

# Transduced Bovine Articular Chondrocytes Affect the Metabolism of Cocultured Nucleus Pulposus Cells *In Vitro*: Implications for Chondrocyte Transplantation Into the Intervertebral Disc

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**Study Design.** Biologic study on the effects of coculture of bovine articular chondrocytes transduced *ex vivo* with genes expressing bone morphogenetic proteins (BMPs) on nucleus pulposus (NP) cells.

**Objective.** To evaluate the effects of bovine articular chondrocytes transduced with adenoviruses expressing various BMPs on proteoglycan and collagen production, and cellular proliferation of NP cells *in vitro*.

**Summary of Background Data.** Matrix synthesis by intervertebral disc cells is promoted by exposing the cells to growth factors or delivering genes that permit sustained expression of growth factors. We propose a novel therapeutic approach involving delivery of autologous chondrocytes, transduced *ex vivo* with bioactive proteins, to provide both the cells and proteins required to stimulate disc healing.

**Methods.** Adult bovine articular chondrocytes were transduced with adenoviruses (Ads) expressing either BMP-2, 4, 5, 7, 10, or 13 and plated as monolayers. Bovine NP cells encapsulated in alginate beads were cocultured, floating in the medium. Proteoglycan and collagen accumulation, and NP cell proliferation were measured after 6 days of coculture. As a positive control, beads were cocultured with articular chondrocytes in the presence of rhBMP-7.

**Results:** NP cells cocultured with articular chondrocytes transduced with BMPs-2, 4, 7, and 10 accumulated significantly ( $P < 0.05$ ) more proteoglycan than when cocultured with chondrocytes transduced with AdGFP (control) [AdBMP-2: 23.6%; AdBMP-4: 27.0%; AdBMP-7: 129.1%; AdBMP-10: 102.1% increases respectively]. Collagen accumulation was significantly ( $P < 0.05$ ) increased by NP cells cocultured with articular chondrocytes transduced

with BMPs-2, 4, 5, and 7. [AdBMP-2: 104.6%; AdBMP-4: 40.6%; AdBMP-5: 58.6%; AdBMP-7: 55.5% increases respectively]. NP cells proliferated when cocultured with articular chondrocytes transduced with AdBMP-2 and -7.

**Conclusions:** Bovine NP cells are stimulated to produce proteoglycans and collagen when exposed to chondrocytes transduced with genes for various BMPs. If applied to the treatment of disc degeneration, this strategy could provide the disc with not only metabolically active chondrocytes but also promote matrix replenishment by stimulating native NP cells.

**Key words:** disc degeneration, chondrocyte transplantation, bone morphogenetic protein, gene therapy. **Spine 2005;30:2601–2607**

Human intervertebral disc degeneration is a formidable clinical problem, and leading cause of spinal pain and disability.<sup>1</sup> Disc degeneration can cause low back pain and is also an early event in the spinal degenerative cascade.<sup>2</sup> Degenerated discs are characterized by an altered matrix composition and a reduced cell number.<sup>3,4</sup> Intervertebral discs have a limited intrinsic capacity for repair, so, to date, most treatments of symptomatic disc degeneration have revolved around pain-control measures or ablative spinal surgeries. An alternative biologic approach involves treatment of the disc by promoting matrix repair, which could slow or reverse the degenerative process,<sup>5</sup> with growth factors<sup>6</sup> or genes that allow for the sustained expression of growth factors.<sup>7</sup>

One strategy for disc repair involves the delivery of exogenous growth factors to promote proliferation of intervertebral disc cells and extracellular matrix synthesis.<sup>8</sup> In cultured intervertebral disc explants, exogenous growth factors have stimulated proliferation of intervertebral disc cells and extracellular matrix synthesis. A recent *in vivo* study has suggested that the direct intradiscal injection of recombinant human bone morphogenetic protein-7 (rhBMP-7) is able to, for some period, mitigate the deleterious effects of a disc puncture in a rabbit model.<sup>9</sup> However, the exogenous growth factors have short half-lives, limiting their potential usefulness for treating chronic conditions, such as degenerative disc disease. It seems unlikely that a single injection of a growth factor could permanently halt or reverse the intervertebral disc degenerative cascade in human beings, and a treatment strategy requiring repeat intradiscal injections may not be clinically acceptable. An

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alternate strategy for the delivery of growth factors to the disc involves the use of gene therapy techniques. These techniques make possible the sustained expression of the delivered growth factor; indeed, after gene transfer, transgene expression for up to 52 weeks in the rabbit intervertebral disc has been reported.<sup>7</sup>

