Autologous Bone Marrow–Derived Mesenchymal Stem Cells Versus Autologous Chondrocyte Implantation: An Observational Cohort Study

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What is This?

Autologous Bone Marrow–Derived Mesenchymal Stem Cells Versus Autologous Chondrocyte Implantation

An Observational Cohort Study

Hossein Nejadnik,* MD, James H. Hui,*† MBBS, FRCS, FAMS, Erica Pei Feng Choong,‡
Bee-Choo Tai,§ PhD, and Eng Hin Lee,* MD, FRCS
*From the *Department of Orthopedic Surgery, Yong Loo Lin School of Medicine, National
University of Singapore, Singapore, ‡National University Hospital, National University Health
System, Singapore, and the §Department of Epidemiology and Public Health, Yong Loo Lin
School of Medicine, National University of Singapore, Singapore*

Background: First-generation autologous chondrocyte implantation has limitations, and introducing new effective cell sources can improve cartilage repair.

Purpose: This study was conducted to compare the clinical outcomes of patients treated with first-generation autologous chondrocyte implantation to patients treated with autologous bone marrow–derived mesenchymal stem cells (BMSCs).

Study Design: Cohort study; Level of evidence, 3.

Methods: Seventy-two matched (lesion site and age) patients underwent cartilage repair using chondrocytes (n = 36) or BMSCs (n = 36). Clinical outcomes were measured before operation and 3, 6, 9, 12, 18, and 24 months after operation using the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package, which included questions from the Short-Form Health Survey, International Knee Documentation Committee (IKDC) subjective knee evaluation form, Lysholm knee scale, and Tegner activity level scale.

Results: There was significant improvement in the patients' quality of life (physical and mental components of the Short Form-36 questionnaire included in the ICRS package) after cartilage repair in both groups (autologous chondrocyte implantation and BMSCs). However, there was no difference between the BMSC and the autologous chondrocyte implantation group in terms of clinical outcomes except for Physical Role Functioning, with a greater improvement over time in the BMSC group (P = .044 for interaction effect). The IKDC subjective knee evaluation (P = .861), Lysholm (P = .627), and Tegner (P = .200) scores did not show any significant difference between groups over time. However, in general, men showed significantly better improvements than women. Patients younger than 45 years of age scored significantly better than patients older than 45 years in the autologous chondrocyte implantation group, but age did not make a difference in outcomes in the BMSC group.

Conclusion: Using BMSCs in cartilage repair is as effective as chondrocytes for articular cartilage repair. In addition, it required 1 less knee surgery, reduced costs, and minimized donor-site morbidity.

Keywords: chondrocyte; autologous chondrocyte implantation (ACI); bone marrow–derived mesenchymal stem cell

Full-thickness, focal cartilage defects cause knee symptoms such as pain, popping, and swelling[42] and affect patients' quality of life and career. Recent large arthroscopic studies have indicated that the prevalence of cartilage defects is between 11% and 63%.[1,9,14] Treatment of articular cartilage defects remains challenging[8,21,26] because cartilage tissue has a limited capacity for repair.[16,27,28] One of the most promising treatments for cartilage defects is autologous chondrocyte implantation (ACI),[3,6,10,31] which provides

durable, hyaline-like cartilage.[7,36] Some of the limitations of ACI include the need for general anesthesia or at least regional anesthesia (to harvest the chondrocytes and transplant expanded cells) for 2 knee procedures, difficulty in obtaining an adequate number of chondrocytes, a slow rate of chondrocyte proliferation, and donor-site morbidity. Some of these limitations could be solved by using other techniques such as second- or third-generation ACI,[2,12] arthroscopic second-generation ACI, and microfracture,[20,25] or introducing new cell sources such as debrided waste chondrocytes, bone marrow–derived mesenchymal stem cells (BMSCs), or any combination of these cells.[5,6,15,22,42]

The use of BMSCs for cell-based cartilage repair has been suggested by various authors. However, as far as we know, this has not been compared with other cell sources.[5,39-42] In this study, we used autologous BMSCs, which have a better proliferation rate than chondrocytes and have differentiation capacity to various tissues including chondrogenesis.[11,17,23] The purpose of this study was to evaluate and compare the clinical outcomes of patients with full-thickness articular cartilage defects of the knee treated with ACI and autologous BMSCs.

