Evaluation of Intra-Articular Mesenchymal Stem Cells to Augment Healing of Microfractured Chondral Defects

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Purpose: This study evaluated intra-articular injection of bone marrow–derived mesenchymal stem cells (BMSCs) to augment healing with microfracture compared with microfracture alone. **Methods:** Ten horses (aged 2.5 to 5 years) had 1-cm^2 defects arthroscopically created on both medial femoral condyles of the stifle joint (analogous to the human knee). Defects were debrided to subchondral bone followed by microfracture. One month later, 1 randomly selected medial femorotibial joint in each horse received an intra-articular injection of either 20×10^6 BMSCs with 22 mg of hyaluronan or 22 mg of hyaluronan alone. Horses were confined for 4 months, with hand walking commencing at 2 weeks and then increasing in duration and intensity. At 4 months, horses were subjected to strenuous treadmill exercise simulating race training until completion of the study at 12 months. Horses underwent musculoskeletal and radiographic examinations bimonthly and second-look arthroscopy at 6 months. Horses were euthanized 12 months after the defects were made, and the affected joints underwent magnetic resonance imaging and gross, histologic, histomorphometric, immunohistochemical, and biochemical examinations. **Results:** Although there was no evidence of any clinically significant improvement in the joints injected with BMSCs, arthroscopic and gross evaluation confirmed a significant increase in repair tissue firmness and a trend for better overall repair tissue quality (cumulative score of all arthroscopic and gross grading criteria) in BMSC-treated joints. Immunohistochemical analysis showed significantly greater levels of aggrecan in repair tissue treated with BMSC injection. There were no other significant treatment effects. **Conclusions:** Although there was no significant difference clinically or histologically in the 2 groups, this study confirms that intra-articular BMSCs enhance cartilage repair quality with increased aggrecan content and tissue firmness. **Clinical Relevance:** Clinical use of BMSCs in conjunction with microfracture of cartilage defects may be potentially beneficial.

The microfracture technique was developed to enhance chondral resurfacing by taking advantage of the body's own healing capability[1-5] and is the most frequently used technique for first-line treatment of symptomatic lesions of the articular cartilage in the knee, especially smaller lesions.[6] More recently, its usefulness in treating full-thickness cartilage injuries of the shoulder has been reported.[7] This procedure was used initially more than 20 years ago based on anecdotal evidence suggesting that it might provide certain advantages over other techniques such as drilling, including the ability to have perpendicularly oriented holes produced arthroscopically and a peripheral rim that potentially helps repair tissue integration with the subchondral bone.[1,5]

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To study microfracture in large, full-thickness articular cartilage defects, an equine model has been developed to mimic lesions often observed in human patients.[8-12] Full-thickness chondral defects are made arthroscopically in the weight-bearing portion of the medial femoral condyle. This model obviates a noted flaw in many studies where the full-thickness defect penetrated deep into the subchondral bone.[13]

Early work using labeled mesenchymal stem cells (MSCs) has shown that they have an affinity for damaged joint tissue with the ability to localize and participate in repair of damaged joint structures including the cruciate ligaments and menisci, as well as cartilage lesions (if administered in sufficient quantities).[12] Most studies, both in vitro[14] and in vivo,[15,16] have focused on meniscal repair using MSCs by either direct injection or intra-articular injection. Intra-articular injection of MSCs into the joint stimulated meniscal regeneration in a caprine model of osteoarthritis.[16] Studies exploring the ability of MSCs to heal articular cartilage defects have largely focused on the use of a carrier scaffold to localize MSCs within the defect site. However, a recent equine study showed early benefit with MSCs in a fibrin matrix, but no significant differences were noted when MSCs plus fibrin were compared with fibrin alone at 8 months.[17] On the basis of that work as well as recent in vitro studies,[18] there may be a problem with migration and proliferation of MSCs embedded within fibrin. Direct intra-articular injection of MSCs suspended in hyaluronan (HA) was evaluated with partial-thickness defects in the medial femoral condyle of adult minipigs, and the treated groups showed improved cartilage healing both histologically and morphologically at 6 and 12 weeks compared with both control groups (saline solution or HA).[19] In another study designed to evaluate the ability of intra-articular MSCs to enhance cartilage repair with microfracture, after drilling of 4-mm subchondral defects in goats, the postoperative intra-articular injection of autologous marrow aspirate in combination with HA showed better histologic repair.[20] In another study with full-thickness chondral defects in minipigs, MSCs covered with a collagen membrane increased the histomorphologic repair tissue quality at 8 weeks, and there was a higher glycosaminoglycan (GAG) content and type II collagen content shown with immunohistochemistry.[21]

The purpose of this study was to test the potential of bone marrow–derived mesenchymal stem cells (BMSCs) to enhance the healing response in full-thickness cartilage defects that have been microfractured. We hypothesized that intra-articular injection of BMSCs would augment healing with microfracture compared with microfracture alone.

