Articular Cartilage Regeneration With Autologous Peripheral Blood Stem Cells Versus Hyaluronic Acid: A Randomized Controlled Trial

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Purpose: The purpose of this study was to compare histologic and magnetic resonance imaging (MRI) evaluation of articular cartilage regeneration in patients with chondral lesions treated by arthroscopic subchondral drilling followed by postoperative intra-articular injections of hyaluronic acid (HA) with and without peripheral blood stem cells (PBSC). **Methods:** Fifty patients aged 18 to 50 years with International Cartilage Repair Society (ICRS) grade 3 and 4 lesions of the knee joint underwent arthroscopic subchondral drilling; 25 patients each were randomized to the control (HA) and the intervention (PBSC + HA) groups. Both groups received 5 weekly injections commencing 1 week after surgery. Three additional injections of either HA or PBSC + HA were given at weekly intervals 6 months after surgery. Subjective IKDC scores and MRI scans were obtained preoperatively and postoperatively at serial visits. We performed second-look arthroscopy and biopsy at 18 months on 16 patients in each group. We graded biopsy specimens using 14 components of the International Cartilage Repair Society Visual Assessment Scale II (ICRS II) and a total score was obtained. MRI scans at 18 months were assessed with a morphologic scoring system. **Results:** The total ICRS II histologic scores for the control group averaged 957 and they averaged 1,066 for the intervention group ($P = .022$). On evaluation of the MRI morphologic scores, the control group averaged 8.5 and the intervention group averaged 9.9 ($P = .013$). The mean 24-month IKDC scores for the control and intervention groups were 71.1 and 74.8, respectively ($P = .844$). One patient was lost to follow-up. There were no notable adverse events. **Conclusions:** After arthroscopic subchondral drilling into grade 3 and 4 chondral lesions, postoperative intra-articular injections of autologous PBSC in combination with HA resulted in an improvement of the quality of articular cartilage repair over the same treatment without PBSC, as shown by histologic and MRI evaluation. **Level of Evidence:** Level II, randomized controlled trial (RCT).

As clinicians have sought to improve methods for articular cartilage repair, investigation has recently focused on cell therapy. Limitations regarding marrow stimulation alone and mature chondrocyte cell therapy have led to the investigation of stem cells.[1-4] Disadvantages of marrow stimulation include the formation of fibrocartilage and decreasing clinical scores after 24 months in some series.[5,6] Chondrocyte therapy also has produced fibrocartilage in some studies and requires seeding of a matrix/scaffold, expensive cell culture, and a protracted recovery because of open surgery.[7-9] Recent application of stem cell therapy in the setting of cartilage regeneration has animal and clinical studies to support its safety and efficacy.[9-17]

We began our investigations of cell therapy for cartilage regeneration in a goat model using subchondral drilling in 3 groups: one with no postoperative injections, one with postoperative injections of hyaluronic acid (HA) alone, and one with postoperative injections of bone marrow aspirate (BMA) and HA. Histologic grading with the Gill score illustrated best

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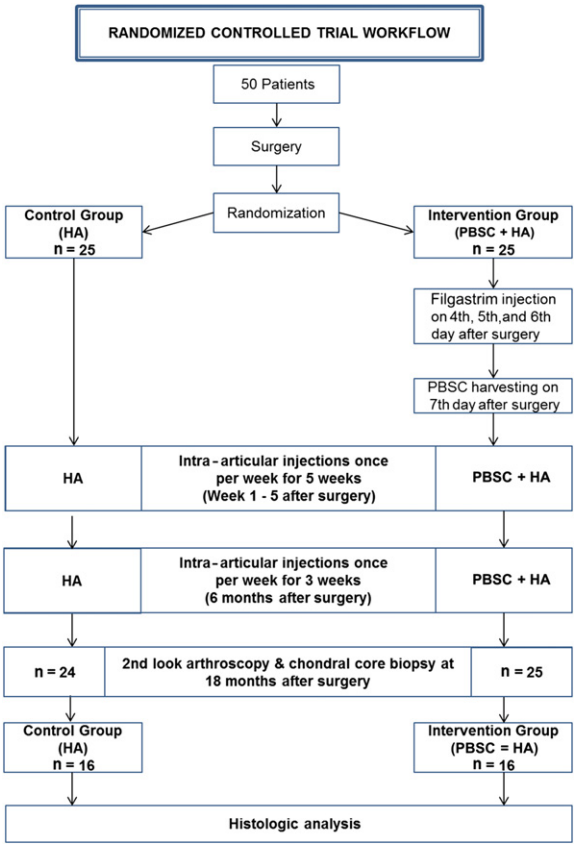

Fig 1. Flowchart of study trial.

outcomes in the group treated with injections of BMA and HA; the worst outcomes were observed in the group with no postoperative injections.[14] This led the first author (K.-Y.S.) to initiate a pilot clinical study. Peripheral blood stem cells (PBSC) were used as opposed to cultured mesenchymal stem cells (MSC) or marrow aspirate because of the ease of harvest[18,19] and the increased potential of this cell line. We recently published the methodology, scientific basis, and results of a case series, including 5 cases with histologic evaluation.[15] We concluded that articular hyaline cartilage regeneration is possible with arthroscopic subchondral drilling followed by postoperative intra-articular injections of autologous PBSC in combination with HA. These results led to our initiating a randomized controlled trial (RCT) comparing postoperative injections of HA alone to postoperative injections of PBSC in combination with HA.

The purpose of this study was to compare histologic and MRI evaluation on articular cartilage regeneration in patients with chondral lesions treated by arthroscopic subchondral drilling followed by postoperative intra-articular injections of HA with and without PBSC. We hypothesized that after arthroscopic subchondral drilling, postoperative intra-articular injections of autologous PBSC and HA would improve the quality of articular cartilage repair, as shown with histologic and MRI evaluations, better than injections without PBSC.

Methods

Institutional Review Board approval was obtained from the Medical Ethics Committee at Universiti Putra Malaysia. This trial was registered under clinicaltrial.gov (NCT01076673).