METHODS

Participants

This ongoing nonrandomized observational cohort study was designed to investigate the effectiveness of chondrocytes and BMSCs as cell sources for repairing full-thickness cartilage defects of the knee. The inclusion criteria were at least 1 symptomatic chondral lesion diagnosed by clinical examination and MRI on the femoral condyle, trochlea, or patella and nonexistent or correctable concomitant pathologic changes. The exclusion criteria were patients with inflammatory arthritis, tricompartmental osteoarthritis, limited range of motion in particular fixed flexion deformity, and those who were 65 years of age or older. Cartilage repair was conducted with informed consent of the patients.

Patients who fulfilled the inclusion and exclusion criteria were treated by the senior author (J.H.H.). Thirty-six consecutive patients underwent BMSCs and were matched with 36 cases of ACI performed earlier, in terms of lesion sites and (10-year) age intervals.

The study protocol was approved by the National Healthcare Group Domain-Specific Review Board (NHG DSRB reference number D/00/814) and the University Hospital Ethic Committee. In addition, cells were processed at the GMP cell processing facility at the National University Hospital of Singapore.

Cell Sources

Chondrocyte (ACI) Preparation. A small amount of cartilage tissue (1 cm × 0.5 cm) was taken from non-weightbearing areas that were deemed macroscopically healthy by arthroscopy. The harvested tissue was transferred into a specimen container filled with sterile saline (about 10 mL) and processed within 60 minutes. The sample was washed twice with phosphate-buffered saline (Gibco BRL, Grand Island, New York) and then minced before being transferred aseptically into a tube with 5 mL collagenase NB6 (Sigma, St Louis, Missouri) for overnight digestion at 37°C in a water bath. Digested chondrocytes were washed with Dulbecco modified Eagle medium (DMEM)/F12 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco) to stop the enzymatic reaction. These cells were then cultured in T-75 cm^2 flasks with DMEM/F12 containing 10% FBS and 50 μg/mL L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma) in a humidified atmosphere of 5% CO_2 at 37°C. Cells were seeded at a cell density of 5000 cells/cm^2. Initial medium change was done after 7 days, when adherent cells were recognized. Subsequent medium change was done 2 to 3 times a week until the preparation of cell sheets, which were formed in the presence of ascorbic acid (passage 1). For each surgery, at least 4 cell sheets were prepared and around 2 million cells/cm^2 were applied.

Autologous BMSC Preparation. With the patient under local anesthesia, 30 mL of bone marrow was aspirated using a Jamshidi needle from the iliac crests of each patient into heparinized syringes and transferred into sterile containers. Seventy or 80 mL of each patient's blood was collected as well. The bone marrow aspirate was processed within 60 minutes. The heparinized bone marrow aspirate was mixed with a one-fifth volume of 6% (w/v) dextran (molecular weight 100 000) (Sigma) and left standing at room temperature for 30 minutes to eliminate erythrocytes. The remaining cells were washed twice with DMEM. These cells were cultured in T-75 cm^2 flasks with an initial culture medium consisting of DMEM containing 10% FBS, 50 μg/ml L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate, and 1% antibiotic-antimycotic (penicillin 100 U/mL, streptomycin 0.1 mg/mL, amphotericin B 0.25 μg/mL) (Sigma) in a humidified atmosphere of 5% CO_2 at 37°C. The cells were seeded at a density of 10 000 cells/cm^2. Initial medium change was done after 5 days when adherent cells were recognized. Subsequently, culture media without antibiotics were used and changed 2 to 3 times a week. Cell sheets were formed in the presence of ascorbic acid (passage 1) and for each surgery; at least 4 cell sheets were prepared and around 2 million cells/cm^2 were applied.

