

Use of Bone Morphogenic Protein-7 as a Treatment for Osteoarthritis

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Abstract Osteoarthritis is a degenerative disorder resulting from breakdown of articular cartilage. Previous work has shown bone morphogenic protein-7 has a potential protective effect on cartilage during the development of osteoarthritis. The purpose of this study was to determine whether bone morphogenic protein-7 could decrease the amount of cartilage degradation in preexisting osteoarthritis. The rabbit ACLT model was used as a model of osteoarthritis. Bone morphogenic protein-7 was delivered via Alzet osmotic pump to the joint 4 weeks after anterior cruciate ligament transection; thus cartilage injury was preexisting. The experimental group showed less cartilage degradation than the controls, with an average Outerbridge score of 1.9 versus 2.6 for the controls. Histomorphometry showed a trend toward less cartilage degradation in the bone morphogenic protein-7 group when compared with controls. Semiquantitative real-time polymerase chain reaction showed a considerably greater expression of aggrecan in the bone morphogenic protein-7-treated cartilage when compared with controls and less expression of matrix metalloproteinase-3 and matrix metalloproteinase-13, important catabolic mediators. The synovial tissue of the experimental group also showed

considerably less expression of matrix metalloproteinase-3, matrix metalloproteinase-13, and aggrecanase. These results indicate bone morphogenic protein-7 may reduce degradation of articular cartilage in osteoarthritis.

Introduction

Osteoarthritis (OA), the most common type of joint disease, is a degenerative disorder resulting from breakdown of articular cartilage in synovial joints. There are many treatments currently available for OA, ranging from the most conservative measures such as physical therapy and nonsteroidal antiinflammatory drugs to more surgical extremes, which range from arthroscopic procedures to total joint arthroplasty [3]. A relatively new treatment is injection of hyaluronan, which improves joint lubrication and can decrease pain [24]. Although current treatments target the symptoms of OA, they are not disease-modifying because they do not address the fundamental mechanism behind OA, which is the destruction of articular cartilage.

Previous studies in our laboratory used bone morphogenic protein (BMP)-7 (osteogenic protein-1) as a novel tissue-engineering solution to protect articular cartilage from degeneration during the development of arthritis [2]. BMP-7, a member of the transforming growth factor- β superfamily, is best known for its osteogenic properties [6]. However, we chose to use BMP-7 because studies have shown it has many major benefits on cartilage tissue in vitro. BMP-7 has a strong anabolic effect on cartilage by stimulating synthesis of cartilage matrix components and increasing proteoglycan and collagen synthesis [5, 8]. BMP-7 also has been shown to antagonize catabolic mediators of cartilage, leading to a possible protective effect. Treatment of human articular chondrocytes with

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Each author certifies that his or her institution has approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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BMP-7 blocked downregulation of proteoglycan synthesis caused by interleukin (IL)-1 [12]. BMP-7 also blocked the cartilage damaged caused by fibronectin by preventing proteoglycan degradation [15] and reduced matrix metalloproteinase (MMP)-13 expression in chondrocytes, which normally plays a pathologic role in the cartilage destruction of arthritis [13].

In a previous study, we determined whether BMP-7 would protect articular cartilage from degeneration during the development of OA in an *in vivo* animal model [2]. We used the rabbit anterior cruciate ligament transection (ACLT) model, in which the ACL was transected, leading to OA. BMP-7 was delivered to the joint for approximately 6 weeks by implantation of an osmotic pump into the medial thigh with a catheter threaded from the pump into the knee. The pump was inserted at the same time as the ACLT so delivery of BMP-7 would begin at the same time initial cartilage degradation began. In that study, the experimental group showed considerably less OA on a gross level and a dramatic decrease in the number of condyles showing full-thickness cartilage loss [2]. On a microscopic level, BMP-7 treatment considerably decreased the amount of articular cartilage loss measured histologically. Semiquantitative real-time polymerase chain reaction (RT-PCR) showed a considerably greater expression of key anabolic mediators in the BMP-7-treated cartilage when compared with controls as well. These results led to the conclusion that BMP-7 may have a potential benefit in protecting articular cartilage during the development of OA.

The purpose of the current study was to determine whether BMP-7 could decrease the amount of cartilage degradation in a model of preexisting OA, a situation more clinically applicable than the previous study. The fundamental hypothesis was that BMP-7 would reduce the progression of preexisting OA but not to the same degree as treatment before the initiation of OA. On a macroscopic level, BMP-7 should decrease the amount and severity of articular cartilage destruction in OA. On a microscopic level, treatment with BMP-7 should result in less degradation of the articular cartilage surface as seen by histomorphometry. Biochemically, BMP-7 should increase the amount of key anabolic mediators of cartilage synthesis and decrease the amount of catabolic mediators of cartilage destruction. BMP-7 also should have this inhibitory effect on the same relevant proteolytic enzymes in the synovial tissue.