*In vivo* gene therapy involves the direct delivery of a virus with the therapeutic gene into the disc. The effectiveness of this approach is dependent on transgene expression by native intradiscal cells. The paucity of metabolically active disc cells in degenerative discs may limit the effectiveness of *in vivo* gene transfer. *Ex vivo* or cell-based gene transfer involves the introduction of the bioactive factors into various cells *in vitro*, after which the cells are implanted at the desired regeneration sites. In the degenerated intervertebral disc, *ex vivo* gene transfer techniques have the advantage of providing a population of metabolically active cells expressing the relevant transgene. *Ex vivo* techniques are also considered safer and more effective than *in vivo* transfer because genetic manipulations occur outside of the patient's body.

Various cell types are available for *ex vivo* gene therapies. In the healthy nucleus pulposus, chondrocyte-like cells are the predominant cell type. It would be desirable to use, as the carrier of the gene of interest, cells with a similar phenotype. Chondrocytes are widely available and can be harvested from nonspinal sites with relatively low morbidity and risks.<sup>10</sup> There is also substantial clinical experience with autologous articular chondrocyte harvest, expansion, and then implantation to treat articular chondral defects in the knee and other joints.

We hypothesize that transplantation of autologous chondrocytes transduced *ex vivo* with bioactive proteins will provide both the cells and proteins required to stimulate intervertebral disc healing. Before proceeding to animal *in vivo* studies to test this hypothesis, this approach requires an understanding of the interactions between transduced chondrocytes and the native intervertebral disc chondrocyte-like cells, both of which would likely participate in any healing process. In this study, our specific goal was to evaluate, *in vitro*, the effects of coculturing bovine articular chondrocytes, which had been transduced with adenoviruses expressing 1 of 6 different BMPs, on proteoglycan and collagen accumulation, and cellular proliferation by nucleus pulposus cells.

## ■ Methods

**Viral Constructs.** Recombinant adenoviruses expressing human BMP-2, 4, 5, 7, 10, 13, and green fluorescent protein (GFP), as the control, were constructed using the AdEasy system developed by He *et al*<sup>11</sup> (Figure 1). The complementary deoxyribonucleic acid (DNA) clones for human BMP-2, 4, 5, 10, and 13 were kindly provided by the Genetics Institute (Cambridge, MA). The coding sequences for BMP-7 were amplified from a human osteosarcoma complementary DNA library with the use of the polymerase chain reaction technique. Specifically, the coding regions of the aforementioned BMPs were subcloned into pAdTrack-CMV, resulting in pAdTracker-BMPs. The resultant plasmids were linearized by

digesting with restriction endonuclease *PmeI* and, subsequently, cotransformed into *Escherichia coli*, with an adenoviral backbone plasmid, pAdEasy. Recombinants were selected for kanamycin resistance. Finally, the linearized recombinant plasmids were transfected into the adenovirus packaging cell line, 293.

**Cell Cultures.** Bovine intervertebral discs were dissected, as previously described,<sup>6</sup> from young adult bovine tails (15–18-months-old) obtained from a local slaughterhouse; the nucleus pulposus was isolated with a scalpel. Nucleus pulposus cells were first released by sequential enzyme digestion (0.4% pronase (Calbiochem, La Jolla, CA) for 1 hour followed by 0.025% collagenase-P (*Clostridium histolyticum*; Boehringer Mannheim, Indianapolis, IN) and 0.004% deoxyribonuclease II (DNase II; Sigma Chemical, St. Louis, MO) for 18 hours at 37°C, as previously described.<sup>6</sup> The isolated cells were suspended in 1.2% low-viscosity alginate (Keltone LV, ISP Alginate, San Diego, CA) in 0.15-M sodium chloride at 2 million cells/mL. The alginate beads containing the nucleus pulposus cells were cultured for 8 days in 10-cm plates with 20-mL complete medium (Dulbecco modified Eagle medium and Ham F-12 medium (DMEM/F12) (Mediatech, Herndon, VA), with 360 µg/mL L-glutamate (Mediatech), 50 µg/mL gentamicin (Gibco BRL, Grand Island, NY), and 25 µg/mL ascorbic acid (Sigma Chemical) supplemented with 20% fetal bovine serum (Hyclone, Logan, UT) before being transferred into wells containing articular chondrocytes, as described later.