Seventy milliliters of venous blood from each patient was transferred into two 50-mL tubes for overnight incubation at 4°C. After centrifuging the tube with slow acceleration, the serum was carefully aspirated and transferred to a new tube. Repeated centrifugation with slow acceleration for 3 minutes at 3000 rpm at ambient temperature was performed. The serum was aspirated into a syringe and filtered with a sterile 0.2-μm filter. The filtered serum was tested for sterility, anti–human immunonodeficiency virus, and hepatitis B antigen, and then stored at a temperature of –20°C.

Flow cytometry against CD90$^+$, CD105$^+$, CD14$^-$, and CD34$^-$ was used to confirm that cultured cells were mesenchymal stem cells. Saline that was used for transporting the cartilage biopsy specimen to the laboratory, aspirated bone marrow and culture media (without antibiotic) were tested for sterility and Mycoplasma hominis contamination.

Operation Techniques

Four to 5 weeks after the cells were harvested, ACI surgery was done. For details on ACI, refer to the procedure described previously.[6] In summary, approximately 10 to 15 million cells (with a viability rate of 96%) were returned for implantation. The cell sheets were transported to the operating theater in a sterile container within the patients' own serum. The debrided chondral defect (without damaging subchondral bone) was measured after arthrotomy. Subsequently, periosteal patch harvesting from the proximal part of the tibia or distal part of the femur was done according to the measured size. Next, the harvested periosteum was sutured precisely to the rim of the debrided defect(s). The cultured chondrocytes or BMSCs were implanted beneath the patch and very fine stitches (microsuture 7-0) were used to hold the periosteum to the defected site. To avoid cell leakage, fibrin glue was used to create a watertight seal.

Rehabilitation

To derive maximum benefit from the surgery, patients were advised to strictly follow the rehabilitation protocol, which is one of the most important parts of recovery. The rehabilitation protocol began on the day of surgery and included passive range of motion and isometric muscle contractions. Patients were able to begin active motion and partial weightbearing at 6 weeks, progressing to full weightbearing exercises. The rehabilitation protocol varies according to the location and size of the lesion, concomitant procedures, and patient age and previous activity level. Rehabilitation focuses on 4 areas: walking/weightbearing, range of motion, strength, and cardiovascular capacity.

Postoperation Evaluation

Patients were evaluated preoperatively (preoperative assessment) as well as at 3, 6, 9, 12, 18, and 24 months postoperatively. Assessments were performed by our trained research staff using the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package, which included questions from the Short-Form (SF-36)[43] Health Survey, International Knee Documentation Committee (IKDC) subjective knee evaluation form, Lysholm[24] knee scale, and Tegner[38] activity level scale.

Second-look arthroscopy was performed in 7 patients (4 in the BMSC group and 3 in the ACI group) 9 to 12 months after implantation. A biopsy sample of the repair tissue was obtained in 2 cases (1 in each group). After fixation, paraffin sections were stained with Alcian blue to evaluate aggrecan content and immunohistochemistry staining was done to assess collagen type I, II, and X content.

Statistical Analysis

Statistical analysis was performed by an independent statistician using STATA statistical software (Version 10,

TABLE 1
Demographic Characteristics of Study Patients[a]

Characteristic	ACI (n = 36)	ABMSCs (n = 36)
Sex (%)		
Male	18 (50)	20 (56)
Female	18 (50)	16 (44)
Age (%)		
<45 years	19 (52.8)	16 (44.4)
≥45 years	17 (47.2)	20 (55.6)
Lesion site (%)		
Patellar	13 (36)	13 (36)
Trochlear	4 (11)	4 (11)
Femoral condyle	12 (33)	12 (33)
Multiple lesions	7 (20)	7 (20)
Lesion grade (%)		
Grade 3	25 (70)	24 (67)
Grade 4	11 (30)	12 (33)
Mean defect size, cm^2 (SD)	3.6[b] (2.84)	4.6[b] (3.53)
Diagnosis (%)		
Trauma	19 (53)	14 (39)
OA	15 (42)	20 (56)
Others	2 (5)	2 (5)

[a]ACI, autologous chondrocyte implantation; ABMSCs, autologous bone marrow–derived mesenchymal stem cells; SD, standard deviation; OA, osteoarthritis.
[b]There was no significant difference between the 2 groups ($P = .270$).