Materials and Methods

The model used to study the development of OA in this study was the ACLT model. Previous studies have shown

this method produces reliable and reproducible degradation of articular cartilage after 9 weeks [25, 27].

After approval from the Institutional Animal Care and Use Committee and University of California, San Diego Animal Subjects Committee, 30 New Zealand White female rabbits, each approximately 1 year old, were used for this experiment. They were divided into two equal groups of 15, one control and the other experimental. Each rabbit initially was sedated with 2 mg/kg Xylocaine and 35 mg/kg ketamine (Abbott Labs, North Chicago, IL) and then anesthetized with 1% isoflurane gas (VEDCO Inc, St Joseph, MO). Their right legs were shaved, prepped, and draped in the usual sterile way using Betadine[®] (Purdue Pharma L.P., Stamford, CT). An approximately 3-cm midline incision was made over the knee. After dissection through subcutaneous tissues, the capsule was incised with a median parapatellar incision. The patella was dislocated laterally, exposing the ACL, which then was transected completely. An intraoperative Lachman test was performed to verify instability had been created. The capsule was closed by a running 2-0 Vicryl[®] layer and the skin was closed with an additional running 4-0 Vicryl[®] layer.

Four weeks after performing the ACLT, each animal underwent a second surgery for implantation of a sterile Alzet osmotic pump (Model 2004; DURECT Corp, Cupertino, CA) with an attached intraarticular catheter to deliver BMP-7 (Stryker Biotech, Hopkinton, MA) to the joint. The animals were anesthetized in the same way as described previously and an identical surgical approach was made to the knee. The pumps were buried under the muscle of the medial thigh with the catheter from the pump passing into the joint through a 2-mm drill hole in the metaphyseal bone of the medial condyle and eventually entering the joint in the intraarticular notch. The catheter was secured in place proximally and distally by polyethylene stents. The capsule then was closed by a running 2-0 Vicryl[®] layer and the skin was closed with an additional running 4-0 Vicryl[®] layer.

The experimental group of 15 rabbits received a continuous infusion of BMP-7 in 5% lactose buffer solution at a volume of 0.25 $\mu\text{L}/\text{hour}$. In a previous study, it was shown the average volume of synovial fluid in the rabbit knee is equal to 0.35 mL based on joint space measurements [1]. The concentration of BMP-7 in the buffered solution was 70 $\mu\text{g}/\text{mL}$, resulting in a constant synovial fluid concentration of approximately 50 ng/mL. This number was targeted because a similar study performed by Jelic et al. [14] in a sheep osteochondral defect model had good results with this concentration. The total amount of BMP-7 in the pump was approximately 250 μL , and at the previously stated flow rate, the pump would last for approximately 6 weeks; the animals were euthanized 5 weeks after implantation. The pump remained in place

until the animal was euthanized. The control group of 15 rabbits received the same pumps and delivery rate; however, the pumps were loaded with only 5% lactose buffer solution with no BMP-7. These pumps also were capable of delivering the buffer solution for approximately 6 weeks and remained in the animal until it was euthanized. The single time of 9 weeks postACLT was chosen because previous studies showed a significant amount of arthritis is formed in this short time [25, 27], so all animals were sacrificed at this point to generate a strong sample size. When the animals were euthanized, each pump used was examined carefully and no kinks or leaks were found in the tubing, nor was there any appreciable amount of fluid remaining inside.

Before beginning the study, the effectiveness of the Alzet osmotic pump was first tested *in vivo* by implanting it into a rabbit thigh with an intraarticular catheter as described previously. This test pump was filled with India ink and the animal was euthanized 2 weeks after implantation; gross extravasation of ink into the knee was observed. The previously stated flow rate and actual synovial concentration also were tested by weekly synovial fluid aspirations on two additional rabbits that received the same ACLT surgery and implantation of BMP-7 pumps. BMP-7 content and percent of oxidized species in BMP-7 samples were determined by reduced reverse-phase high-pressure liquid chromatography (rpHPLC) using a Waters Alliance 2695 Separations Module with a Waters 2487 Dual λ Absorbance Detector (Waters Corp, Milford, MA). Dried samples were solubilized in 8 mol/L urea, 100 mmol/L NaCl, 50 mmol/L Tris, pH 8.0, buffer and were reduced with β -mercaptoethanol. Separation was achieved on an ACE C4 (4.6-mm \times 50-mm) column (MAC-MOD Analytical, Chadds Ford, PA) under a gradient of 0.1% trifluoroacetic acid (TFA) in water and 0.075% TFA in acetonitrile (70% to 45% 0.1% TFA and water over 11 minutes). Detection was at 215 nm. The BMP-7 content of each sample was determined by comparison of the main peak area to a standard curve generated with the BMP-7 reference standard. The relative percent of oxidized species also was determined from the peak areas of species present. The procedure AM005-M03 "Reduced rpHPLC with C4 column" was used as guidance. Data were processed using EmpowerTM 2 software (Waters Corp). These synovial fluid collections showed a concentration of BMP-7 near the target of 50 ng/mL over 5 weeks, confirming BMP-7 was being pumped into the joint.