METHODS

Experimental Design

Ten skeletally mature horses, free of musculoskeletal abnormalities, were entered into this 12-month study. We performed a power calculation based on a previous study with microfractured defects, and n = 10 provided a power of 81% based on this previous study.[10]

Defects measuring 1 cm^2 were arthroscopically created on the medial femoral condyle of both medial femorotibial joints of the stifle joints (analogous to the human knee) as previously described.[8,9] Defects were debrided through the calcified cartilage layer down to the level of the subchondral bone plate, and both defects were subjected to standard subchondral bone microfracture (Fig 1). One month after defect creation, each horse had 1 joint randomly treated by intra-articular injection of BMSCs with 22 mg of HA (Hyvisc [hyaluronate sodium], 3×10^6 Da; Anika Therapeutics, Woburn, MA)

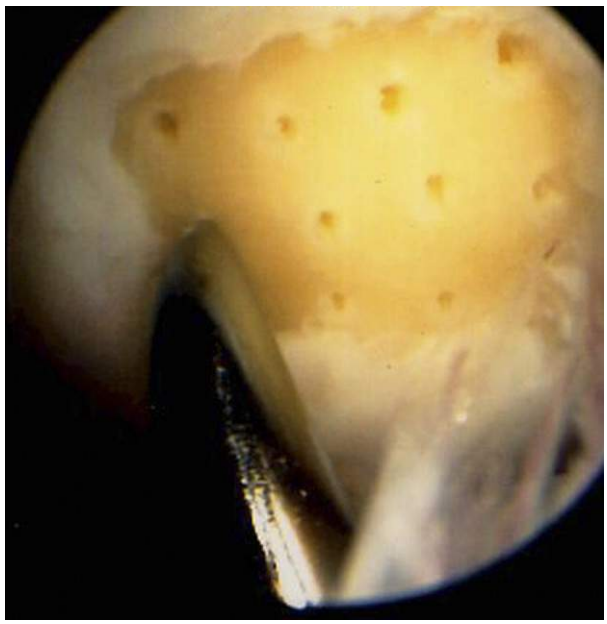

Figure 1. Arthroscopic view of a chondral defect in the medial femoral condyle after microfracture.

(BMSC + HA) whereas the opposite joint received 22 mg of HA alone (OHA). A routine postoperative rehabilitation protocol was followed, and the horses were subjected to a strenuous treadmill exercise protocol. During the study, the horses underwent routine musculoskeletal and radiographic examinations every other month until the end of the study, as well as second-look arthroscopy at 6 months after defect creation. All examinations (before death and post mortem) were done in a blinded fashion so that evaluators were unaware of treatment assignments. The horses were humanely euthanized 12 months after defect creation (11 months after experimental treatment), and all study joints were subjected to magnetic resonance imaging (MRI) examination. Gross examination and collection of tissue for histologic, histomorphometric, immunohistochemical, and biochemical procedures were then performed.

The timing of injection of the stem cells was based on the pragmatics of requiring 3 weeks to culture 20 million BMSCs. Furthermore, we reasoned that for this technique to be used in clinical orthopaedics, the surgeon would make a decision at arthroscopy that BMSCs were indicated; thus bone marrow would be drawn at this time, and a period of at least 3 weeks would be required before intra-articular therapy could be performed. The selection of the number of MSCs was based on generalizations in the literature showing that cell numbers of greater than 5 million are more associated with positive outcomes, as well as the observation that early flares occurred with cell doses in the 30 to 60 million range. Clearly, dose titration studies in small laboratory animals would be useful.

Bone Marrow Harvest and MSC Isolation

MSC cultures were established by previously optimized techniques for equine bone marrow.[19] Approximately 10 mL of bone marrow was aseptically harvested from the iliac crest of each horse into syringes preloaded with 10,000 U of heparin. The nucleated cells were separated from the red blood cells through centrifugation at 100*g* for 2 to 5 minutes and then seeded into low-glucose Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Hyclone, Logan, UT) in tissue culture flasks (Corning, Lowell, MA) at a concentration of 0.25×10^6 cells/cm^2. The medium was changed after 24 hours to remove nonadherent cells. Over the next 6 to 8 days, adherent MSCs proliferated in colonies to near confluence, at which point the MSCs were trypsinized by use of 0.25% trypsin/ethylenediaminetetraacetic acid (Invitrogen). The MSCs were subcultured by seeding at 10×10^3 cells/cm^2 in Alpha Minimal Essential Medium (Invitrogen) plus 10% fetal bovine serum and 2 ng/mL of fibroblast growth factor 2 (R&D Systems, Minneapolis, MN). One to three passages were required to obtain a 20 million cell count.