StataCorp, College Station, Texas). The MIXED effect model (with random intercept) was used to evaluate the effect of cell type and time on the quality of life and other functional or pain outcomes of patients, depending on gender. This method of analysis appropriately accounts for the possible correlation between repeated measurements of an individual. All statistical evaluations were made based on an assumption of a 2-sided test at the conventional 5% level of significance.

RESULTS

Seventy-two patients who fulfilled the inclusion and exclusion criteria were treated using ACI (n = 36) and BMSCs (n = 36) from 2001 to 2005 and 2005 to 2007, respectively. All patients were followed for 2 years. Table 1 shows the demographic characteristics of the patients. As anticipated, patients in the 2 groups had similar age and gender distributions because they were matched by age and gender. There were equal numbers of men and women in the ACI group, with a mean age of 42.5 years (standard deviation [SD], 11.2 years). Correspondingly, in the BMSC group, there were 20 men and 16 women with a mean age of 44.0 years (SD, 11.4 years). However, the mean defect sizes of the ACI and BMSC groups were 3.6 cm^2 (SD, 2.84 cm^2) and 4.6 cm^2 (SD, 3.53 cm^2), respectively ($P = .270$). Concomitant procedures included patella realignment (6 cases in ACI and 5 cases in BMSC group), high tibial osteotomy (5 cases in BMSC group), partial meniscectomy (1 in each group), and anterior cruciate ligament reconstruction (1 in each group).
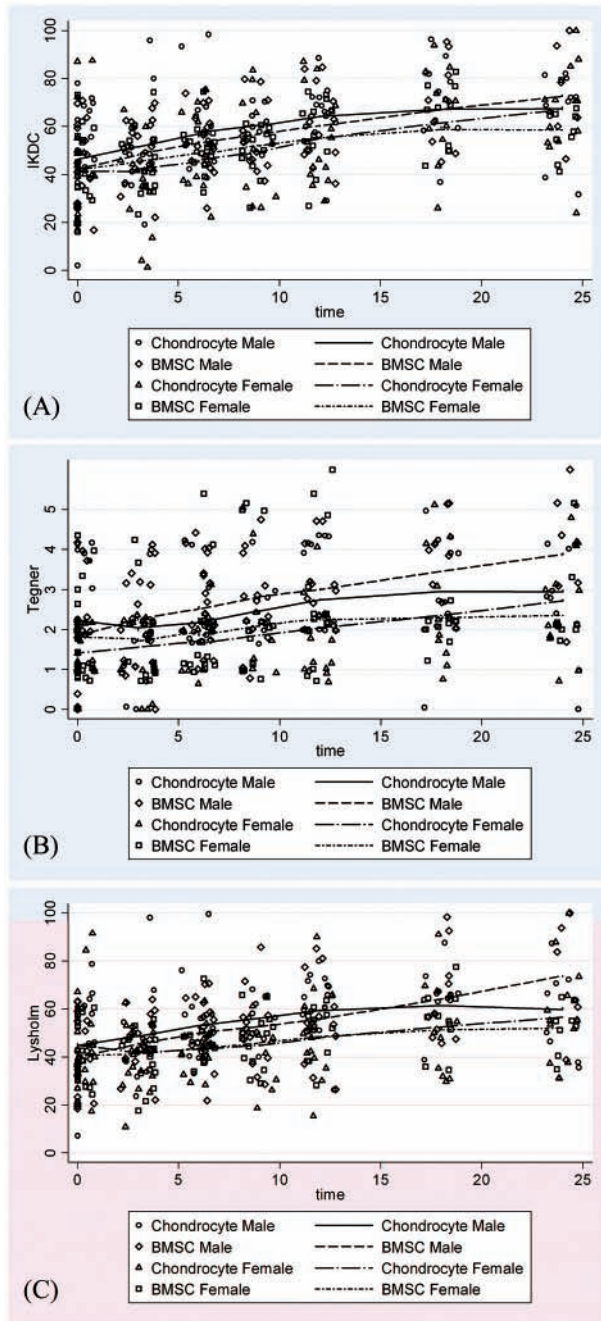
Figure 1. International Knee Documentation Committee (A), Tegner (B), and Lysholm (C) scores preoperatively and at 3, 6, 9, 12, 18, and 24 months after surgery for the bone marrow–derived mesenchymal stem cell (BMSC) and chondrocyte groups in both genders.

