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Treatment Outcomes of Alginate-Embedded Allogenic Mesenchymal Stem Cells Versus Autologous Chondrocytes for the Repair of Focal Articular Cartilage Defects in a Rabbit Model

Liang Xin Tay,*† BSc, Raja Elina Ahmad,‡ PhD, Havva Dashtdar,† MSc, K.W. Tay,† MD, T. Masjuddin,† MD, S. Ab-Rahim,§ PhD, Pan Pan Chong,† MSc, L. Selvaratnam,‖ PhD, and T. Kamarul,† MS(Orth)
Investigation performed at Tissue Engineering Group, NOCERAL, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Background: Mesenchymal stem cells (MSCs) represent a promising alternative form of cell-based therapy for cartilage injury. However, the capacity of MSCs for chondrogenesis has not been fully explored. In particular, there is presently a lack of studies comparing the effectiveness of MSCs to conventional autologous chondrocyte (autoC) treatment for regeneration of full-thickness cartilage defects in vivo.

Hypothesis: Treatment with allogenic undifferentiated MSCs (alloMSCs) results in superior cartilage tissue regeneration profiles when compared with autoC for repair of focal articular cartilage defects.

Study Design: Controlled laboratory study.

Methods: Full-thickness articular cartilage defects were created on the weightbearing surface of the medial femoral condyles in both knees of New Zealand White rabbits (N = 30). Six weeks after the defect was induced, the right knee was treated with either alloMSCs (n = 12) or autoC (n = 18), while the left knee remained untreated (control). The rabbits were sacrificed at 6 months after treatment for assessment of cartilage tissue regeneration, which included the Brittberg morphologic score, histologic grading by O'Driscoll score, and quantitative analysis of glycosaminoglycans per total protein content.

Results: Apart from significantly higher Brittberg scores in the alloMSC treatment group (8.8 $\pm$ 0.8) versus the autoC treatment group (6.6 $\pm$ 0.8) (P = .04), both treatments showed similar cartilage regenerative profiles. All outcome measures were significantly higher in the treatment groups compared with their respective controls ($P < .05$).

Conclusion: AlloMSCs have similar effectiveness as autoC for repair of focal cartilage defects. Both treatments resulted in superior tissue regeneration compared with untreated defects.

Clinical Relevance: The results have an implication of supporting the potential use of MSCs for cartilage repair after sports injuries or diseases, in view of similar efficacy but less patient morbidity and potential cost savings as compared with conventional autoC therapy.

Keywords: mesenchymal stem cell; chondrogenic differentiation; cartilage repair; chondrocyte

Cellular therapy has been considered a successful treatment modality for the repair of damaged articular cartilage, producing superior tissue repair quality as compared with the standard surgical approach.[11] The first clinical trial reporting the efficacy of autologous chondrocyte implantation/transplantation (ACT/ACI), the conventional form of cellular therapy, was published in 1994[12] after its success in animal studies in the preceding years.[20,50] Since then, ACI has been studied in many clinical trials with good outcomes in the majority of cases.[4,12,34,36,41] Despite a successful historical performance for more than 15 years, the use of ACI has not been without limitations. Its recognized shortcomings include donor-site morbidity, limited supply of chondrocytes, cellular dedifferentiation into fibroblast-like phenotype when cultivated in vitro, and inability to maintain good tissue repair in the long term.[10,28] It has been speculated that the use of an alternative cell source such as mesenchymal stem cells (MSCs) might overcome these issues. Mesenchymal stem cells may be harvested from many potential donor sites including bone marrow, adipose

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tissue, trabecular bone, and other tissues without causing damage to the unaffected cartilage.[40] In addition, they have a high proliferative capacity, and owing to their multipotency, these cells can conveniently be manipulated in vitro to differentiate into chondrocytes for subsequent use in cartilage regeneration.[14,18,31,43]

There have been many previous reports involving in vivo experiments describing good repair outcomes after transplantation of MSCs in cartilage defects. Wakitani et al[49] in 1994 were among the first research groups to report the successful transplantation of bone marrow–derived MSCs in osteochondral defects in rabbit models. Other researchers have also studied the application of allogenic or autologous bone marrow–derived MSCs using different scaffolds with or without the addition of growth factors to treat cartilage defects in various animal models.[19,22,37] While the use of autologous chondrocytes (autoC) and allogenic undifferentiated MSCs (alloMSCs) to repair damaged cartilage have been forthcoming in many studies, it has been demonstrated that additional use of scaffolds enhances the tissue repair.[2,13,22,48,55] These scaffolds not only provide a convenient method for delivering cells into focal defect sites, but also provide structural support to the construct and induce cartilage matrix formation within the defective sites.[47,48,55] Alginate is a well-established biomaterial for use in these conditions.[8,23,30,33,48,52] Not only is this material biocompatible, the embedded chondrocytes maintain their phenotype and produce more depositions of extracellular cartilaginous matrix in alginate.[16]

