 days without change of medium. Cell counts for each group were performed at day 1 and 3 to enable normalization of the data. TGF-β1 concentrations in each group were measured sequentially by enzyme-linked immunosorbent assay. Newly synthesized proteoglycans were assessed by 35S-sulfate incorporation using chromatography on Sephadex G-25 in PD-10 columns. One-way analysis of variance with Newman-Keuls post-hoc test was conducted to compare the amount of newly synthesized proteoglycans. Significance level was set at p<0.05.

RESULTS
Initial cell viability upon isolation was 95% to 100%. Following gene transduction and incorporation procedures, cell viability remained high (90% to 95%). Figure 1 shows the concentration of TGF-β1 in culture medium vs. time following treatment with either exogenous TGF-β1 or Ad/TGF-β1. Note that TGF-β1 concentration decreased rapidly with time following administration of exogenous TGF-β1. In contrast, TGF-β1 concentration initially increased following treatment with Ad/TGF-β1, then remained constant for up to 3 days. Cells from the saline control and viral control groups produced only negligible (<0.05ng/ml) amounts of TGF-β1.

DISCUSSION
Three-dimensional culture of disc cells in alginate beads has a number of advantages compared to monolayer culture systems and also appears to be appropriate for elucidating the metabolism of genetically modified disc cells. In the present study, intervertebral disc cells were first transduced with adenovirus TGF-β1 construct, and then were successfully incorporated into alginate beads. The anabolic effects of gene transfer (encoding TGF-β1) and exogenous growth factor (TGF-β1) were then compared. The increase in the rate of proteoglycan synthesis was more pronounced in disc cells transduced with Ad/TGF-β1 than in cells treated with exogenous TGF-β1. This observation implies that genetically modified disc cells are able to produce and secrete endogenous TGF-β1 of sufficient quality and quantity to effect increased proteoglycan synthesis in-vitro. In summary, gene therapy proved to be an effective mechanism for increasing endogenous TGF-β1 production and upregulating proteoglycan synthesis in human intervertebral disc cells cultured in an alginate bead three-dimensional culture system.

REFERENCES

ACKNOWLEDGMENTS
The authors thank Dr. Savio L-Y. Woo, Dr. Freddie H. Fu, and Dr. Nam-Hyun Kim for their generous guidance and support of this study.