Musculoskeletal Examinations

Horses were evaluated clinically by use of the American Association of Equine Practitioners' grading scale. Examinations were carried out 2 weeks before surgical defects were made, at the time of articular defect creation, and at 2, 9, 16, 23, 28, 36, 45, and 52 weeks. Specifically, horses were evaluated for degree of pain (from 0 [normal] to 5 [non–weight bearing]), degree of synovial effusion (from 0 [normal] to 4 [severe change]), response to pain after flexion of the affected joint (from 0 [normal] to 4 [severe pain]), and range of motion of the affected joint (from 0 [normal] to 3 [severely reduced]).

Radiographic Evaluation

Both left and right medial femorotibial joints were assessed radiographically by use of lateromedial, caudocranial, and craniolateral-caudomedial oblique views in 4 categories: proliferation, lysis, osteophyte formation, and opacity of defect. All 4 criteria were graded on a scale from 0 to 4, where 0 was normal and 4 was severe.

Six-Month Arthroscopic and End Point Evaluation of Repair Tissue

The degree of repair tissue firmness was graded on a scale from 0 to 4 (where 0 is similar to the surrounding normal cartilage and 4 indicates marked softening compared with the surrounding normal articular cartilage).

The overall grade of tissue repair (this category takes into account all aspects of the repair including attachment to bone and surrounding cartilage, repair tissue volume, firmness, and undulation of repair tissue surface, as well as any degeneration of original defect margins) was evaluated on a scale from 0 to 4 (where 0 is poor and 4 is excellent).

MRI Evaluation

MRI was performed with a GE Signa 1.5-T system (GE Healthcare, Milwaukee, WI). Proton den-

sity with and without fat suppression and T2-weighted fast spin echo images were acquired in the sagittal, frontal, and transverse planes. The femorotibial joints were graded by use of the following MRI criteria on a scale from 0 to 4, where 0 was normal and 4 was severe: osteophytes, enthesophytes, synovial proliferation, and joint capsule thickness. In addition, we graded the subchondral bone of the medial femoral condyle, as well as the presence of bone edema or sclerosis, the amount of repair tissue in the defect, the character of repair tissue, and the interface of repair tissue and surrounding cartilage, using the same scale.

Histologic Analysis of Synovial Membrane

The synovial membrane was evaluated for cellular infiltration, intimal hyperplasia, subintimal edema, subintimal fibrosis, and vascularity. Each of these categories was graded 0 to 4, where 0 is normal and 4 indicates marked change. A cumulative total was also calculated based on the outcome for each of the 5 categories, with a total possible range from 0 to 20.

Histologic Evaluation of Repair Tissue

The nature of the repair tissue was graded on a scale from 0 to 4, with 0 being some fibrocartilaginous mostly non-chondrocytic cells and 4 being hyaline cartilage. Surface regularity was graded on a scale from 0 to 3, with 0 being severe disruption including fibrillation and 3 being smooth and intact. Structural integrity was graded on a scale from 0 to 2, with 0 being severe disintegration and 2 being normal. Thickness was graded on a scale from 0 to 2, with 0 being 0% to 50% of normal cartilage and 2 being 100% of normal adjacent cartilage. Integration with adjacent cartilage was graded on a scale from 0 to 2, with 0 being not bonded and 2 being bonded at both ends of the repair tissue.

Hypocellularity was graded on a scale from 0 to 2, with 0 being moderate hypocellularity and 2 being normal cellularity. Chondrocyte clustering was graded on a scale from 0 to 2, with 0 being 25% to 100% of the cells and 2 being no clusters. Freedom from degenerative changes in adjacent cartilage was graded on a scale from 0 to 3, with 0 being severe hypocellularity with poor or no staining and 3 being normal cellularity with no clusters and with normal staining. Reconstitution of subchondral bone was graded on a scale from 0 to 3, with 0 being no subchondral bone reconstitution and 3 being normal.

Inflammatory response in the subchondral bone region was graded on a scale from 0 to 2, with 0 being severe and 2 being none/mild. The cumulative histology score is the cumulative sum of all of the above categories, with a range of 0 to 28.[22,23]

Histomorphometry of Repair Tissue and Surrounding Tissue

Histomorphometric analysis was performed on a single H&E-stained section. Digital pictures were taken of slides with a capture program (QCapture; QImaging, Surrey, British Columbia, Canada). Then, the picture was opened in the Rincon program (Imaging Planet Research Microscopy Group, Goleta, CA) for measurement. The Rincon measurement tool was calibrated with a photo of a stage micrometer taken at the same power and bin as the tissue pictures. With the Rincon measurement tool, a line was drawn around the area of interest and the program simultaneously gave the measurement of that area. For these pictures, the area was measured in square millimeters, with 2 places past the decimal point (e.g., 26.59 mm^2).