A sample-sized study was performed before initiation of the trial based on a cohort study involving standard marrow stimulation and retrospective review of pilot data.[15] Based on subjective International Knee Documentation Committee (IKDC) clinical scores, the minimum recruitment quota for each group to achieve the desired statistical power of 80% at 48 months was calculated as 50 patients. For histologic evaluation, to achieve the desired statistical power of 80%, a sample size of 8 for each group was calculated using biopsy sample histologic characteristics with reference to the International Cartilage Repair Society Visual Assessment Scale II (ICRS II) scores[20] and histologic scores from the previous goat model.[14] Since the purpose of this study was evaluation with a histologic endpoint at 18 months, recruitment of 25 patients per group was deemed sufficient.

From November of 2009 to December of 2010, enrollment was offered to patients presenting to the first author (K.-Y.S.) for treatment of knee pain and who had clinical and radiographic confirmation of a cartilage defect. A summary of this study methodology is shown in the flowchart presented in Fig 1.

Patient Selection

We recruited 50 patients. The diagnosis of chondral injury to the knee joint was made after clinical and radiologic evaluation. The inclusion criteria included male and female patients aged 18 to 50 years, with ICRS[21] grade 3 and 4 lesions of the knee joint. Preenrollment data collection involved a clinical examination, radiographs and MRI scans, medical screening, a baseline subjective IKDC score, and informed consent. Exclusion criteria included a history of more than one surgery on the knee in question or previous lower extremity amputation or significant peripheral vascular disease, a calculated body mass index (BMI) of 35 or greater, a varus/valgus deformity of more than 10°, a deformity requiring osteotomy or complex surgery, a positive pregnancy test, or the inability to speak English/local language, ambulate, tolerate MRI, answer subjective IKDC questionnaires, or provide informed consent. Patients were not excluded based on number of cartilage lesions or size. The chondral lesions were degenerative in nature.

Surgical Procedures and Postoperative Rehabilitation

All surgical procedures were performed by a single surgeon (K.-Y.S) and involved standard arthroscopic procedure with the patient in the supine position and

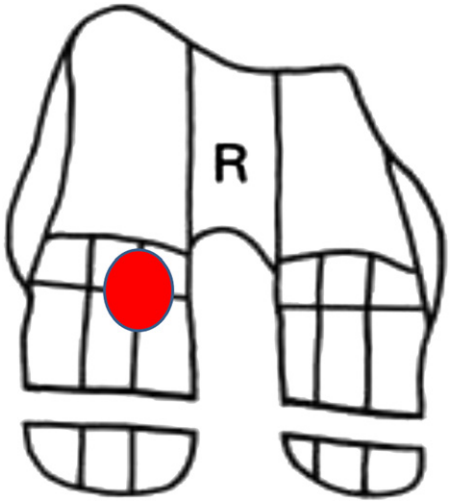

Fig 2. An example of a lesion (red circle) mapped over 4 quadrants using ICRS mapping system. (R, right.)

without the use of a tourniquet. Chilled saline irrigation solution was used to minimize bleeding during the arthroscopic procedure. Subchondral drilling and abrasion chondroplasty were performed over the chondral defects. After surgery, continuous passive motion was used on the operated knee 2 hours per day for a period of 1 month. The details of the surgical procedure and postoperative rehabilitation have previously been published.[15,22]

ICRS Articular Cartilage Injury Mapping

We used the ICRS articular cartilage injury mapping system and outlined 48 quadrants per knee for documentation.[21] We documented the areas after subchondral drilling according to the ICRS mapping system and archived them. For example, a lesion that is mapped as shown in Fig 2 was counted as 4 quadrants. This allowed for identification and comparison during second-look arthroscopy and biopsy.

Randomization

Fifty patients were randomized after surgery by using a computer system that generated sequentially numbered sealed envelopes with a randomized sequence; 25 patients were randomized to the control group (HA) and 25 patients to the intervention group (PBSC + HA). Because we harvested cells in the intervention group, patient blinding was not possible.

Filgrastim Administration, Apheresis, and Cryopreservation

Twenty-five patients in the intervention group (PBSC + HA) underwent autologous PBSC harvesting through apheresis. Stem cell harvesting in this group was performed 1 week after surgery and stimulation with filgrastim, which contains recombinant human granulocyte colony-stimulating factor. The PBSC were then divided, placed in vials, and cryopreserved. The details of the harvesting procedure and cell preparation have been outlined in our previous reports.[15,22]

Intra-articular Injections

All patients received 8 postoperative intra-articular injections. This regimen is based on the HA protocol for osteoarthritis, as well as the suggestion from preclinical animal studies involving BMA and HA, which found that an increased number of intra-articular injections is more efficacious.[14] The first 5 injections began at 1 week and continued on a weekly basis. At 6 months, 3 additional intra-articular injections were administered at weekly intervals. The control group received 2 mL of HA in each injection. The intervention group received 8 mL of PBSC in combination with 2 mL of HA. Before the intra-articular injections, the operated knee was first aspirated for hemarthrosis.

Subjective IKDC Scores

We obtained preoperative subjective IKDC scores[21] as well as serial subjective IKDC scores at 6, 12, 18, and 24 months after surgery.

MRI Scans

We obtained MRI scans preoperatively and postoperatively on day 1 and 6 months, 12 months, and 18 months after surgery. Postoperative MRI was performed in a 1.5-T extremity MRI (ONI MSK Extreme; GE Healthcare, Waukesha, WI) with the use of a transmit-and-receive phased-array radiofrequency coil. Intermediate weighted fast spin-echo images were acquired in the sagittal and axial planes to assess articular cartilage with the use of a previously validated cartilage-sensitive pulse sequence. All images were acquired with a repetition time of 2,000 to 2,500 msec, echo times of 30 to 40 msec, a field of view of 16 cm^2, a matrix of 512×488, and an echo train length of 6, acquiring images at 4 mm with a 10% gap. The MR images at the 18-month time point were evaluated by a single blinded musculoskeletal radiologist using a scoring system developed from morphologic MRI evaluation as described by Mithoefer et al.[6] The scoring system had a maximum score of 12 and is outlined in Table 1.