Despite a multitude of available experimental reports on a cell-based approach for articular cartilage repair, there is presently limited exploratory research comparing alloMSCs with other sources of chondrocytes, particularly those obtained from the autologous transplantation procedure (autoC). To date, there is only 1 prospective clinical study comparing the effectiveness of MSCs against autoC for repairing damaged cartilage in humans.[38] The study revealed similar clinical outcomes between MSCs and autoC assessed postoperatively by a multifaceted questionnaire-based clinical evaluation carried out throughout a temporal course of 2 years. While it may be appropriate to use the validated knee cartilage outcome clinical instruments to assess the overall function of the repaired cartilage tissue, it is equally important to ascertain the changes in tissue repair quality in response to both treatments. However, these forms of investigation are usually confined to animal models since the invasiveness of the tissue biopsy procedure may not necessarily be tolerated by human subjects in the clinical setting. A study incorporating more objective assessments of tissue repair is therefore required to determine the cellular changes that may exist during the transplantation process. This may demonstrate a clear distinction in the physical features of the repaired cartilage tissue resulting from the individual treatment. No parallel comparative analysis of tissue-healing outcome has been previously performed to evaluate the effectiveness of alloMSCs compared with autoC in animal studies. Therefore, the aim of the current study is to compare in a more objective manner the effectiveness of the 2 different forms of cell-based therapy (alloMSCs vs autoC) in repairing focal cartilage defects in an in vivo rabbit model, by analyzing the gross morphology, histology, and extracellular matrix protein content such as glycosaminoglycan in response to transplantation of chondrocytes from these distinct sources into the defective areas in the joint.

MATERIALS AND METHODS

Rabbit Focal Articular Cartilage Defect Model

Thirty male New Zealand White (NZW) rabbits aged between 5 and 6 months and weighing approximately 2.5 kg were randomly assigned into 2 treatment groups: ACI group (autoC; n = 18) and allogenic MSC group (alloMSC; n = 12). The sample size of each treatment group was calculated based on 2 independent studies previously conducted in our laboratory comparing MSCs and autoC treatments with their respective control groups,[15,29,30] in view of the lack of reference data involving direct parallel comparison between the 2 treatment groups using similar objective measurements. The stated number of subjects in each group was required to detect 2-fold difference in Brittberg scores between the treated and untreated groups with 80% power at a .05% significance level. In addition, 3 rabbits were sacrificed separately to harvest bone-marrow–derived MSCs for use in the alloMSC-treated group. This study was approved by University of Malaya animal ethics committee and the experiments were carried out in accordance to the regulations imposed by the university and the appropriate Malaysian government regulatory body (OS/05/07/2006/TKZ/A[R] and PM/24/06/2008/TKZ[c][R]).

The rabbits were anesthetized before both femorotibial joints were surgically exposed. A 5-mm–diameter full-thickness articular cartilage defect was created in both knees using a custom-made chondrotome as previously described by Kamarul et al in 2008.[30] All defects were created on the weightbearing portion of the medial femoral condyle. Cartilage from the operated sites were removed from both knees and collected aseptically in 1× phosphate-buffered saline solution (PBS; pH 7.4) (Gibco/Invitrogen, Carlsbad,

*Address correspondence to Liang Xin Tay, BSc, Tissue Engineering Group, National Orthopaedic Surgery Centre of Excellence for Research and Learning (NOCERAL), Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia (e-mail: liangxin28@gmail.com).

†Tissue Engineering Group, NOCERAL, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

‡Department of Physiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

§Faculty of Medicine, UiTM, Shah Alam, Selangor, Malaysia.

||School of Medicine and Health Sciences, Monash University, Sunway Campus, Selangor, Malaysia.

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California) supplemented with 4% penicillin-streptomycin (Invitrogen). The cartilage specimens were kept at 4°C before further processing of chondrocytes. The wounds were surgically closed in layers with 5-0 Vicryl absorbable sutures (Ethicon, Johnson & Johnson, Somerville, New Jersey) and bandages. All surgeries were done in accordance with the standard procedures under sterile surgical conditions. Postoperatively, the animals were transferred into the cages without immobilization. All animals were closely monitored for infections and other complications.