Immunohistochemical Evaluation of Repair Tissue and Surrounding Tissue

Antibodies that recognized major articular cartilage matrix proteins were used for immunolocalization by use of tissue collected and then embedded at optimal cutting temperature (Tissue-Tek; Electron Microscopy Sciences, Hatfield, PA) and snap frozen at the time of death. Seven-micrometer serial sections of repair tissue were obtained for immunohistochemistry. Sections were placed on positively coated slides by use of an automated stainer (model 760-001; Ventana Medical Systems, Tucson, AZ); sections were exposed to primary antibodies of type I and type II collagen as well as aggrecan.

Biochemical Analysis

Repair tissue was evaluated for total GAG content of articular cartilage by use of the dimethylmethylene blue assay.

RESULTS

Musculoskeletal Examinations

On average throughout the study, BMSC + HA limbs were graded with slight pain (1.3 ± 0.08) compared with OHA limbs (1.3 ± 0.08), but there was no significant effect of treatment. The degree of lameness changed over the study period ($P < .0001$). On average throughout the study, the degree of effusion was slight to mild in both the BMSC + HA–treated joints (1.7 ± 0.01) and OHA-treated joints (1.7 ± 0.01), and

there was no significant effect of treatment. On average throughout the study, the response to joint flexion was slight to mild in both the BMSC + HA–treated joints (1.4 ± 0.1) and OHA-treated joints (1.4 ± 0.1), but it did change significantly over the study period ($P < .0001$), decreasing over time. There was no significant effect of treatment on the response to joint flexion, although it did change significantly over the study period ($P < .0001$). There was no significant effect of treatment on the range of motion for the stifle joint, although it did change significantly over the study period ($P < .0001$).

Radiographic Evaluation

The results of radiographic evaluation are depicted in Table 1. Although various radiographic changes progressed over time, there was no significant difference between treatment groups.

Six-Month Arthroscopic and End Point Evaluation of Repair Tissue

At the termination of the study BMSC + HA compared with OHA joints had significantly firmer repair tissue, whereas no difference existed at the 6-month second-look arthroscopy (Fig 2). No deterioration in firmness was noted in the BMSC + HA–treated joints from 6 to 12 months, but the OHA-treated repair tissue became significantly softer compared with the surrounding cartilage in the same comparison.

There was no significant effect of time on overall grade of repair tissue. There was a trend for treatment group to affect the overall grade of repair tissue, with BMSC + HA joints having a numerically better score (2.2 ± 0.2) than OHA joints (1.75 ± 0.2). The degree of repair tissue surface irregularity was not significantly different by either treatment group or evaluation period. On average, at both time points, the repair tissue was graded as slightly to mildly undulating. The surface area of repair tissue was significantly different based on both treatment ($P = .02$) and time period ($P = .04$). Specifically, BMSC + HA defects had

Table 1. *Results of Radiograph Grading Categories*

Radiograph Grading Categories	Mean Value During Study Period		
	BMSC + HA	OHA	*P* Value
Bone proliferation	2.34 ± 0.2	2.24 ± 0.2	.20
Lysis	2.3 ± 0.2	2.2 ± 0.2	.20
Osteophyte formation	0.8 ± 0.2	0.7 ± 0.2	.38
Opacity of defect	2.2 ± 0.1	2.2 ± 0.1	.70

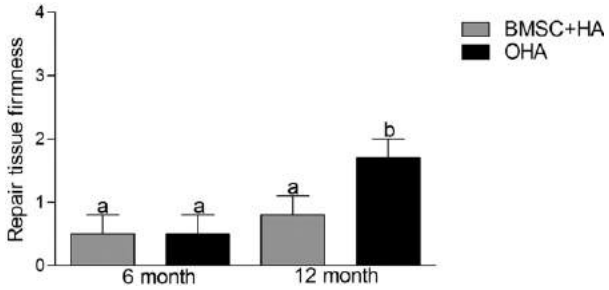

Figure 2. Plot of repair tissue firmness at 6-month second-look arthroscopy and 12-month necropsy evaluation by treatment group. Different letters indicate a significant difference between groups ($P < .05$).

84.7% ± 6.4% of the defect covered with repair tissue compared with 71% ± 6.4% for OHA (Fig 3). There was no significant treatment effect of repair tissue volume, nor was there a significant effect of time. On average, at both time points, the BMSC + HA defects were 54.5% ± 5.6% filled compared with the OHA defects, which were 53.7% ± 5.6% filled.

MRI Evaluation

Evaluation of the magnetic resonance images yielded no significant treatment differences between groups (Table 2, Fig 4).