Second-Look Arthroscopy With Chondral Core Biopsy

An informed consent for second-look arthroscopy and chondral biopsy was requested of all patients in the study. The procedure was performed 18 months after the initial surgery on a volunteer basis and after obtaining a second informed consent pertaining to the biopsy. We procured a chondral core biopsy specimen from the center of each regenerated articular cartilage lesion. The number of biopsy specimens obtained per patient varied because the number of cartilage lesions

Table 1. Criteria, Findings, and Score System for MRI Evaluation

Criteria	Findings	Scores
Repaired cartilage signal	Isointense	2
	Hyperintense	1
	Hypointense	0
Repaired lesion morphologic features	Flush	2
	Proud	1
	Depressed	0
Repaired cartilage fill	Good (67%-100%)	2
	Moderate (34%-66%)	1
	Poor (0%-33%)	0
Peripheral repaired cartilage integration	No gap	2
	Small (gap of ≤2 mm)	1
	Large (gap of >2 mm)	0
Subchondral edema	None	3
	Mild (<1 cm^2)	2
	Moderate (1-3 cm^2)	1
	Severe (>3 cm^2)	0
Osseous overgrowth	No	1
	Yes	0

MRI, magnetic resonance imaging.

and size of lesions varied among patients. Biopsy samples were obtained from all areas of cartilage repair that were readily accessible. Some areas were not accessible, such as the central patella, which underwent initial treatment in conjunction with a lateral release. It would have been unethical to perform a lateral release for the purpose of biopsy, and thus not all patellar lesions were biopsied. Typically, a 2-mm-diameter specimen of cartilage together with a core of bone up to 1 cm in length was obtained.[15]

Histologic Evaluation and Grading Using the ICRS II

We stained histologic samples with H&E to visualize overall morphologic features and with Safranin O to highlight proteoglycans. We performed immunohistochemical staining with anti—collagen type I mouse Ab I-8H5 (catalog No. CP 17; Calbiochem Merck, Darmstadt, Germany) to highlight collagen type I, and with anti—collagen type II mouse monoclonal antibody Ab 3 (clone 6B3) (catalog No. MAB8887; Millipore, Billerica, MA) to highlight collagen type II. Optimal dilution and predigestion with pepsin were determined by the investigator, with the protocol saved using the software of an automated immunohistochemical slide preparation system (Ventana BenchMark; Ventana Medical Systems, Tucson, AZ). The biopsy specimens were graded by 2 independent blinded histopathologists using all 14 components of the ICRS II. Light and polarized microscopy were used during the grading process. For each of the 14 ICRS II parameters, a score between 0 and 100 was given as described by the initial ICRS II publication. To obtain an overall histologic quality score of the repaired tissue, we calculated a total score by summation of these 14 histologic parameters for each of the biopsy samples. This scoring system had a maximum score of 1,400. We then obtained an average score for each patient for those who had more than one biopsy sample.

Statistics

Because the subjective IKDC and ICRS articular cartilage injury mapping and ICRS II scores were distributed normally, we used an independent *t* test to evaluate for a difference among the averages of all the scores as well as the demographic data. Because the MRI and biopsy area results were not normally distributed, the nonparametric Wilcoxon rank-sum test was used to investigate for a difference in scores between the 2 groups; $P < .05$ was considered statistically significant in this study. For a given patient with more than one lesion, an average MRI score was calculated from all the lesions. Similarly, for a patient with multiple biopsy samples, an average ICRS II score was calculated from all the biopsy specimens and used in the final *t* test. Hence, for the total ICRS II scores, 16 scores were produced per group. For the MRI scores, 24 scores were produced for the control group and 25 scores were produced for the intervention group. One patient in the control group was lost to follow-up. For subjective IKDC scores, we obtained 25 scores from both groups for 0 and 6 months. At months 12 and 18, 24 scores were produced for the control group and 25 scores were produced for the intervention group, and at month 24, 20 scores were obtained from both groups.

Results

Group Comparison

Demographics of the study participants are presented in Table 2. One patient in the control group was lost to follow-up. There was a significant difference in the age of the subjects in the 2 groups. The average age of the control group was 42 years and the average age of the intervention group was 38 years ($P = .031$).

Subjective IKDC Scores

At the 24-month time point, the average subjective IKDC score for the control group was 71.1 and it was 74.8 for the intervention group. The Student *t* test evaluation produced $P = .844$, which is not statistically significant. Figure 3 shows the progressive improvement of the subjective IKDC scores.

MRI Results

On morphologic grading of the MRI data obtained at 18 months with the 12-point scale outlined earlier, the control group averaged 8.5, whereas the intervention group averaged 9.9, with $P = .013$, which is statistically significant (Fig 4, Appendix Table 1). Comparison of the 2 groups according to the individual elements of the scoring system is presented in Table 3.

Table 2. Demographics for the Control and Intervention Groups

Characteristics	Control Group (HA)	Intervention Group (PBSC + HA)	*P* value
Total number	24	25	—
Sex	7 (30%) male 17 (70%) female	10 (40%) male 15 (60%) female	—
Age (yr)	42 (5.91) [22-50]	38 (7.33) [22-48]	.03
Height (m)	1.64 (0.10)	1.65 (0.11)	.76
Weight (kg)	66.89 (13.92)	67.86 (15.30)	.82
BMI (kg/m^2)	24.83 (4.04)	24.91 (4.15)	.96

NOTE. Data are presented as mean (standard deviation) [range].
BMI, body mass index; HA, hyaluronic acid; PBSC, peripheral blood stem cell.

ICRS Articular Cartilage Injury Mapping

During the initial surgery, the areas of chondral lesion that underwent subchondral drilling were documented with the ICRS mapping system.[21] The distribution of cartilage lesions is shown in Table 4. Comparing the 2 groups, $P = .569$. The average number of quadrants involved according to ICRS mapping for the intervention group was 5.1 (range, 1 to 12) and for the control group it was 6.0 (range, 2 to 13).