After surgery, rabbits were given appropriate postoperative care and analgesia, consisting of twice daily subcutaneous injections of 0.03 mg/kg buprenorphine for 3 days postoperatively. All animals were allowed normal cage activity and were euthanized 9 weeks after the initial ACLT surgery (5 weeks after implantation of the pump). At the time of euthanasia, there was no appreciable

evidence of bone formation in the synovium or surrounding tissues. The synovium was grossly normal without obvious synovitis in all animals. Therefore, the synovium was not analyzed further microscopically. The synovium was used for RT-PCR to analyze levels of catabolic enzymes of cartilage degradation, but there was no attempt to quantify osteogenic enzymes because the purpose of this study was to assess the effect of BMP-7 on cartilage. The meniscus also was not assessed because it was not the goal of the study. No other side effects of treatment were noted.

The rabbit knees were harvested and initially placed for 1 hour in a phosphate-buffered solution with protease inhibitors: 2 mmol/L disodium ethylenediaminetetraacetic acid, 1 mmol/L phenylmethanesulfonylfluoride, 5 mmol/L benzamidine hydrochloride, and 10 mmol/L N-ethylmaleimide. The femoral condyles then were stained with Higgins[®] Waterproof India Ink (Sanford Corp, Bellwood, IL). The ink was applied to the surface of the condyle with a small paintbrush and the excess was washed off with a quick rinse of sterile water. The condyles then were photographed with a Nikon Dx 5 megapixel digital camera (Nikon Corp, Tokyo, Japan) for analysis.

Each condyle was assessed by three individuals (NB, YO, RC) who were blinded to which group the condyle was in. They graded the condyles using the Outerbridge classification, which is a well-known method of grading cartilage after India ink staining [19–21, 27], with the following criteria: Grade 1 (intact surface), surface appears normal and does not retain any ink; Grade 2 (minimal fibrillation), site appears normal before staining but retains ink as elongated specks or light gray patches; Grade 3 (overt fibrillation), the cartilage is velvety in appearance and retains ink as intense black patches; and Grade 4 (erosion), loss of cartilage exposing the underlying bone. Therefore, a lower grade indicates less damage to the cartilage surface. Using these grades, an average score for each condyle and for each entire group was calculated. The average Outerbridge scores for each condyle also were separated into groups from one to four to determine a frequency of each score for each group.

The condyles were placed in 10% buffered neutral formalin with 1% cetylpyridinium chloride at the time of harvest. The medial femoral condyle of each animal was used for histology. The specimens remained in the fixative for approximately 3 days. After the cartilage/bone core had been fixed, it was decalcified with 30% formic acid. The end point of decalcification was determined radiographically. After the cartilage-bone was completely decalcified, the specimens were dehydrated with ascending alcohol grades: 70% (two changes), 95% (two changes), and 100% (two changes), cleared with Histo-Clear[®] (National Diagnostics, Atlanta, GA), and embedded in paraffin blocks. The blocks were sectioned on a rotary microtome at 6 μ m

and placed on Superfrost Gold™ slides (Erie Scientific, Portsmouth, NH). The medial femoral condyles were sectioned in the sagittal plane perpendicular to the bone. The slides were allowed to dry at room temperature overnight. The slides-sections were heated in a slide dryer at approximately 62°C for 25 minutes and then allowed to return to room temperature before being stained with safranin O and hematoxylin and eosin.

Hematoxylin and eosin staining was done by first placing the slides in hematoxylin for 3 minutes followed by washes with ethanol and water and briefly lithium carbonate. The slides then were placed in eosin for 30 seconds followed by additional ethanol and water washes.

Safranin O staining was done by first treating the samples with xylene followed by ethanol and water washes and then treatment with fast green and safranin O stain with a wash of acetic acid in between. Final washing was done with ethanol and xylene before mounting.