Histologic Analysis of Synovial Membrane

None of the individual categories showed a significant treatment effect. Likewise, the total cumulative score for synovial membrane pathology had no significant treatment effect; BMSC + HA joints had a mean score of 5.0 ± 0.6, and OHA joints had a mean score of 4.7 ± 0.6.

Histologic Evaluation of Repair Tissue

There was no significant treatment effect on nature of the repair tissue. BMSC + HA–treated joints had a mean value of 0.5 ± 0.2 and OHA a mean value of 0.7 ± 0.2. There was no significant treatment effect on surface regularity. BMSC + HA joints had a mean value of 1.3 ± 0.3 and OHA a mean value of 1.4 ± 0.3. There was no significant treatment effect on structural integrity. BMSC + HA had a mean value of 1.0 ± 0.2 and OHA a mean value of 0.9 ± 0.2. There was a significant treatment effect observed ($P = .04$) on thickness. BMSC + HA had a mean value of 0.4 ± 0.1 and OHA a mean value of 0.8 ± 0.14. This finding is in contradiction to the arthroscopic, gross, and MRI results and may be a result of finite (2 histologic locations) sampling error

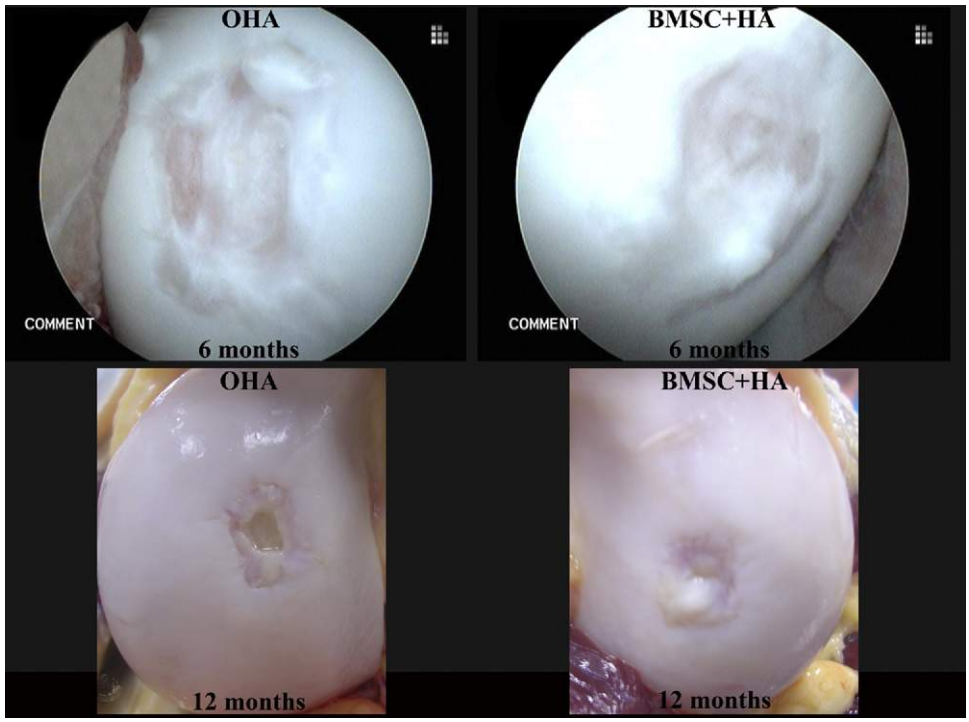

FIGURE 3. Arthroscopic and gross photographs of OHA-treated and BMSC + HA–treated joints at 6 and 12 months (all from the same horse).

because sites of histology were based on exact positioning within the defect rather than visual appraisal of what appeared to be the best repair. There was no significant treatment effect on integration with adjacent cartilage. BMSC + HA had a mean value of 1.4 ± 0.2 and OHA a mean value of 1.3 ± 0.2. There was no significant treatment effect on hypocellularity. BMSC + HA had a mean value of 2.0 ± 0.1 and OHA a mean value of 1.8 ± 0.14. There was no significant treatment effect on chondrocyte clustering. BMSC + HA had a mean value of 1.8 ± 0.2 and OHA a mean value of 1.6 ± 0.2. There was no significant treatment effect on freedom from degenerative changes in adjacent cartilage. BMSC + HA had a mean value of 1.5 ± 0.2 and OHA a mean value of 1.4 ± 0.12. There was no significant treatment effect on reconstruction of subchondral bone. BMSC + HA had a mean value of 1.6 ± 0.3 and OHA a mean value of 1.5 ± 0.3. There was no significant treatment effect on inflammatory response. All values were equal to 2 in all categories. Safranin O staining was graded on a scale from 0 to 3, with 0 being none and 3 being normal. There was a significant treatment effect ($P = .04$), with BMSC + HA having a mean value of 0.5 ± 0.3 and OHA having a mean value of 1.2 ± 0.3. There was no significant treatment effect in the cumulative histology