International Cartilage Repair Society Package SF-36 Components Clinical Outcomes

The Appendix (see online Appendix for this article at http://ajs.sagepub.com/supplemental/) shows the physical and mental components of the SF-36 questionnaire included in the

TABLE 2
Effect of Cell Type, Time, and Gender on IKDC,
Lysholm, and Tegner Outcomes[a]

Outcome/Parameter	Estimate	95% Confidence Interval	P Value
IKDC			
Cell type	−0.46	−5.61 to 4.69	.861
Time, years	1.08	0.90 to 1.25	<.001
Sex	−6.03	−11.18 to −0.88	.022
Lysholm			
Cell type	−1.16	−5.83 to 3.52	.627
Time, years	0.79	0.63 to 0.95	<.001
Sex	−6.44	−11.17 to −1.71	.008
Tegner			
Cell type	0.25	−0.13 to 0.64	.200
Time, years	0.06	0.05 to 0.07	<.001
Sex	−0.55	−0.98 to −0.13	.011

[a]IKDC, International Knee Documentation Committee.

ICRS package. Generally, there was a significant improvement in these quality of life outcomes after ACI over time. However, there were no differences between patients treated with BMSCs and chondrocytes in terms of these clinical outcomes ($P > .05$) except for Physical Role Functioning, which suggested greater improvements in patients treated with BMSCs as compared with chondrocytes for both men and women ($P = .044$ for interaction effect).

Within groups, there were significant differences in outcomes between genders. In particular, men demonstrated greater improvements in scores for Physical Functioning, Physical Health Summary, Physical Role Functioning, and Mental Health Summary ($P < .05$). On the other hand, gender did not have any effect on scores for Vitality, Social Functioning, Emotional Role Functioning, Mental Health, and Bodily Pain.

There were no differences in physical and mental component scores between the age groups (<45 years vs ≥45 years) within each cell type.

International Knee Documentation Committee Subjective Knee Evaluation Outcomes

The postoperative IKDC scores (Figure 1A) indicated a significant improvement in performance over time throughout the follow-up period. Patients treated with chondrocytes and BMSCs did not differ significantly with regard to improvements in the IKDC subjective knee evaluation. However, men did show significantly better improvement than women (Table 2). Moreover, there were no differences in IKDC scores between patients younger than 45 years and those who were at least 45 years of age within the ACI group ($P = .070$) and BMSC group ($P = .671$).

Tegner Activity Level Outcomes

The sport activity level was evaluated by the Tegner score. As illustrated in Figure 1B and Table 2, there was notable improvement in the Tegner score during the 2-year

follow-up period ($P < .001$), and the scores did not differ between the 2 cell type groups ($P = .200$). However, men had more improvement in sports activity than women ($P = .011$). Although there was a significant difference in Tegner scores between patients who were $\geq$45 years and those who were younger than 45 years among those treated by ACI ($P = .038$), no evidence of difference was noted in the BMSC group ($P = .307$).