Histologic sections were observed with a Nikon Microphot microscope and an attached Nikon E5700 5 megapixel digital camera and then scanned at high resolution (3600 dpi) for image analysis using a previously described technique for histomorphometry [10]. The entire medial femoral condyle was sectioned for each animal and the slide with the greatest depth of cartilage loss from each condyle was chosen for analysis. All of these chosen sections were analyzed by three individuals (NB, RH, RC) who were blinded to which group each slide was from. Measurements were made using ImageJ software (Version 1.34 s; NIH, Bethesda, MD). A profile of the cartilage surface and subchondral plate was generated and the x-y

coordinates copied into a Microsoft® Excel® spreadsheet (Microsoft Corp, Redmond, WA). The profile data were used to define two idealized third-order polynomial curves, which described the upper and lower limits of the cartilage area. The ratio of the area of the zone filled with cartilage and the area of the idealized zone were presented as the percentage repair (%rep). The percentage of deterioration was calculated as $1 - \%rep$. Cartilage depression in absolute terms was determined as the average distance from the idealized cartilage surface curve to the actual condylar surface [10] (Fig. 1).

mRNA expression levels of anabolic and catabolic genes were determined by semiquantitative RT-PCR [11, 26]. Articular cartilage was taken from the trochlea of each femur and synovial tissue from the suprapatellar area. This cartilage and synovial tissue were pulverized in liquid nitrogen, and total RNA was isolated using the acid-guanidinium-thiocyanate-phenol extraction procedure [11]. Starting with 1 µg total RNA, first-strand cDNA was synthesized using oligo (dT)₁₅ primers.

Based on published sequences (Basic Local Alignment Search Tool [BLAST]), human RT-PCR primer sets were constructed specific to selected coding regions of aggrecan, Type II collagen, aggrecanase, MMP-3, MMP-13, IL-1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Table 1). Cycle studies were done for all genes to determine the linear range of amplification for each gene. The appropriate cycle and temperature then were used for each primer. NIH image analysis software (Version 1.61) was used to quantitatively scan RT-PCR profiles after agarose gel electrophoresis and for observation of ethidium

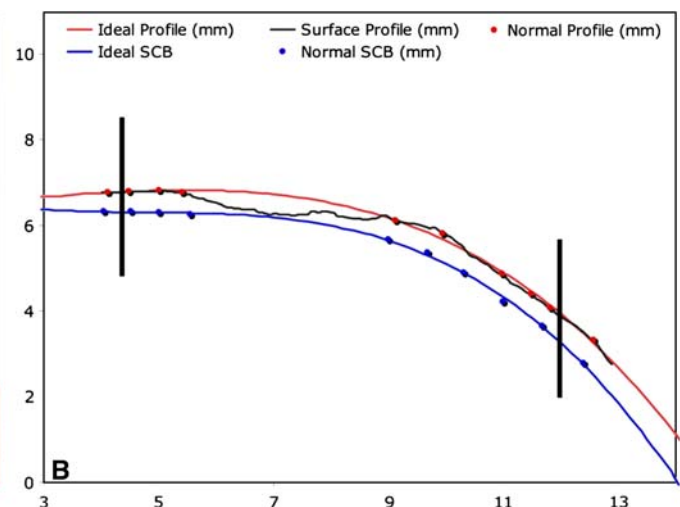
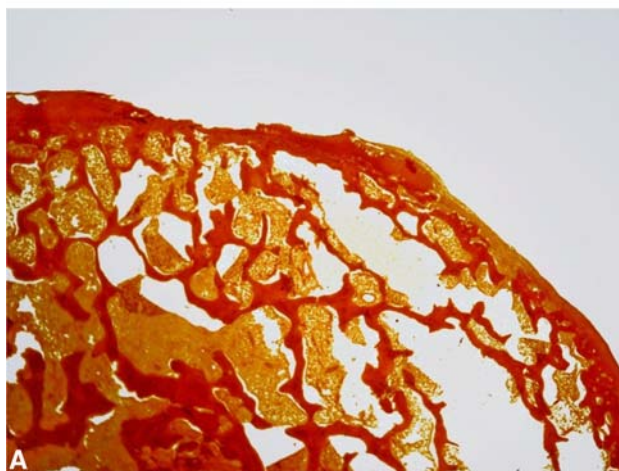


Fig. 1A–B This figure illustrates the calculation of percent deterioration and average defect size. **(A)** An actual histologic section of cartilage and **(B)** projection of that section for calculations are shown. **(B)** The red line represents the ideal cartilage surface, the blue line represents the ideal subchondral plate, and the black line represents the actual cartilage surface. The calculated area between the red and

blue lines is the ideal cartilage area, whereas the calculated area between the black and blue lines is the actual cartilage area. One minus the ratio of actual area to ideal area is the percent deterioration. The average defect is calculated as an average amount of depression from the red to black lines.