TABLE 2. *Results of MRI Grading Categories*

MRI Grading Categories	BMSC + HA (Mean)	OHA (Mean)	*P* Value
Osteophytes	1.9 ± 0.3	2.0 ± 0.3	.76
Enthesophytes	1.6 ± 0.2	1.7 ± 0.2	.68
Synovial proliferation	1.6 ± 0.2	1.2 ± 0.2	.17
Joint capsule thickness	2.1 ± 0.3	2.0 ± 0.3	.68
Subchondral bone evaluation	2.3 ± 0.34	2.2 ± 0.3	.84
Bone edema	2.1 ± 0.3	2.2 ± 0.3	.73
Bone sclerosis	3.2 ± 0.3	3.4 ± 0.3	.70
Amount of repair tissue in defect	1.1 ± 0.3	1.0 ± 0.3	.84
Character of repair tissue	1.9 ± 0.7	2.0 ± 0.7	.34
Interface of repair tissue and surrounding cartilage	3.1 ± 0.3	2.6 ± 0.3	.26

NOTE. All categories were graded as slight (1), mild (2), moderate (3), or severe (4).

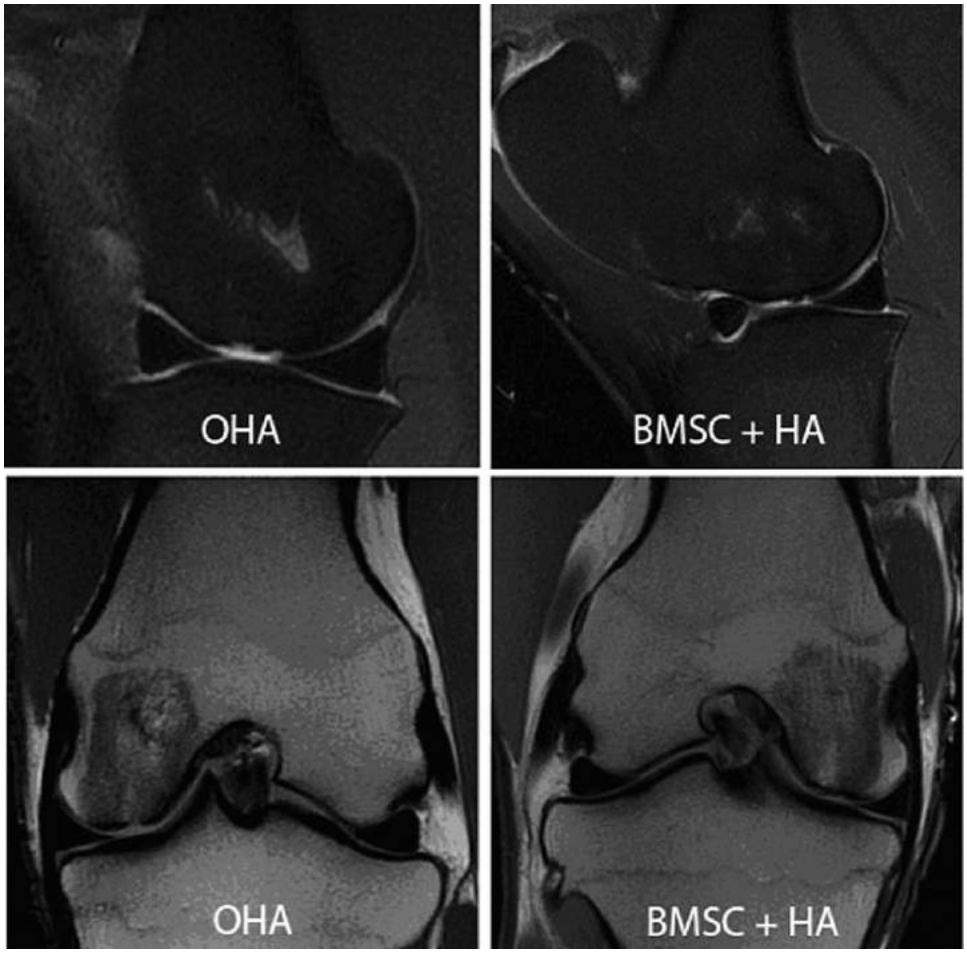

FIGURE 4. MRI proton density fat-saturated sagittal and proton density frontal images showing subchondral bone and articular cartilage defects in the medial femoral condyle. There is sclerosis and osteonecrosis in the adjacent trabecular bone.

score, with BMSC + HA having a mean value of 14.0 ± 1.2 and OHA having a mean value of 14.6 ± 1.2.