Second-Look Arthroscopy With Biopsy

Thirty-two patients consented to second-look arthroscopy and biopsy at 18 months, with 16 patients in each group. The control group (HA) consisted of 34 biopsies and the intervention group (PBSC + HA) consisted of 31 biopsies. Table 5 lists the areas from which the biopsy specimens were procured. Comparing the 2 groups, $P = .224$. The average number of biopsies for the intervention group was 1.8 (range, 1 to 4) and for the control group the number was 2.1 (range, 1 to 4). Figures 5 and 6 present 2 opposing cases illustrating second-look arthroscopy and biopsy findings of the medial femoral condyles. Both patients were 47-year old women with lesions of approximately 250 mm^2 at the medial femoral condyle.

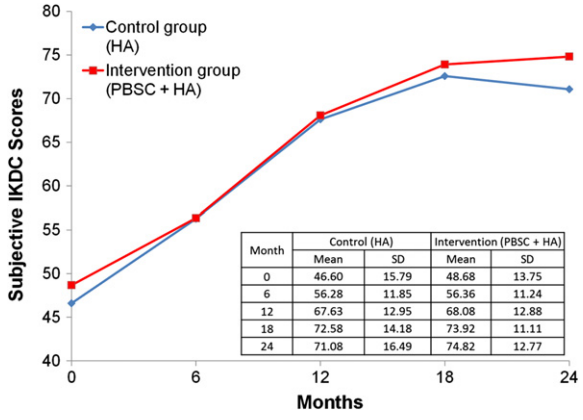

Fig 3. Subjective IKDC outcomes. (SD, standard deviation.)

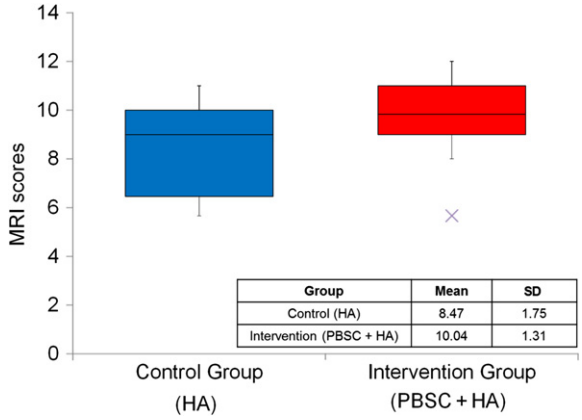

Fig 4. Box plot of MRI score by treatment groups, with the line in the middle of the box representing the median and "X" representing the outlier. Refer to Appendix Table 1 for the data points.

Histologic Evaluation and Grading Using the ICRS II

In evaluating the histologic results, the average total ICRS II score for the control group was 957 and was 1,066 for the intervention group ($P = .022$), which is statistically significant (Fig 7; Appendix Table 2).

Complications and Adverse Events

One patient in the control group had a deep vein thrombosis below the knee diagnosed by duplex ultrasonography 1 day after surgery. This was treated with a standard anticoagulation regimen without any long-term sequelae. There were no postoperative infections. Table 6 shows a compilation of adverse events related to the postoperative injections for the first 24 hours (acute response) and thereafter (delayed response). Both responses showed no statistical significance, with $P = .513$ for acute response and $P = .554$ for delayed response.

Discussion

The results of this RCT evaluating postoperative intra-articular injections of PBSC and HA after arthroscopic subchondral drilling showed a significant statistical improvement in histologic and MRI scores. This confirmed our hypothesis that after arthroscopic subchondral drilling, postoperative intra-articular injections of autologous PBSC in combination with HA can improve the quality of articular cartilage repair compared with the injections without PBSC.

In assessing the results of articular cartilage regeneration, second-look arthroscopy with chondral core biopsy and MRI scan are synergistic. Second-look arthroscopy allows for articular surface visualization of the repaired cartilage, and chondral core biopsy evaluates a 2-mm diameter area through the subchondral plate. However examination of the biopsy sample assesses only a small proportion of the repaired cartilage, whereas MRI has the advantage of assessing the entire repair area and the

Table 3. Comparison of Control to Intervention Group With MRI Morphologic Scoring

Finding	No. (%)* Control Group (HA only)	Intervention Group (PBSC + HA)
Repaired cartilage signal		
Isointense	34 (58%)	24 (43%)
Hyperintense	3 (5%)	4 (7%)
Hypointense	22 (37%)	28 (50%)
Repaired lesion morphologic features		
Flush	32 (54%)	38 (68%)
Proud	8 (14%)	8 (14%)
Depressed	19 (32%)	10 (18%)
Repaired cartilage fill		
Good (67%-100%)	35 (59%)	46 (82%)
Moderate (34%-66%)	10 (17%)	6 (11%)
Poor (0%-33%)	14 (24%)	4 (7%)
Peripheral repaired cartilage integration		
No gap	35 (59%)	44 (79%)
Small (gap of ≤2 mm)	15 (25%)	9 (16%)
Large (gap of >2 mm)	9 (15%)	3 (5%)
Subchondral edema		
None	31 (53%)	36 (64%)
Mild (<1 cm^2)	22 (37%)	19 (34%)
Moderate (1-3 cm^2)	3 (5%)	1 (2%)
Severe (>3 cm^2)	3 (5%)	0 (0%)
Osseous overgrowth	1 (2%)	0 (0%)

HA, hyaluronic acid; PBSC, peripheral blood stem cells.
*Percentage of total in parentheses.

whole joint in a noninvasive manner. The 49 patients from our RCT assessed by MRI at 18 months showed no evidence of adverse synovial or osseous changes.

When evaluating stem cell therapy for articular cartilage regeneration, this is the first RCT, to our knowledge, involving PBSC. Although there are animal studies, we are unaware of any clinical study comparing stem cell therapy to the absence of stem cell therapy as was compared in this study.[10,11,14] There is one clinical comparison trial involving MSC. Nejadnik et al.[13] prospectively compared stem cell therapy using autologous bone-marrow–derived MSC to chondrocyte cell therapy using autologous chondrocyte implantation (ACI). Similar clinical outcomes were found with measured questionnaires at 2 years, suggesting that stem cell therapy with MSC is as effective as ACI.