Table 1. Real-time PCR primers*

Gene	Primers	Characteristics
GAPDH	Sense: 5'-TCA CCA TCT TCC AGG AGC GA-3' Antisense: 5'-CAC AAT GCC GAA GTG GTC GT-3'	293 bp (289–581), 55°C, 26 cy
Type II collagen	Sense: 5'-GCA CCC ATG GAC ATT GGA GGG-3' Antisense: 5'-GAC ACG GAG TAG CAC CAT CG-3'	367 bp (1–367), 65°C, 28 cy
Aggrecan	Sense: 5-GAG GTC GTG GTG AAA GGT GT-3' Antisense: 5-GTG TGG ATG GGG TAC CTG AC-3'	206 bp (247–452), 58°C, 34 cy
MMP-3	Sense: 5'-GCC AAG AGA TGC TGT TGA TG-3' Antisense: 5'-AGG TCT GTG AAG GCG TTG TA-3'	363 bp (429–791), 65°C, 34 cy
MMP-13	Sense: 5'-AAC GAG GAT GAT GAT TTG GTC CG -3' Antisense: 5'-TTG GCC AGG AGG AAA AGC GTG AG -3'	300 bp (742–1241), 55°C, 30 cy
Aggrecanase	Sense: 5'-CTG TGC CGT GAT TGA AGA TG-3' Antisense: 5'-GCT GAG TGG CAT CGT AGG TTT-3	308 bp (50–357), 56°C, 34 cy
IL-1	Sense: 5'-TAC AAC AAG AGC TTC CGG CA -3' Antisense: 5'-GGC CAC AGG TAT CTT GTC GT -3'	354 bp (255–582), 58°C, 34 cy

* Each gene used is listed followed by the number of base pairs (bp), temperature of reaction, number of cycles (cy), and the actual gene sequences used; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; MMP = matrix metalloproteinase; IL-1 = interleukin-1.

bromide. This software measures relative mean density over a fixed gray scale range after correction for background. All values were normalized to GAPDH. Data are expressed as mean mRNA expression \pm standard deviation (SD) and evaluated using unpaired Student's *t* test. Statistical significance was set at $p < 0.05$.

Results

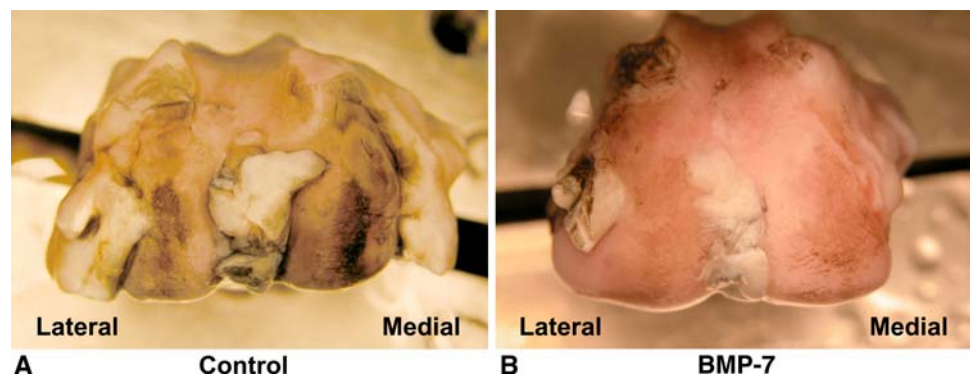
The BMP-7-treated condyles showed less macroscopic cartilage degradation and a lower percentage of condyles with severe erosion of the articular surface when compared with controls (Fig. 2). The mean Outerbridge score for the control group was 2.6 (SD, 1.2), whereas the BMP-7 group had a mean score of 1.9 (SD, 1.0) (Fig. 3A). In the control group, 46% of the condyles were graded 3 or 4, indicating they had a high degree of cartilage fibrillation or loss. In the BMP-7-treated group, only 22% of the condyles were graded either 3 or 4. Moreover, in the BMP-7-treated

group, 44% of condyles showed Grade 1 or near-normal cartilage compared with just 15% in the control group (Fig. 3B).

On a microscopic level, the control group showed a greater loss of cartilage than the BMP-7 group (Fig. 4). In percentage terms, the average percent deterioration of the control group was 30.8% (SD, 8%) of the ideal cartilage surface versus a deterioration of only 23.5% (SD, 10%) for the BMP-7-treated condyles (Fig. 5A) ($p > 0.05$). In absolute terms, the average depression of the cartilage surface of the control group was 0.141 mm (SD, 0.057 mm), whereas the BMP-7-treated group had an average depression of 0.103 mm (SD, 0.062 mm) (Fig. 5B) ($p > 0.05$). Additionally, no bony overgrowth was noted microscopically.