Histomorphometry of Repair Tissue and Surrounding Tissue

No significant treatment effects were observed; and the following outlines the assessed categories, with mean values: degree of repair tissue filling the defect (78%); percent repair tissue consisting of granulation (2%) or fibrous tissue (19%), fibrocartilage (35%), or hyaline-like cartilage (16%); amount of new bone (19%) and calcified cartilage (5%) in the defect; and finally, percent attachment of the repair tissue to the surrounding cartilage (77%) and bone (69%).

Immunohistochemical Evaluation of Repair Tissue and Surrounding Tissue

There was no significant treatment effect on type I or II collagen staining in either the repair tissue or surrounding articular cartilage. On average, less than 1% of either the repair or surrounding cartilage stained positive for type I procollagen, and approximately 76% of the surrounding tissue and 67% of the repair tissue stained positive for type II collagen. Likewise, aggrecan staining was shown in approximately 75% of the surrounding and repair tissue after either treatment. However, significantly ($P = .02$) greater aggrecan staining was observed in the repair tissue of BMSC + HA–treated joints (75.2% ± 8%) compared with OHA-treated joints (57% ± 8%) (Fig 5).

Biochemical Analysis

There was no significant effect of treatment on total GAG content of articular cartilage dry weight, with BMSC + HA cartilage having a mean value of 97.4 ± 16 μg/mg dry weight compared with 100.15 ± 16 μg/mg dry weight for OHA cartilage.

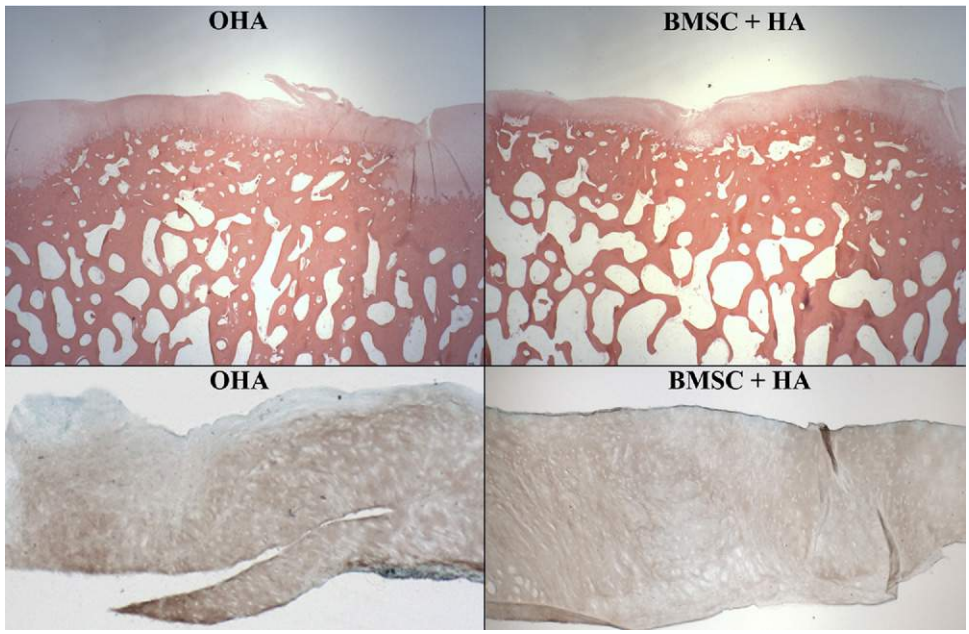

FIGURE 5. Histologic (H & E staining, original magnification ×1) and immunohistochemical (for aggrecan) (original magnification ×4) photographs of OHA-treated and BMSC + HA–treated joints at 12 months. One should note the overall increase in staining for aggrecan in the BMSC + HA group. (These images are from the same horse depicted in Fig 3.)