Lysholm Knee Scale Outcomes

The Lysholm score (Figure 1C) showed significant improvement over time. However, there was no difference in scores between the BMSC and ACI groups. As described for Tegner and IKDC scores, men had more improvement than women (Table 2). Younger patients (<45 years) in the ACI group had better outcomes ($P = .010$); however, there was no difference between age groups for those treated with BMSCs ($P = .459$).

Of the 72 patients, 7 patients (4 in the BMSC group and 3 in the ACI group) underwent second-look arthroscopy (Figure 2) as a result of removal of realignment screw, or symptoms of pain and swelling. The surface of the repaired cartilage was smooth in 3 BMSC cases and 2 ACI cases. One case in each group had some irregularities. There was no evidence of abnormal calcification or necrosis in either group. Histologic evaluation of the biopsy samples taken from patients showed hyaline-like cartilage tissue (Figure 3).

DISCUSSION

The objective of this study was to compare the effectiveness of BMSCs to chondrocytes as a cell source for treatment of symptomatic full-thickness articular cartilage defects.

The strengths of this study were (1) selection of patients according to established inclusion and exclusion criteria, (2) surgeries performed by a single surgeon, (3) use of validated knee cartilage outcomes instruments, (4) use of matched data to decrease the confounding effect of site and age, (5) use of the same outcome evaluation scales from baseline and different time points, and (6) use of a trained independent observer for data collection.

In many comparative studies, ACI has been found to be a promising method for cartilage repair.[3,6,7,10,31] Fu et al[10] reported significantly greater improvement in function and pain relief in patients treated with ACI compared with those treated with debridement of cartilage defects in the knee. Bentley et al[3] demonstrated better results for cartilage repair with ACI compared with mosaicplasty. Saris et al[35] and Robinson and Nevo[32] found that ACI produced significantly better repair tissue compared with microfracture. However, Knutsen at al[18,19] reported similar clinical outcomes. Moreover, new ACI techniques like matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI),[2,12] by using biomaterials seeded with chondrocytes as a scaffold instead of a periosteal patch, lead to less surgical time, morbidity, and periosteal patch hypertrophy.
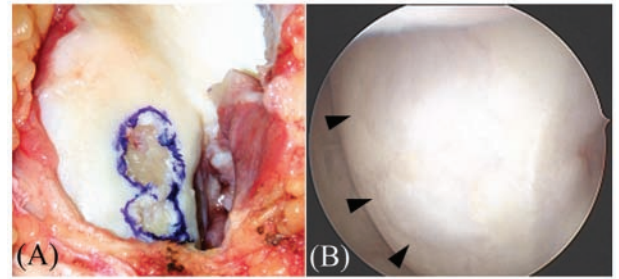

Figure 2. A, femoral condyle cartilage defect after debridement. B, second-look arthroscopic evaluation 1 year after autologous bone marrow–derived mesenchymal stem cell implantation.
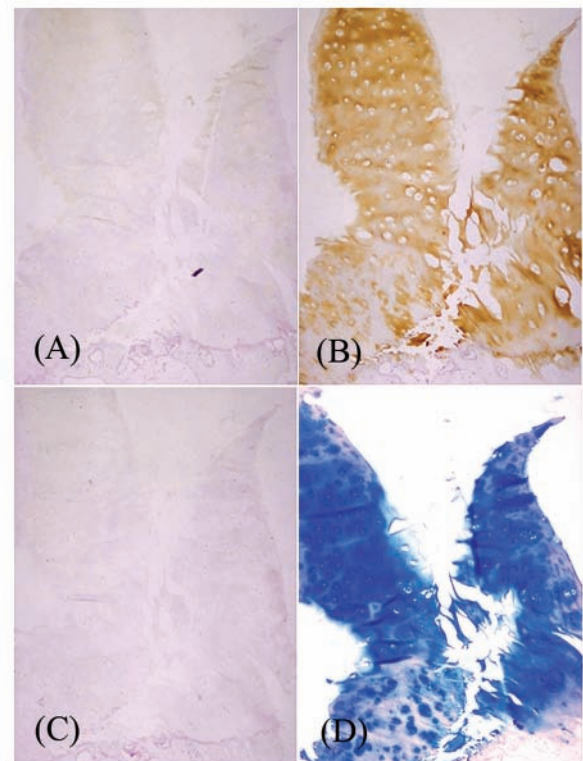