Biochemically, the BMP-7-treated cartilage showed some increased expression of anabolic genes and decreased expression of catabolic genes when compared with the control group. The anabolic gene, aggrecan, had a higher expression in the BMP-7 group when compared with the

Fig. 2A–B India ink staining of (A) control and (B) BMP-7-treated knees after euthanasia shows more areas of wear and greater depths of fibrillation in the control group.



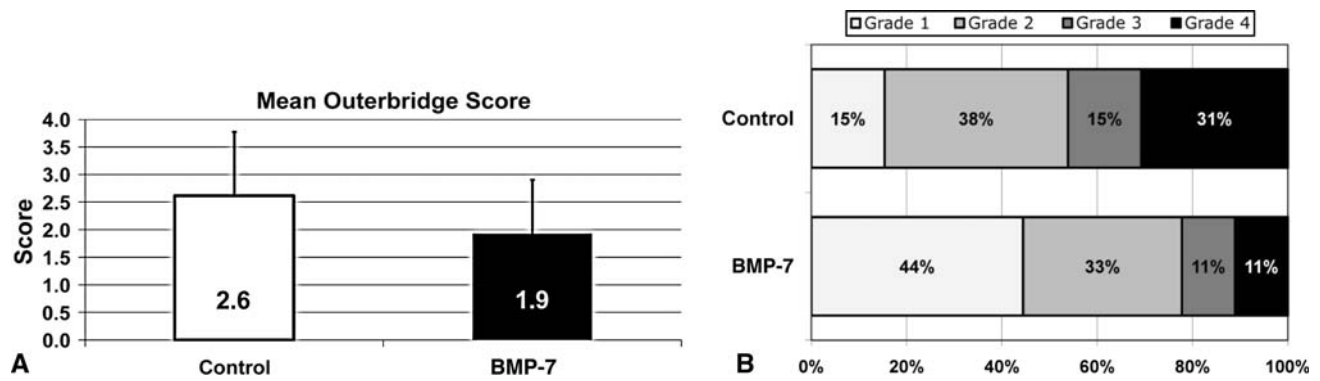


Fig. 3A–B (A) The mean Outerbridge score of the control group (2.6) was higher than the score of the BMP-7-treated group (1.9). (B) The frequency of each Outerbridge score for control and BMP-7 groups shows a far higher percentage of severely worn condyles in the control group.

Fig. 4A–B Sample histologic sections of (A) control group condyles and (B) BMP-7-treated condyles, taken perpendicular to the bone in the sagittal plane from the medial femoral condyles, show greater loss of articular cartilage in the control group sections.

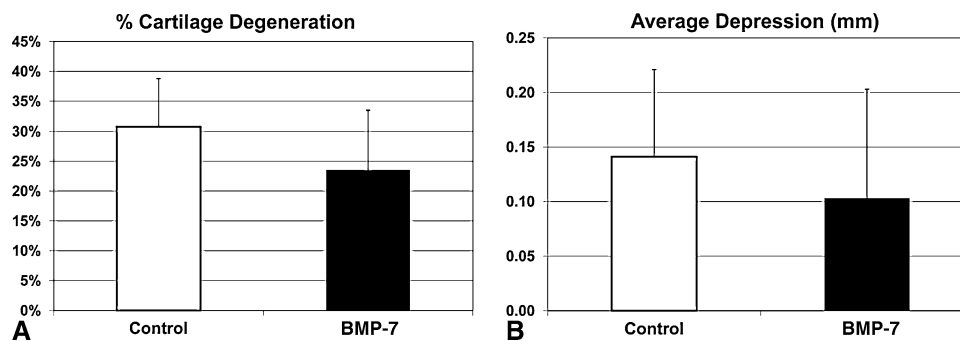
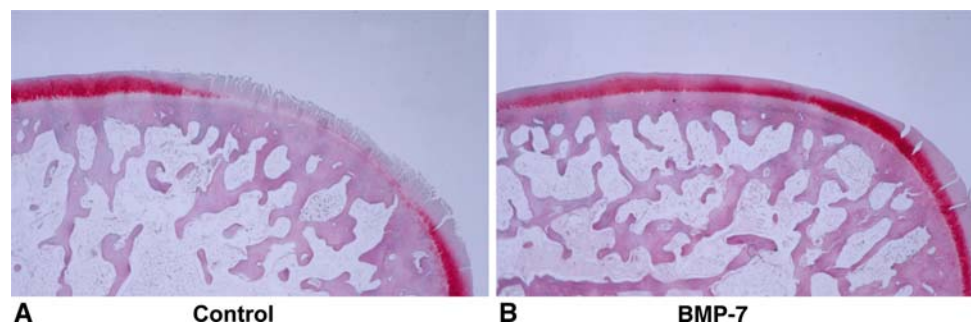