Articular chondrocytes were released by sequential enzyme digestion (0.2% pronase for 1 hour and 0.025% collagenase-P for 18 hours) from young adult bovine metacarpophalangeal joints (15–18-months-old) obtained from a local slaughterhouse and then cultured in monolayer at 200,000 cells per 5 cm<sup>2</sup> well in 1.5-mL complete medium, as described previously, supplemented with 20% fetal bovine serum. The chondrocyte cultures were assigned to 9 groups (triplicate cultures in each group): cells only; rhBMP-7 (positive control); chondrocyte-adenovirus GFP (negative control); and chondrocyte-adenovirus BMP-2, 4, 5, 7, 10, and 13 groups. Adenovirus expressing BMPs were included in the cultures at plating for 16 hours at an optimized multiplicity of infection. On the second day, the medium was then replaced by fresh medium, and 9 alginate beads containing nucleus pulposus cells were added to each well, floating in the culture media. The cocultures were maintained for 7 days at 37°C with daily changes of medium.

To summarize, beads in the cells only group were cocultured with articular chondrocytes without any exogenous gene. Beads in the adenovirus GFP group were cocultured with articular chondrocytes transduced with adenovirus GFP, and beads in the adenovirus BMP group were cocultured with articular chondrocytes transduced with either adenovirus BMP-2, 4, 5, 7, 10, or 13. As a positive control, beads were cocultured with articular chondrocytes without any exogenous gene, in the presence of rhBMP-7 (also referred to as osteogenic protein-1), at 100 ng/mL for 1 day. After 7 days of coculture, beads were dissolved and, after digestion with papain, analyzed for contents of total sulfated proteoglycans and DNA, as described later. The cell-associated matrix accumulated by the nucleus pulposus cells was also visualized by the erythrocyte exclusion assay described later. Each experiment was repeated 3–4 times.