Table 4. ICRS Mapping Results Presented by Anatomic Subregion

Lesion Location	Control Group (HA)	Intervention Group (PBSC + HA)
Total ICRS areas	143	127
Patella	77 (54%)	73 (57%)
Trochlear	30 (21%)	29 (23%)
Medial femoral condyle	20 (14%)	10 (8%)
Lateral femoral condyle	3 (2%)	5 (4%)
Medial tibial plateau	8 (5%)	7 (6%)
Lateral tibial plateau	5 (4%)	3 (2%)

NOTE. Percent of total in parentheses.
HA, hyaluronic acid; ICRS, International Cartilage Repair Society; PBSC, peripheral blood stem cells.

Although clinical data are limited, evidence from animal models continues to support the theory that stem cell therapy can effectively augment cartilage regeneration. The histologic findings of this RCT are similar to those of our previously published animal model.[14] In that study, the best outcomes were seen in a group treated with injections of BMA and HA after subchondral drilling compared with a group treated with injections of HA alone after subchondral drilling and a group with no injections after subchondral drilling. Poor results were encountered in the group with no injections after subchondral drilling, including histologic features suggestive of fibrocartilage and poor fill of the treated defects. This evidence from our animal model led to the 2-group design of our RCT, because a group without postoperative injection was deemed unethical.[14]

Additional animal studies have supported the use of stem cell therapy to augment cartilage repair and guided our use of PBSC. Culture-expanded bone-marrow–derived MSC were first evaluated in 1994 in a rabbit model.[17] This study illustrated the differentiation of cultured MSC embedded in a collagen I gel to chondrocytes at 2 weeks.[17] A second study involving culture-derived MSC in a mini-pig model showed improved histologic and morphologic assessment in a group treated with one postsurgical injection of MSC and HA in the first week and 2 injections of HA alone in the subsequent 2 weeks compared with HA injections alone. This study also localized fluorescence-labeled MSC to the cartilage repair tissue.[11] Additional study compared undifferentiated MSC to MSC differentiated toward the chondrocyte lineage in a porcine model, showing better results in the undifferentiated group. The undifferentiated nature of PBSC, the large numbers of cells that are available through apheresis, and the proliferative/multipotentiality properties that they have shown in animal studies[23] led us to use PBSC in clinical practice, with more encouraging histologic results, which we have published previously.[15]

The results of this RCT show that the histologic features of the intervention group were more consistent with normal articular cartilage when compared with the control group using the ICRS II histologic grading system ($P = .022$). When comparing the total areas of

Table 5. Biopsy Areas Presented by Anatomic Subregion*

Lesion Location	Control Group (HA)	Intervention Group (PBSC + HA)
Total biopsy areas	34	31
Patella	15 (44%)	10 (32%)
Trochlea	12 (35%)	13 (42%)
Medial femoral condyle	4 (12%)	6 (20%)
Lateral femoral condyle	2 (6%)	1 (3%)
Medial tibial plateau	1 (3%)	1 (3%)
Lateral tibial plateau	0 (0%)	0 (0%)

HA, hyaluronic acid; PBSC, peripheral blood stem cells.
*Percent of total in parentheses.

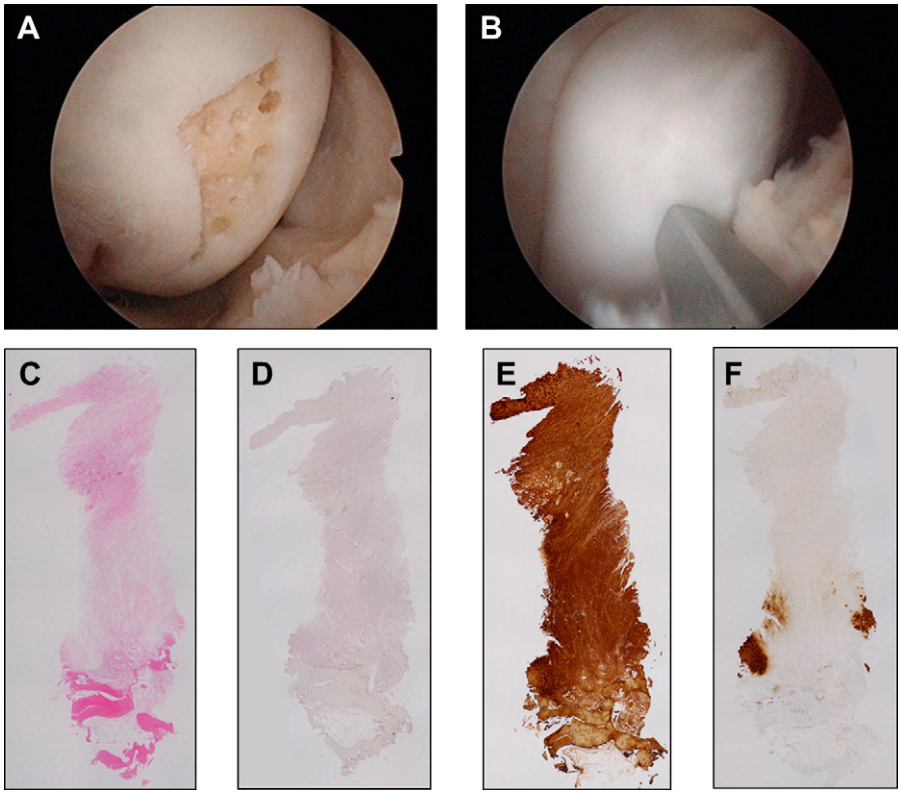

Fig 5. A 47-year-old woman in the control group (HA) with a cartilage lesion of the right medial femoral condyle. (A) Arthroscopic view from the anterolateral portal after subchondral drilling. (B) The process of chondral core biopsy over the center of the regenerated cartilage at 18 months. (C) H&E staining of the biopsy specimen showing mainly fibrous tissue. (D) Safranin O staining shows absence of proteoglycans. (E) Collagen type I staining was extensive. (F) Collagen type II staining was minimal.