Fig. 5A–B (A) Histomorphometry data of the percent deterioration of cartilage in the control and BMP-7-treated groups show the percent deterioration of the control group was 30.8% (SD, 8%) of the ideal cartilage surface versus deterioration of only 23.5% (SD, 10%) for the BMP-7-treated condyles ($p > 0.05$). (B) Histomorphometry data of

the average depression or defect of cartilage in the control and BMP-7-treated groups show the average depression of the control group was 0.141 mm (SD, 0.057 mm), whereas the BMP-7-treated group had an average depression of 0.103 mm (SD, 0.062 mm) ($p > 0.05$).

control group ($p < 0.05$). Four catabolic genes were tested in the cartilage: MMP-3, MMP-13, IL-1, and aggrecanase. MMP-3 and MMP-13 showed lower expression in the BMP-7-treated group when compared with the control ($p < 0.05$), but there was no difference in expression of aggrecanase or IL-1 (Fig. 6) ($p > 0.05$).

Expression of the same four catabolic genes, MMP-3, MMP-13, IL-1, and aggrecanase, was tested in the synovial tissue of the BMP-7-treated group and the control group, and three, MMP-3, MMP-13, and aggrecanase, showed a

decrease in expression in the BMP-7 group ($p < 0.05$) (Fig. 7).

Discussion

The aim of this study was to test the success of BMP-7 as a therapy for preexisting OA and to begin to elucidate a mechanism behind its potential protective effect on articular cartilage. After ACLT and 4 weeks of progression of

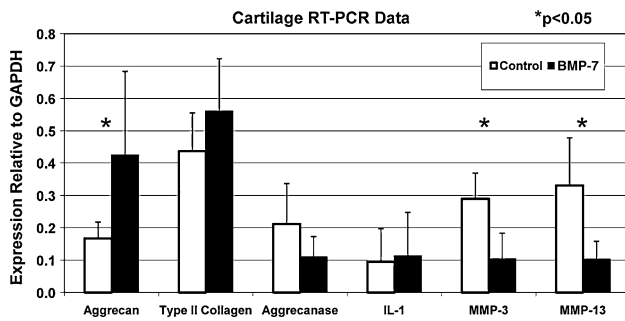


Fig. 6 RT-PCR data of the expression of anabolic and catabolic genes in the cartilage tissue of the control and BMP-7-treated groups are shown. The BMP-7-treated cartilage showed an increase in aggrecan expression and decrease ($p < 0.05$) in MMP-3 and MMP-13 expression when compared with the control group.

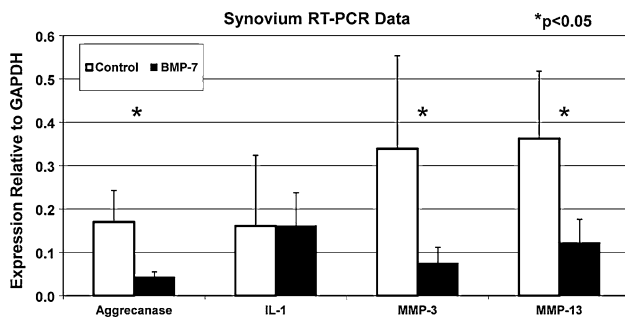


Fig. 7 RT-PCR data of the expression of catabolic genes in the synovial tissue of the control and BMP-7-treated groups show the BMP-7-treated synovium had a decrease ($p < 0.05$) in aggrecanase, MMP-3, and MMP-13 expression when compared with the control group.

arthritis, BMP-7 treatment reduced the number of severely worn condyles from 46% to 22% on a gross morphologic level. Microscopically, there was also a trend toward a decrease in cartilage loss of the articular surface with BMP-7 treatment. BMP-7 treatment also increased expression of aggrecan and decreased expression of key catabolic mediators of cartilage degradation, including aggrecanase, MMP-3, and MMP-13.

A limitation of this study was that only one time of 9 weeks post-ACLT was chosen. This was thought to be sufficient because previous studies showed this is enough time to generate significant arthritis with full-thickness cartilage wear in many specimens [25, 27]. Therefore, all animals were sacrificed at this time rather than at varying times, which would reduce the sample size of each and make drawing statistical conclusions more difficult. Additionally, the small size of the animal made it impossible to use a pump that could hold more fluid to allow for a longer treatment time; however, this challenge can be addressed in future studies.