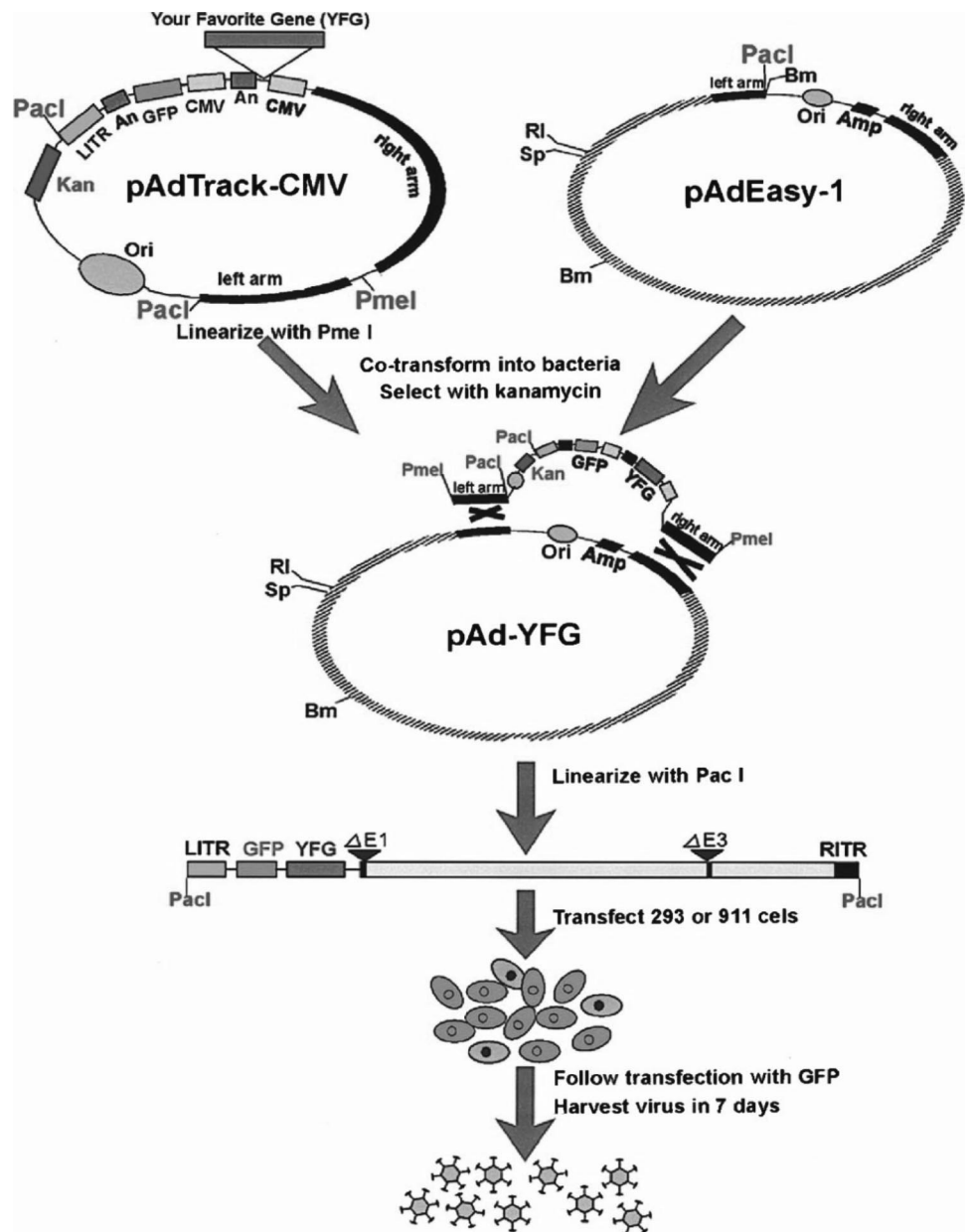


Figure 1. Adenoviral vector expressing BMPs. The coding regions of YFG (your favorite gene, in our case, human BMP genes) were cloned into pAdTrack-CMV. Resultant pAdTrack-BMPs were subsequently used to generate adenoviral recombinants, through homologous recombination with the adenoviral backbone vector, pAdEasy-1, in BJ5183 bacterial cells. The pAdEasy is an adenovirus 5 backbone vector containing all adenovirus 5 sequences except E1 and E3. The resulting adenovirus carries GFP and BMPs (YFG genes), but no E1 and E3 regions, which render it replication deficient. A recombinant adenoviral vector expressing GFP alone was also constructed as a control vector (adenovirus GFP). The adenoviral constructs were amplified by transducing 293 cells and were harvested 7 days following the transduction.

**Measurement of Proteoglycan Accumulation in Alginate Beads.** At the end of each culture period, alginate beads were collected, solubilized in dissolving buffer (0.055-M sodium citrate, 0.03-M ethylenediaminetetraacetic acid, 0.15-M sodium chloride, pH 6.8), and the cells and extracellular matrix digested with papain, as previously described.<sup>12</sup> The papain digests were analyzed for contents of total sulfated proteoglycan by the dimethyl methylene blue (Polysciences, Inc., Warrington, PA) dye-binding method. Alginate gives a minor positive reactivity with the dye, which is constant over a broad range of values. To circumvent this problem, alginate was maintained at a minimum concentration of 0.03% in all solutions.<sup>13</sup> Purified bovine nasal septum-D1 proteoglycan was used as a standard.

**Erythrocyte Exclusion Assay.** Briefly, horse red blood cells (MG Scientific, Buffalo Grove, IL) were fixed in 1.5% formaldehyde in calcium-free and magnesium-free phosphate buffered saline (PBS) overnight at room temperature, washed exten-

sively, and stored in PBS with antibiotics at 4°C.<sup>14</sup> Alginate beads were collected and dissolved. The cells with their cell-associated matrix, which had been recovered by centrifugation at 100 g for 5 minutes, were resuspended in DMEM, which was then replaced with a 750  $\mu$ L suspension of the fixed red blood cells ( $10^8$ /mL) in PBS containing 0.1% bovine serum albumin. The red blood cells were allowed to settle for approximately 10 minutes. The presence of a cell-associated matrix excludes the erythrocytes from adhering to the culture plate. The cells were visualized and photographed with an inverted phase-contrast microscope.

**Measurement of Hydroxyproline Content as an Indicator of Collagen Content.** Hydroxyproline, as a measure of collagen, was quantified by reverse-phase high-pressure liquid chromatography after hydrolysis with 6-M hydrochloric acid for 16 hours at 120°C and derivatization with phenylisothiocyanate.<sup>15,16</sup>

**Measurement of DNA Content as an Indicator of Cell Proliferation.** The papain digests of cells were analyzed for contents of DNA using the Hoechst dye method (Hoechst 33258; Polysciences, Inc.), as previously described.<sup>12</sup> Briefly, 100  $\mu$ L of the papain-digested samples were mixed with 1- $\mu$ g/mL Hoechst dye solution. The dye/sample complex was excited with ultraviolet light (wavelength 360 nm/L), and the emission at 460 nm was measured. Calf thymus DNA type I (Sigma Chemical) was used as a standard.