Figure 3. Histologic evaluation of biopsy specimens taken 1 year after autologous bone marrow–derived mesenchymal stem cell implantation of femoral condyle. A, immunohistochemistry (IHC) staining against collagen I; B, IHC against collagen II; C, IHC against collagen X; and D, Alcian blue staining against aggrecan.

Previous studies showed that BMSCs and synovium-derived mesenchymal stem cells (SMSCs) are promising stem cell sources for cartilage repair.[30] Although some in vitro[34,37] and animal[30] studies demonstrated that SMSCs had greater chondrogenic potential than BMSCs, SMSCs have not been used clinically. In addition, the process of harvesting SMSCs requires the use of arthroscopy, which is

more invasive than bone marrow biopsy for BMSCs. The advantages of using BMSCs compared with chondrocytes as a cell source for cartilage repair are the ability to harvest a sufficient number of cells, acceptable cellular proliferation capacity,[29,39] trophic effect on neighbor cartilage tissues, acceptable capacity for chondrogenic differentiation,[4] and less donor-site morbidity. The second-look arthroscopy and histologic evaluations looked promising, but the limited number of arthroscopies precluded statistical comparison between tissue derived from BMSCs and chondrocytes.

Our results confirmed that patients treated by ACI and BMSCs had a comparable improvement in quality of life, health, and sport activity, with men reporting greater improvements than women. In addition, patients older than 45 years of age had less significant improvements than younger patients in the ACI group. This differed from the study by Rosenberger et al,[33] which showed minimal differences between patients older than 45 years of age and younger patients treated by ACI.

Autologous chondrocyte implantation using a periosteal patch, a procedure approved by the US Food and Drug Administration, was chosen as a control group to increase the validity of this study method. Moreover, to evaluate the effectiveness of BMSCs, results were compared with chondrocytes, which are widely used as a cell source. Wakitani et al[40] demonstrated better arthroscopic and histologic results in BMSCs for femoral condyle cartilage defect repair compared with the control group (no cell) in his studies, with similar results in different anatomic sites.[41,42] Our study showed that patients in the BMSC group had similar improvement in clinical outcomes as the ACI group. Moreover, patients older than 45 years in the BMSC group had the same results as younger patients.

The limitations of this study are that possible biases might be introduced because the patients were not randomized and there were variations in patient characteristics between the 2 groups. However, patients were matched to minimize the variations in age and site implantation among the cohorts and, therefore, limit the effect of these important factors on the outcomes. Also, concomitant high tibial osteotomy, a potential confounder, was only performed in the BMSC cohort. Another limitation of the current study was that objective data that may be obtained with second-look arthroscopy and biopsy for histology or MRI evaluation were not included. Although MRI can be used to determine defect filling, integration of repaired cartilage to the adjacent cartilage and subchondral bone, bone marrow edema, and lesion osteophytes, the lack of MRI scans had minimal effect on interpretation of results because there is poor correlation between MRI and patient-reported clinical outcomes.[13] Certainly, a randomized control trial with objective outcomes is needed to provide a higher level of evidence.

In summary, this study demonstrated that the use of BMSCs was as effective as chondrocytes for articular cartilage repair. The use of BMSCs in cartilage repair undoubtedly offers advantages. Bone marrow biopsy is less invasive than knee arthroscopy, normal articular cartilage is not damaged, and 1 less general or regional anesthesia application is required; consequently the cost is less.

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