The primary aim of this study was to assess the effect of BMP-7 on articular cartilage. Synovial tissue was analyzed

for relevant inflammatory mediators, but there was no histologic analysis of the synovium or meniscus, which can be considered another limitation. Also, there was no gross bone formation noted, but future studies can further analyze this question by quantifying osteogenic proteins in the surrounding tissues.

A previous study showed BMP-7 has efficacy in reducing the development of OA in the rabbit ACLT model [2]. However, in that study, BMP-7 treatment was initiated at the same time as the ACLT, whereas in the current study, there was a lag time of 4 weeks between the ACLT and initiation of BMP-7 treatment to allow for formation of arthritis before treatment, which is a situation far more clinically relevant. Comparing the two studies confirms BMP-7 does have a protective effect on articular cartilage; however, initiation of treatment at an earlier time results in a greater effect, as one might intuitively expect. In a previous study, BMP-7 treatment showed a reduction in Grades 3 and 4 condyles from 46% in the control to only 12% in the experimental group [2] and microscopically showed a 10.9% surface deterioration versus 22.3% for the controls [2]. In the current study, gross morphology results were similar with BMP-7 treatment, reducing the number of Grades 3 and 4 condyles from 46% to 22%, and histomorphometry, showing surface deterioration of 23.5% compared with 30.8% in the control group. Therefore, although BMP-7 treatment was effective in reducing the amount of cartilage loss whether treatment began with the initial ACLT or 4 weeks later as in the current study, starting BMP-7 treatment at a later time had a slightly smaller protective effect.

Expression of several vital anabolic and catabolic mediators of cartilage was analyzed using semiquantitative RT-PCR. In a previous study, there was considerably greater expression of the anabolic genes, aggrecan and collagen Type II, in the BMP-7-treated cartilage when compared with controls [2], and this result was duplicated in the current study with higher expression of aggrecan in the BMP-7-treated cartilage, and collagen Type II. Some in vitro studies showed BMP-7 has a strong anabolic effect on cartilage by stimulating synthesis of cartilage matrix components and increasing proteoglycan and collagen synthesis [5, 8]. Additional work was done with intervertebral disc cells that showed BMP-7 treatment increases total DNA, proteoglycan, and collagen levels in rabbit nucleus pulposus and annulus fibrosus cells cultured in vitro [18]. Our studies are some of the only in vivo studies using BMP-7. Two other in vivo studies had similar results with injection of BMP-7 into degenerated intervertebral discs resulting in an increase in disc height and an associated increase in collagen and proteoglycan content in a rabbit model [17, 22].

The current study showed an even more dramatic decrease in the key catabolic mediators aggrecanase,

MMP-3, and MMP-13 in cartilage and synovial tissue in the BMP-7-treated group. Similar effects of BMP-7 on catabolic mediators of cartilage have been observed in previous in vitro studies. BMP-7 inhibited the hyaluronan hexasaccharide-induced cartilage matrix depletion in one study [23]. BMP-7 also blocked cartilage damage caused by fibronectin by preventing proteoglycan degradation [15] and reduced MMP-13 expression in chondrocytes, which normally plays a pathologic role in the cartilage destruction of arthritis [13]. In an in vivo rat model of intervertebral disc degeneration, BMP-7 injection effectively decreased expression of key catabolic mediators such as aggrecanase and MMP-13 [4]. In the current study, BMP-7 treatment correlated with a reduction of MMP-3 and MMP-13 expression of three to four times in cartilage and synovial tissue when compared with the control.

Only a few other in vivo studies have used BMP-7 to treat cartilage lesions, but none have been OA models. Treatment of an osteochondral defect in sheep knees with a similar dose of BMP-7 in an acetate solution by means of the same osmotic pump for 2 weeks resulted in consistent filling of the defect with new cartilage at 3 months postoperatively whereas controls showed no evidence of new cartilage formation [14]. BMP-7 delivered on bone-derived Type I collagen particles press-fitted into large focal defects in rabbit knees showed improved repair of the cartilage lesions over controls [9]. BMP-7-induced hyaline cartilage-like repair of full-thickness osteochondral defects in a dog model were maintained 52 weeks postoperatively with no significant degradation of the BMP-7-induced repair tissue [7]. Subchondral defects in goat knees treated with BMP-7 mixed with autologous blood and autologous particles of perichondrium and covered with a periosteal flap resulted in formation of new cartilage after 4 months [16]. All of these results support the ability of BMP-7 to regenerate articular cartilage. The current study is unique because of the use of BMP-7 without other scaffolds, perichondrium, or collagens as in some of these previous studies, and perhaps more importantly, it is the most clinically applicable model of OA to display this similar positive effect of BMP-7 on cartilage.

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