**Statistical Analyses.** Each experiment was repeated 3–4 times with triplicate cultures for each condition. Therefore, the sample number (n) is the number of experiments multiplied by 3 (representing triplicate cultures). The results are expressed as the mean  $\pm$  standard error of the mean (SEM). Analysis of variance was used to assess the effects of coculturing nucleus pulposus cells with articular chondrocytes transduced with various adenovirus BMPs on proteoglycan accumulation, collagen accumulation, and DNA content. The *P* values were obtained by the Student *t* test. The level of statistical significance was *P* < 0.05.

## ■ Results

### Proteoglycan Accumulation

Proteoglycan accumulation by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus expressing various BMPs were compared in this study (Figure 2). The total sulfated proteoglycan content of alginate beads at the end of the 15-day culture period represents the net amount of proteoglycan accumulated. Nucleus pulposus cells cocultured with articular chondrocytes transduced with BMP-2, 4, 7, and 10 accumulated significantly more proteoglycan compared to nucleus pulposus cells cultured with chondrocytes transduced with adenovirus GFP (control) (adenovirus BMP-2: 23.6% increase [*P* = 0.0261]; adenovirus BMP-4: 27.0% increase [*P* = 0.0097]; adenovirus BMP-7: 129.1% increase [*P* < 0.0001]; and adenovirus BMP-10: 102.1% increase [*P* = 0.0056]). The

increase in proteoglycan accumulation by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-5 and 13 did not reach statistical significance (adenovirus BMP-5: 11.6% increase [*P* = 0.1267]; and adenovirus BMP-13: 42.6% increase [*P* = 0.2270]).

Nucleus pulposus cells stimulated with rhBMP-7 at 100 ng/mL for 1 day of culture accumulated 145.9% more proteoglycan than cells cocultured with untreated articular chondrocytes (*P* = 0.0008). It is noteworthy that coculturing with articular chondrocytes transduced with adenovirus BMP-7 resulted in essentially as high a stimulation in proteoglycan accumulation as nucleus pulposus cells maintained in the presence of rhBMP-7 in the medium (*P* = 0.7428). Spot checks, with trypan blue dye staining, of nucleus pulposus cells cultured in alginate beads indicated that cell viability was more than 95% at all times. Consequently, it is unlikely that changes in nucleus pulposus cell viability in some of the cultures could have contributed significantly to the effects on proteoglycan production.

### Collagen Content

Hydroxyproline, as a measure of collagen, was quantified by reverse-phase high-pressure liquid chromatography at the end of the 15-day culture period (Figure 3). Collagen accumulated by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus expressing various BMPs was compared in this study. Nucleus pulposus cells cocultured with articular chondrocytes transduced with BMP-2, 4, 5, and 7 accumulated significantly more collagen compared to nucleus pulposus cells cultured with chondrocytes transduced with adenovirus GFP (adenovirus BMP-2: 104.6% increase [*P* = 0.0202]; adenovirus BMP-4: 40.6% increase [*P* = 0.0362]; adenovirus BMP-5: 58.6% increase [*P* = 0.0399]; and adenovi-

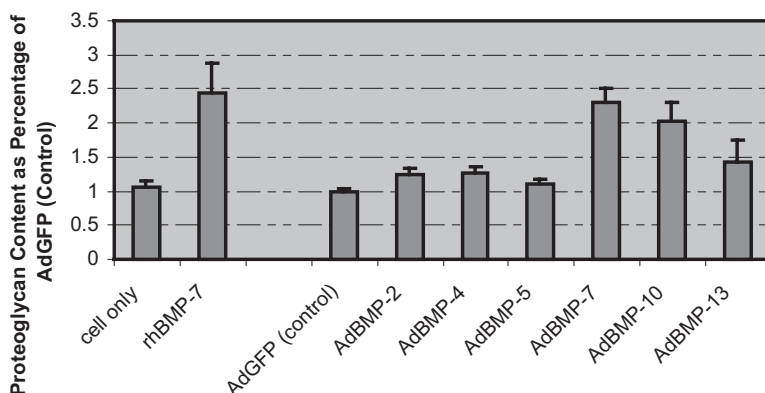


Figure 2. Comparison of proteoglycan accumulation in beads containing bovine nucleus pulposus cells treated with rhBMP-7 or cocultured with articular chondrocytes transduced with various adenovirus (Ad) BMPs. Nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-2, 4, 7, and 10 accumulated significantly more proteoglycan compared to nucleus pulposus cells cultured with chondrocytes transduced with adenovirus GFP (control) (adenovirus BMP-2: 23.6% increase [*P* = 0.0261]; adenovirus BMP-4: 27.0% increase [*P* = 0.0097]; adenovirus BMP-7: 129.1% increase [*P* < 0.0001]; and adenovirus BMP-10: 102.1% increase [*P* = 0.0056]). The increase in proteoglycan accumulation by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-5 and 13 did not reach statistical significance (adenovirus BMP-5: 11.6% increase [*P* = 0.1267]; and adenovirus BMP-13: 42.6% increase [*P* = 0.2270]). The results are expressed as the mean  $\pm$  SEM.