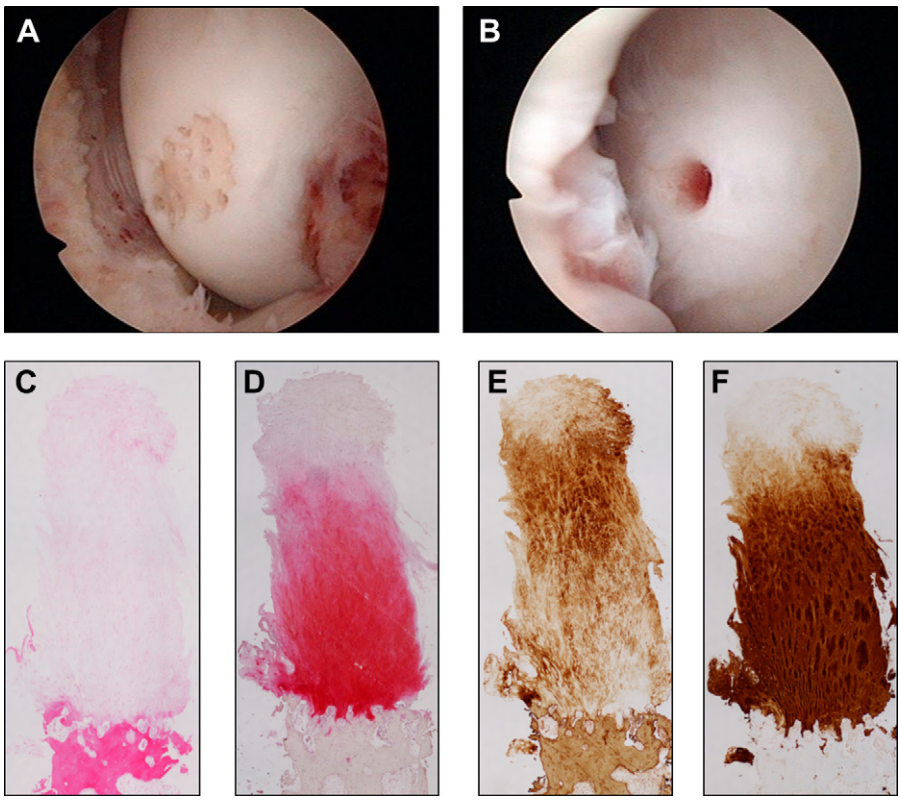

Fig 6. A 47-year-old woman in the intervention group (PBSC + HA) with a cartilage lesion of the left medial femoral condyle. (A) Arthroscopic view from the anterolateral portal after subchondral drilling. (B) View after chondral core biopsy over the central area of the regenerated cartilage at 18 months. (C) H&E staining shows the presence of chondrocytes. (D) Safranin O staining showed extensive deposition of proteoglycans. (E) Collagen type I staining mainly in the upper half of the biopsy specimen. (F) Collagen type II staining was extensive over the lower two thirds of the chondral biopsy specimen.

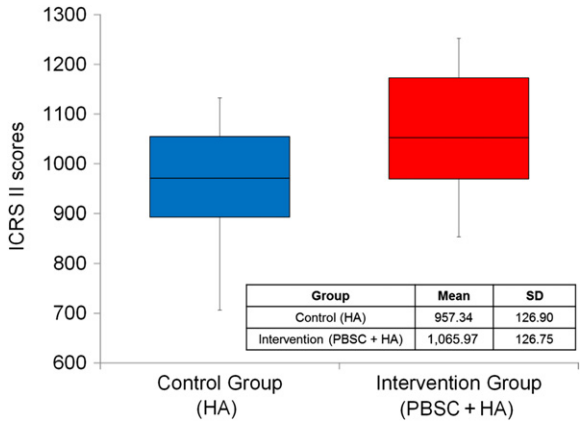

Fig 7. Box plot showing ICRS II scores for control group (HA) and intervention group (PBSC + HA). Refer to Appendix Table 2 for the data points.

biopsy samples between the control and the intervention groups, the groups are matched ($P = .224$). Although the ICRS II scoring system does not involve immunohistochemical staining for collagen type I and type II, we included these in our observation of the quality of articular cartilage repair. For example, when comparing the chondral core biopsy specimens from the medial femoral condyle of the control and intervention groups (Figs 5 and 6), the quality of the repair tissue of the intervention group more closely resembled hyaline cartilage (Fig 6). When stained with Safranin O, this specimen from the intervention group showed an abundance of proteoglycans throughout the regenerated articular cartilage. Additionally, collagen type I was distributed mainly over the superficial layer, and collagen type II was present throughout the deeper layers. These features approached what is seen with normal articular cartilage obtained from a normal biopsy specimen (Fig 8) as opposed to fibrocartilage.

Our morphologic MRI scores showed statistical significance ($P = .013$) comparing the control group (HA) with the intervention group (PBSC + HA). When comparing the 2 groups with the individual components of the scoring system, the intervention group scored 14% higher with flush morphologic features, 23% higher on good repaired cartilage fill, and 20% higher on no gap integration. When comparing our findings with a study involving a standard microfracture technique, a notable difference is seen regarding subchondral edema. We consider this significant, because subchondral edema represents subchondral bone stress. [24,25] In our control group, 10% of patients had moderate to severe edema and 90% had no to mild subchondral edema. In contrast, 2% of patients in the intervention group had moderate to severe edema and 98% had no to mild edema. Mithoefer et al.[6] reported 71% with moderate to severe edema and 29% with no to mild edema. We theorize that the lowest incidence of subchondral edema seen in our intervention group can be explained by the quality of the repaired tissue. Repaired tissue that approaches native hyaline cartilage provides more protection to the underlying subchondral bone. Mithoefer et al. also reported osseous overgrowth of 25% in their study. We noted no osseous overgrowth in our intervention group (PBSC + HA) and one case (2%) of osseous overgrowth in the control group (HA). Although we used a different technique, we theorize that the HA lowered the incidence of osseous overgrowth in the control group when compared with the study of Mithoefer et al.[6]