DISCUSSION

Microfracture has been previously shown to enhance the amount of repair tissue in full-thickness articular cartilage defects on the medial femoral condyle in the horse, and type II collagen content is significantly increased.[8,9] It has also been shown that removal of calcified cartilage with retention of the subchondral bone plate in microfractured defects increased the overall amount of repair tissue as assessed by arthroscopic (4 months) and gross evaluation (12 months); furthermore, there was improved attachment of the repair tissue to the underlying subchondral bone and the adjacent normal cartilage.[10] Our study showed improvement in aggrecan content in microfractured defects that received intra-articular MSCs, a critical part of achieving cartilage repair. Previous work in evaluating defects treated with microfracture at 12 months showed good content and staining for type II collagen but inadequate content and staining for aggrecan.[8,10] This finding probably explains why no differences were seen between the MSC-treated groups and the controls with regard to type II collagen, because that is already increased with microfracture alone. On the other hand, enhancement of aggrecan content could be very beneficial to the durability and quality of the repair tissue and, in particular, its ability to resist compression. The increased aggrecan content parallels the observation of increased firmness in the repair tissue. However, not all outcome parameters were positively improved by the BMSC + HA treatment. Indeed, there were contradictions in the thickness measured through histomorphology when compared with the subjective estimation of defect area filling at gross examination. We assume that the gross examination is more reliable because it estimates the defect area filling as a whole, whereas the histomorphology is limited to estimating the thickness based on two 5-μm sections. Another apparently contradictory point revolves around the safranin O and fast green staining, which indicates more normal staining in the OHA group when compared with the BMSC + HA group; however, the aggrecan staining showed more aggrecan in the BMSC + HA group when compared with the OHA group. It is suggested that the histology results could be associated with sampling error and the safranin O and fast green changes need to be balanced with the quantitative measurement of GAG levels in the tissue where there were no significant differences. There was a modest but definite improvement with BMSC + HA compared with HA alone with quantitative measurement of GAG levels in the tissue. There were no significant differences in the clinical categories, but this finding was not surprising because the degree of lameness is relatively subtle, and it is difficult to discern differences in such studies. This study showed that BMSC + HA provided improvement over microfracture alone. Previous studies have shown improvement with microfracture in full-thickness debrided defects compared with debridement alone; therefore, it is log-

ical that only moderate improvements would be seen in this study.

The positive findings in this study contrast with a recent equine study that showed early benefit with MSCs in a fibrin matrix but noted no significant differences when MSCs plus fibrin were compared with fibrin alone at 8 months.[17] Previous work indicates that there may be difficulty with migration and proliferation of MSCs embedded within full-strength fibrin,[18] and we have evidence that with dilution, there is an increased ability to migrate and proliferate.[18] However, results of a study with partial-thickness defects in the medial femoral condyle of adult minipigs indicate the potential for intra-articular injection of stem cells to improve cartilage healing at 6 and 12 weeks compared with a control group of saline solution or HA.[18] The morphologic and histologic results in that pig study are more dramatic than in our equine study, but the porcine study only went out to 12 weeks whereas we have performed a 12-month study, and much can change in that time period difference. In addition, the horses in our study were subjected to an athletic exercise regimen from 4 to 12 months.

The concept of simple intra-articular injection of MSCs promoting healing confirms the multiple potential ways in which these cells could influence repair in addition to their ability to differentiate into a target cell and synthesize new tissue. It has been previously documented that MSCs secrete a variety of cytokines and growth factors that have both paracrine and autocrine effects, including suppression of the local immune system, inhibition of fibrosis, and apoptosis, as well as stimulation of mitosis and differentiation of stem cells.[24] These effects have been referred to as trophic effects and are distinct from direct differentiation of MSCs into repair tissue. In addition, it has been suggested that endogenous mesenchymal stromal cells could be augmented by these paracrine effects of MSCs themselves.[25] In a recent equine study using an osteochondral fragment with bone and cartilage debris to induce osteoarthritis (rather than relying on joint instability to create secondary osteoarthritis), there was a significant reduction in synovial fluid prostaglandin levels (prostaglandin E_2) in response to treatment with BMSCs injected intra-articularly[26]; however, this effect was not seen with adipose-derived stem cells. That study also showed a negative response through an increase in synovial fluid tumor necrosis factor concentrations in response to intra-articular injection of adipose-derived cells. Positive effects on cartilage repair could therefore be gained by inhibition of catabolism as well as promotion of anabolism through cytokine mediators. In addition, a recent publication has shown that BMSCs produced longer core protein and longer chondroitin sulfate chains and fewer short core protein molecules than chondrocytes from 2- to 5-year-old horses. This observation suggests that BMSC–produced aggrecan has a phenotype more characteristic of young tissue than chondrocyte–produced aggrecan.[27] These findings are in agreement with our in vivo demonstration of increased aggrecan content in equine chondral defects.

The positive findings from this study led us to speculate that the clinical use of BMSCs in conjunction with microfracture of cartilage defects may be potentially beneficial. Further research, including longer-term in vivo animal research and human clinical trials, needs to be done to determine the clinical efficacy of BMSCs in conjunction with microfracture for the treatment of cartilage defects.

CONCLUSIONS

There were no significant clinical improvements or histologic differences between the groups. It is noteworthy that there was statistically significant enhancement in repair tissue firmness and repair quality based on improved aggrecan staining after intra-articular administration of bone marrow–derived culture-expanded MSCs. However, the clinical significance of these findings is yet to be established. Our hypothesis was affirmed in part.

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