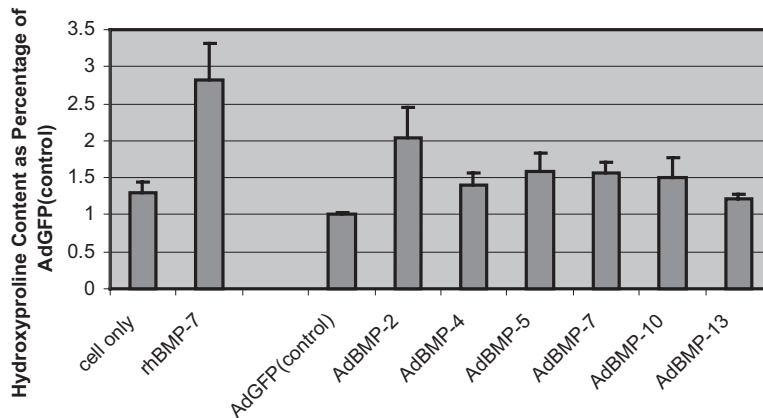


Figure 3. Comparison of collagen accumulation in beads containing bovine nucleus pulposus cells treated with rhBMP-7 or cocultured with articular chondrocytes transduced with various adenovirus BMPs. Nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus (Ad) BMP-2, 4, 5, and 7 accumulated significantly more collagen compared to nucleus pulposus cells cultured with chondrocytes transduced with adenovirus GFP (adenovirus BMP-2: 104.6% increase [ $P = 0.0202$ ]; adenovirus BMP-4: 40.6% increase [ $P = 0.0362$ ]; adenovirus BMP-5: 58.6% increase [ $P = 0.0399$ ]; and adenovirus BMP-7: 55.5% increase [ $P = 0.0036$ ]). A trend toward increased collagen accumulation by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-10 and 13 that did not reach statistical significance was observed (adenovirus BMP-10: 49.5% increase [ $P = 0.0938$ ]; and adenovirus BMP-13: 21.2% increase [ $P = 0.0734$ ]). The results are expressed as the mean  $\pm$  SEM.

rus BMP-7: 55.5% increase [ $P = 0.0036$ ]). A trend toward increased collagen accumulation by nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-10 and 13 that did not reach statistical significance was observed (adenovirus BMP-10: 49.5% increase [ $P = 0.0938$ ]; and adenovirus BMP-13: 21.2% increase [ $P = 0.0734$ ]). Nucleus pulposus cells stimulated with rhBMP-7 at 100 ng/mL during the first day of coculture accumulated 88.9% more collagen than cells not stimulated with rhBMP-7 ( $P = 0.0051$ ).

#### Cell-Associated Matrix

The presence of extracellular matrix surrounding a cell excludes erythrocytes from adhering to the culture plate in that location. Therefore, the size of the cell-associated matrix can be inferred by a halo surrounding the cells of interest after the erythrocyte exclusion assay. Nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-7 appear to have accumulated a larger cell-associated matrix compared with cells cultured with articular chondrocytes transduced with adenovirus GFP (Figure 4).

#### Nucleus Pulposus Cell Proliferation

It is known that rhBMP-7 has mitogenic effects on bovine intervertebral disc cells.<sup>6</sup> In this study, as expected, rhBMP-7 at 100 ng/mL significantly stimulated proliferation of nucleus pulposus cells (91.3% increase,  $P = 0.0198$ ). Nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-2 and 7 also proliferated significantly (adenovirus BMP-2: increase by 41.4% [ $P = 0.0405$ ]; and adenovirus BMP-7: increase by 54.5% [ $P = 0.0041$ ]), while nucleus pulposus cells cocultured with adenovirus BMP-4, 5, 10, or 13 did not proliferate significantly (Table 1).