Our 24-month subjective IKDC clinical scores (Fig 3) show a significant increase similar to studies in the literature documenting overall outcomes with marrow stimulation and chondrocyte implantation; however comparison is difficult at the 24-month time point.[6,26-31] Clinical outcomes in our RCT measured by subjective IKDC questionnaires at 24 months showed continued improvement in both groups, with no statistical significance. Based on our previous pilot study,[15] this is an expected trend and observation. Nevertheless, a longer follow-up is necessary to determine whether a statistical significance and clinical difference will be seen in these 2 groups as time progresses. We hypothesized that as our RCT matures, a significant difference will emerge in the clinical

Table 6. Compilation of Adverse Events (Reported in Number of Patients) Related to the Postoperative Injections*

	Acute Intra-articular Response		Delayed Intra-articular Response	
Event	Control Group (HA)	Intervention Group (PBSC + HA)	Control Group (HA)	Intervention Group (PBSC + HA)
Pain at injection site	7	7	3	2
Persistent bleeding	0	0	0	0
Swelling in knee	8	15	8	12
Warmth in knee	9	16	8	12
Difficulty moving knee	11	10	7	8
Infection in knee	0	0	0	0
Other	0	2	0	1
Total	35	50	26	35
P value	.513		.554	

HA, hyaluronic acid; PBSC, peripheral blood stem cells.
*Acute refers to first 24 hours and delayed refers to after 24 hours.

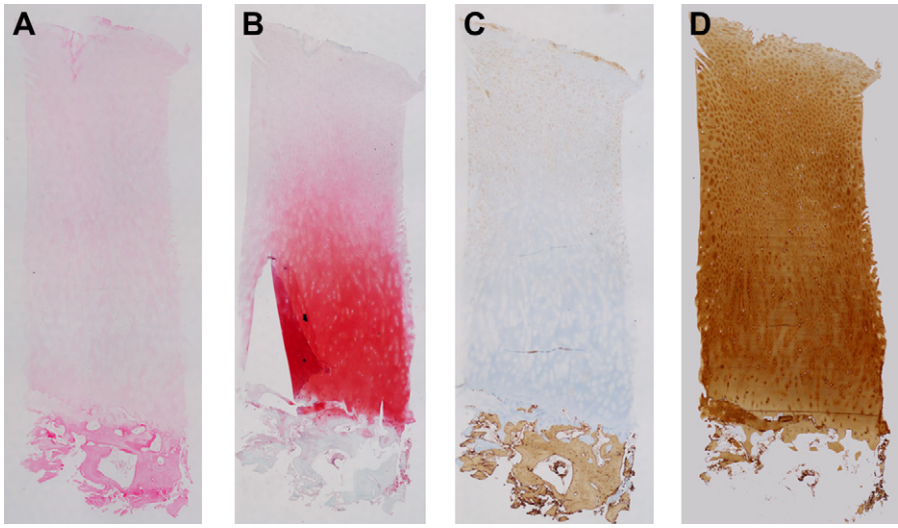

Fig 8. Histologic features of normal articular cartilage showing (A) H&E staining; (B) Safranin O staining for proteoglycans; (C) collagen type I staining, which is almost absent other than the surface of the biopsy specimen; and (D) collagen type II staining, which was extensive over the whole specimen.

outcome scores between the control group and the intervention group.

There is no statistical significance in the reported adverse events for the 2 groups, but it was noted that the symptoms of discomfort were more pronounced in the intervention group (PBSC + HA). This could be explained by the trophic effects of the PBSC after injection. We theorize that PBSC perform 2 functions during the process. The first function is PBSC integration and differentiation toward the osteocyte and chondrocyte line.[23] The second function is the activation of PBSC to release trophic factors into the local environment after intra-articular injections.[32] The localized warmth resulting and discomfort experienced in the intervention group may be explained by this theory.

Limitations

The statistical significance observed for the mean age between the 2 groups was an unforeseen limitation. Although this represents a confounding variable, we do not believe it is responsible for the differences seen on histologic and MRI evaluation, as this was an RCT. A study investigating MSC availability based on age illustrated differences with an age gap of more than 10 years.[33] In addition, a recent study conducted to evaluate the relationship between age and the ability of colony-forming capacity in PBSC found that there was no statistical significance when comparing the apheresis cycles and colony-forming capacity between young (<60 years) and older (>60 years) patients.[34] With these facts in mind, we do not feel that the 4-year difference between the 2 groups would affect the outcome of our study to the extent observed in the histologic and MRI scores. Nevertheless, the age difference highlights the fact that the current sample size of the study may mask some minor differences that truly exist between the groups. The use of a new MRI morphologic scoring system in this study, although developed from a well-cited source,[6] is another limitation; nonetheless, the results still reflect true findings. We consider the 24-month IKDC value an early time point and thus an additional limitation for clinical outcome assessment. These patients will be followed to obtain 5- and 10-year data that will help provide further definitive information.

Conclusions

After arthroscopic subchondral drilling into grade 3 and 4 chondral lesions, postoperative intra-articular injections of autologous PBSC in combination with HA resulted in an improvement of the quality of articular cartilage repair over the same treatment without PBSC, as shown by histologic and MRI evaluation.

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References

1. Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell* 1978;15:1313-1321.