#### Discussion

The nucleus pulposus is essential for normal disc biomechanical functioning, and there is evidence that disc degeneration may begin in the nucleus pulposus.<sup>17</sup> As disc degeneration progresses, the distinguishing features of the nucleus pulposus are lost. The synthesis of proteoglycans and type II collagen in the nucleus pulposus declines, while, in parallel, chondrocytic cells are replaced by fibrocytes synthesizing less-compliant type I collagen.<sup>2,3</sup> In addition, an overall decrease in disc cell density with age and degeneration is seen.<sup>4</sup> In the current study, native intervertebral disc nucleus pulposus cells were stimulated *in vitro* to proliferate and produce proteoglycans and collagen by exposure to autologous chondrocytes transduced *ex vivo* with genes for BMPs.

To date, preclinical studies of gene therapy in the intervertebral disc have involved *in vivo* strategies in which adenovirus carrying the genes of interest, such as transforming growth factor (TGF)- $\beta$ 1 or tissue inhibitor of metalloproteinase-1, are injected into the disc.<sup>7,18</sup> These studies have shown acceptable levels of gene expression

Table 1. Nucleus Pulposus DNA Content After Coculture With Transduced Articular Chondrocytes

DNA Content	Ratio	$P$
AdBMP-2/AdGFP	1.414	0.0405
AdBMP-4/AdGFP	1.337	0.1617
AdBMP-5/AdGFP	1.209	0.3121
AdBMP-7/AdGFP	1.545	0.0041
AdBMP-10/AdGFP	1.153	0.3461
AdBMP-13/AdGFP	0.831	0.2508
rhBMP-7/cell only	1.913	0.0198
Cell only/AdGFP	0.906	0.5725

Ad indicates adenovirus.

for prolonged periods, and also accumulation of proteoglycans and collagens within the disc. Although *in vivo* techniques are simpler and less time-consuming, the *ex vivo* method for gene transfer selected for the current study is considered safer because the host is not directly exposed to the virus. In addition, *ex vivo* manipulations are more controlled and can be verified before application. The *ex vivo* technique also provides a source of metabolically active cells to the hypochondrocyte degenerating intervertebral disc.

We have previously identified adenovirus BMP-2, 4, 5, 7, 10, and 13 to be the most effective of the BMPs for stimulating proteoglycan or collagen accumulation in the intervertebral disc, therefore, these adenovirus BMPs were chosen for the current study.<sup>19</sup> Articular chondrocytes transduced with adenovirus BMP-7 and 10 were most effective in promoting proteoglycan synthesis by the cocultured nucleus pulposus cells. The magnitude of increased proteoglycan production observed compares favorably with previous findings on the direct stimulation of disc cells with recombinant BMP-7<sup>6</sup> and BMP-2.<sup>20</sup> Although the stimulatory effects of BMP-7 on proteoglycan metabolism have been well studied,<sup>6,7,21,22</sup> the effects of BMP-10 on intervertebral disc cells have not been previously described. BMP-10 is a member of the TGF- $\beta$  family of growth factors first described by Neuhaus *et al.*<sup>23</sup> Structurally, BMP-10 is a 424 amino acid protein with 1 TGF- $\beta$  N-terminal motif. In this study, we have shown, to our knowledge, for the first time, the effects of adenovirus BMP-10 on proteoglycan accumulation by nucleus pulposus cells.

Nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-7 had as high a stimulation of proteoglycan accumulation as nucleus pulposus cells maintained in the presence of rhBMP-7 in the medium. This finding highlights the advantage of gene therapy in which a 1-time delivery of the therapeutic gene makes possible the sustained expression of the bioactive proteins. This process avoids the need for frequent and repeated administration of the recombinant protein, which is unlikely to be clinically feasible.

The maintenance of high concentrations of competent type II collagen in the nucleus pulposus is thought to be important for normal disc function. When the intervertebral disc degenerates, type II collagen production in the nucleus pulposus declines.<sup>2</sup> In the current study, nucleus pulposus cells cocultured with articular chondrocytes transduced with BMP-2, 4, 5, and 7 accumulated significantly more total collagen than nucleus pulposus cells cultured with chondrocytes transduced with adenovirus GFP (control). The significance of stimulation of type II collagen for treating disc degeneration has been suggested by Paul *et al.*,<sup>24</sup> who found that the up-regulation of type II collagen by *in vivo* Sox9 gene transfer retarded disc degeneration after a disc stab injury in a rabbit model.