2. Hunziker EB. Articular cartilage repair: Basic science and clinical progress: A review of the current status and prospects. *Osteoarthritis* 2002;10:432-463.
3. Magnussen RA, Dunn WR, Carey JL, Spindler KP. Treatment of focal articular cartilage defects in the knee: A systematic review. *Clin Orthop Relat Res* 2008;466: 952-962.
4. Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: Generations of autologous chondrocyte transplantation. *Eur J Radiol* 2006;57:24-31.
5. LaPrade RF, Bursch LS, Olson EJ, Havlas V. CS Carlson. Histologic and immunohistochemical characteristics of failed articular cartilage resurfacing procedures for osteochondritis of the knee: A case series. *Am J Sports Med* 2008;36:360-368.
6. Mithoefer K, Williams RJ III, Warren RF, et al. The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. *J Bone Joint Surg Am* 2005;87:1911-1920.
7. Koga H, Engebretsen L, Brinchmann JE, Muneta T, Sekiya I. Mesenchymal stem cell-based therapy for cartilage repair: A review. *Knee Surg Sports Traumatol Arthrosc* 2009;17:1289-1297.
8. Bhosale AM, Richardson JB. Articular cartilage: Structure, injuries and review management. *Br Med Bull* 2008;87:77-95.
9. Minas T, Chiu R. Autologous chondrocyte implantation. *Am J Knee Surg* 2000;13:41-50.
10. Fortier LA, Potter HG, Rickey EJ, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am* 2010;92:1927-1937.
11. Lee KB, Hui JH, Song IC, Ardany L, Lee EH. Injectable mesenchymal stem cell therapy for large cartilage defects—A porcine model. *Stem Cells* 2007;25: 2964-2971.
12. McIlwraith CW, Frisbie DD, Rodkey WG, et al. Evaluation of intra-articular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy* 2011;27:1552-1561.
13. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. *Am J Sports Med* 2010;38: 1110-1116.
14. Saw KY, Hussin P, Loke SC, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: An experimental study in a goat model. *Arthroscopy* 2009;25:1391-1400.
15. Saw KY, Anz AW, Merican S, et al. Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: A report of 5 cases with histology. *Arthroscopy* 2011; 27:493-506.
16. Tay LX, Ahmad RE, Dashtdar H, et al. Treatment outcomes of alginate-embedded allogenic mesenchymal stem cells versus autologous chondrocytes for the repair of focal articular cartilage defects in a rabbit model. *Am J Sports Med* 2012;40:83-90.
17. Wakitani S, Goto T, Pineda SJ, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;76:579-592.
18. Korbling M, Anderlini P. Peripheral blood stem cell versus bone marrow allotransplantation: Does the source of hematopoietic stem cells matter? *Blood* 2001;98:2900-2908.
19. Besinger WI, Weaver CH, Appelbaum FR, et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. *Blood* 1995;85:1655-1658.
20. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. *Am J Sports Med* 2010;38:880-890.
21. ICRS Cartilage Injury Evaluation Package. Available at: www.cartilage.org/_files/contentmanagement/ICRS_evaluation.pdf. Accessed December 10, 2012.
22. Saw KY, Anz AW, Stabile K, et al. Articular cartilage regeneration with stem cells. In: Dragoo JL, ed. *Modern arthroscopy*. Rijeka, Croatia: InTech, 2011.
23. Cesselli D, Beltrami AP, Rigo S, et al. Multipotent progenitor cells are present in human peripheral blood. *Circulation Res* 2009;104:1225-1234.
24. Kijawski R, Stanton P, Fine J, De Smet A. Subchondral bone marrow edema in patients with degeneration of the articular cartilage of the knee joint. *Radiology* 2006;238:943-949.
25. Elias I, Zoga AC, Raikin SM, et al. Bone stress injury of the ankle in professional ballet dancers seen on MRI. *BMC Musculoskel Disord* 2008;9:39.
26. Blevins FT, Steadman JR, Rodrigo JJ, Silliman J. Treatment of articular cartilage defects in athletes: An analysis of functional outcome and lesion appearance. *Orthopedics* 1998;21:761-767.
27. Gobbi A, Nunag P, Malinowski K. Treatment of full thickness chondral lesions of the knee with microfracture in a group of athletes. *Knee Surg Sports Traumatol Arthrosc* 2005;13:213-215.
28. Knutsen G, Drogset JO, Engebretsen L, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. *J Bone Joint Surg Am* 2007;89:2105-2112.
29. Kreuz PC, Steinwachs MR, Erggelet C, et al. Results after microfracture of full-thickness chondral defects in different compartments in the knee. *Osteoarthritis Cartilage* 2006;14:1119-1125.
30. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: Average 11-year follow-up. *Arthroscopy* 2003;19:477-484.
31. Knutsen G, Engebretsen L, Ludvigsen TC, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. *J Bone Joint Surg Am* 2004;86:455-464.
32. Caplan AI, Correa D. PDGF in bone formation and regeneration: New insights into a novel mechanism involving MSCs. *J Orthop Res* 2011;29:1795-1803.
33. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies. *Mech Ageing Dev* 2008;129:163-173.
34. Civriz Bozdag S, Bay M, Ayyildiz E, Topcuoglu P, Ilhan O. Older age and capacity of colony forming unit in autologous peripheral derived hematopoietic cells. *Transfusion Apheresis Sci* 2012;47:113-116.

Appendix

Appendix Table 1. Patient Average of ICRS II Total Scores From 14 Parameters

Cases	Control Group (HA)	Intervention Group (PBSCs + HA)
1	706.25	853.75
2	720.00	917.50
3	860.00	940.00
4	870.00	960.00
5	900.00	972.50
6	908.75	975.00
7	932.50	984.00
8	958.75	1035.00
9	983.33	1070.00
10	1012.50	1145.00
11	1030.00	1166.50
12	1054.17	1171.25
13	1056.25	1177.50
14	1070.00	1190.00
15	1122.50	1245.00
16	1132.50	1252.50

Appendix Table 2. Patient Average of MRI Total Scores

Cases	Control Group (HA)	Intervention Group (PBSCs + HA)
1	5.67	5.67
2	6.00	8.00
3	6.00	8.00
4	6.00	8.33
5	6.00	8.50
6	6.33	9.00
7	6.50	9.00
8	7.67	9.00
9	8.33	9.20
10	8.50	9.50
11	8.83	9.50
12	9.00	9.67
13	9.00	9.67
14	9.00	10.00
15	9.00	10.00
16	9.00	10.00
17	9.67	11.00
18	10.00	11.00
19	10.00	11.00
20	10.33	11.00
21	10.33	11.50
22	10.50	12.00
23	10.50	12.00
24	11.00	12.00
25	-	12.00