In the current study, the effects of the individual adenovirus BMPs on collagen and proteoglycan production by nucleus pulposus cells varied. Articular chondrocytes trans-

duced with BMP-2 were most effective in promoting nucleus pulposus collagen production, whereas articular chondrocytes transduced with BMP-7 were most effective in promoting nucleus pulposus proteoglycan production. These findings might suggest a role for transductions with multiple genes to optimize disc matrix repair.

The mitogenic effects on intervertebral disc cells by rhBMP-7 have been shown previously.<sup>6,7</sup> In this study, we found that nucleus pulposus cells cocultured with articular chondrocytes transduced with adenovirus BMP-2 or 7 also proliferated. This moderate increase in cell number observed in the adenovirus BMP-2 and 7 groups may have contributed to the increase in extracellular matrix accumulation. The mitogenic effects of coculture with chondrocytes transduced with adenovirus BMP-4, 5, 10, and 13 did not reach statistical significance.

Although nucleus pulposus cells are strongly stimulated by media containing 20% fetal bovine serum, it is remarkable that adenovirus BMP-2, 4, 7, and 10 were able to stimulate additionally proteoglycan accumulation, and adenovirus BMP-2, 4, 5, 7 were able to stimulate further collagen accumulation. It is noteworthy that only those proteoglycans and collagens entrapped within the alginate beads in coculture were measured to reflect changes in response to exogenous genes. We chose to report the accumulation within the alginate beads because the coculture medium contains a mixture of extracellular matrix molecules synthesized by both the articular chondrocytes in monolayer and the nucleus pulposus cells encapsulated in the alginate beads. The alginate bead culture system used in the current study has been advantageous for studying the metabolism of phenotypically stable chondrocytes.<sup>25</sup>

Bovine tissue was selected for this study because the biology of the bovine intervertebral disc has been well characterized.<sup>26</sup> The animals that contributed cells for this study were between 15 and 18 months old. By 15 months of age, bovine intervertebral discs no longer contain a significant proportion of notochordal cells and contain predominantly adult intervertebral disc cells.<sup>27</sup> The quantitative effects of the various BMPs observed in this study may be species specific and not necessarily transferable to human cells. Before moving toward application in an animal model, studies to confirm which adenovirus BMPs are the most appropriate candidates in that particular species cell line are required.

Although the effects of various growth factors on disc repair have received significant attention, strategies for replenishing cells in the degenerating disc have been the subject of only a few studies. To date, most studies of cell transplantation for the treatment of degenerative disc disease have used autologous disc cells. Nishimura and Mochida<sup>28</sup> reported on the effectiveness of reinsertion of autologous nucleus cells into the disc in the treatment of disc degeneration. However, in the clinical setting, a healthy disc would have to be damaged to harvest autologous disc cells to be implanted, unless the disc cells were harvested from the degenerative disc to be treated. The latter ap-

proach would be hampered by the fact that there are fewer metabolically active chondrocyte-like cells in a degenerated disc.<sup>4</sup> We believe that an alternate source of chondrocytic cells, such as nonweight bearing articular cartilage, would be preferable and could provide a large number of metabolically active cells. Bone marrow stromal cells and other multipotential cells might also be considered for this application, and have the advantage of being harvested with relative ease and low morbidity. Zhang *et al*<sup>29</sup> have shown that bone marrow stromal cells survive when transplanted into the intervertebral disc. However, the use of these more primitive cells requires considerable *ex vivo* manipulation to induce the cells into a more chondrocyte-like phenotype before transplantation.

The delivery of gene products by inserting chondrocytes into the intervertebral disc represents a novel methodology to manipulate the biologic pathways necessary for disc repair. The data reported here are consistent with the view that native intervertebral disc nucleus pulposus cells stimulated *via* a paracrine effect are able to proliferate and produce proteoglycans and collagen when exposed to autologous chondrocytes transduced with genes for various BMPs. If applied to the treatment of disc degeneration, this strategy would provide the disc with not only metabolically active chondrocytes producing growth factors but also promote matrix replenishment by stimulating native nucleus pulposus cells.

### ■ Key Points

- We propose a novel therapeutic approach involving the delivery of autologous chondrocytes transduced *ex vivo* with bioactive proteins to provide both the cells and proteins required to stimulate intervertebral disc healing.
- Nucleus pulposus cells are stimulated to produce proteoglycans and collagen, and proliferate when they are cocultured with autologous chondrocytes transduced with genes for various BMPs.
- Bovine articular chondrocytes transduced with BMP-2 were most effective in promoting nucleus pulposus collagen production, whereas articular chondrocytes transduced with BMP-7 were most effective in promoting nucleus pulposus proteoglycan production.

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