Isolation of AutoC and Allogenic MSCs

Autologous chondrocytes were isolated under sterile conditions from cartilage tissues in accordance with the techniques previously described by Ab-Rahim et al in 2008.[1] The specimens were first digested using 0.6% type II collagenase (Sigma-Aldrich, St Louis, Missouri) at 37°C and rotated overnight followed by centrifugation at 500*g* for 10 minutes. The supernatant was discarded and the cell pellet was resuspended in 1 mL of Dulbecco's Modified Eagle Medium (DMEM; Invitrogen). The resuspended cells were then seeded into a T75 flask (Corning, Lowell, Massachusetts) containing DMEM/Ham's F-12 Nutrient Mixture (1:1) (Invitrogen) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah), 0.0025% ascorbic acid (Sigma), and 1% penicillin-streptomycin. The autoCs were maintained in monolayer culture at 37°C, 95% humidity, and 5% CO_2 for 3 weeks while the growth medium was exchanged every 2 to 3 days.

To obtain alloMSCs for transplantation, 3 rabbits were sacrificed using an intravenous overdose of pentobarbital. Femurs and tibias of both lower limbs were removed and any adherent tissues were discarded. All harvested bones were kept on ice in 1× PBS solution supplemented with 4% penicillin-streptomycin until they were processed. Within 3 hours, bone marrow was harvested from femurs and tibias. AlloMSCs were isolated according to an established protocol described by Pittenger et al in 1999.[42] Briefly, 2 mL of bone marrow was diluted with 1× PBS solution (1:2) and loaded over 3 mL Ficoll-paque (GE Healthcare–Amersham Biosciences, Piscataway, New Jersey) in a 15-mL Falcon tube (Corning). Mononuclear cells were harvested from the interface of plasma and Ficoll-paque after centrifugation at 2200 rpm for 25 minutes. The cells were then washed with prewarmed DMEM and centrifuged for another 10 minutes. The cell pellet was resuspended in DMEM containing 10% FBS and 1% penicillin-streptomycin and seeded into a T75 flask. All cells were maintained and incubated under the same conditions as described above. After 8 days, the nonadherent cells were removed while the adherent cells (alloMSCs) were replenished with fresh medium. The alloMSC culture was also maintained in monolayer for 3 weeks in a similar environment to that of the autoC culture.

Autologous Chondrocytes (AutoC) and AlloMSC Culture in Alginate

Autologous chondrocytes and alloMSC alginate beads were prepared in 1.2% low-viscosity alginate solutions (Sigma-Aldrich) in 0.15 M sodium chloride as previously described by Kamarul et al[30] after 21 days of monolayer culture. Autologous chondrocytes and alloMSCs were first detached from the T75 flask surface using TypLE express (Invitrogen) and pelletized in a separate 15-mL Falcon tube. Cell count was performed, followed by the addition of the appropriate volume of alginate solution to form alginate beads, each containing approximately 5.0×10^5 cells. The cell suspension was mixed thoroughly and expressed slowly through a micropipette tip into a 102-mM calcium chloride solution. After 10 minutes of polymerization in the solution, the alginate beads were washed with 0.15 M sodium chloride to remove the excess calcium chloride. Five autoC-alginate beads were transferred to each of the 24-well culture plates (Becton Dickinson, Durham, North Carolina) and cultured in DMEM/F12 containing 10% FBS, 25 μg/mL ascorbic acid, 50 μg/mL gentamicin (Invitrogen), and 360 μg/mL L-glutamine (Invitrogen). The alloMSC-alginate beads were cultured in DMEM low glucose containing 10% FBS and 1% penicillin-streptomycin. Both cultures in the alginate scaffold were maintained at 37°C in a humidified environment of 5% CO_2 for another 3 weeks.

Implantation of AutoC-Alginate and AlloMSC-Alginate Constructs Into Defective Areas

To avoid dedifferentiation of autoC, implantation of autoC and alloMSC embedded in alginate beads into the rabbits' cartilage defects was carried out after 3 weeks of cell culture in the alginate constructs, culminating in an overall culture period of 6 weeks (ie, 3 weeks in monolayer and 3 weeks in alginate construct) after the creation of the defect. All transplantations were performed only in the right knee of each rabbit. The rabbits were anesthetized and the chondral defects were exposed and identified. A periosteum flap of 7-mm diameter was harvested from the medial aspect of the proximal tibia of the same limb. The periosteum flap was then placed on top of the defect and anchored with 1 suture in each corner using 8-0 Vicryl absorbable sutures. Two beads of either autoC-alginate or alloMSC-alginate (total of 1×10^6 cells) were inserted underneath the flap according to the respective treatment group. The remaining opening of the periosteum flap was then sealed using Tisseel fibrin glue (Baxter AG Industries, Vienna, Austria) and suture (11-0 Vicryl absorbable suture). The wound was closed in layers using absorbable suture and bandages, followed by application of antiseptic solution to prevent infection. The left knees were left untreated (control group). All transplanted rabbits were treated with analgesic and antipyretic, Metacam (Boehringer Ingelheim, Berks, United Kingdom) and Kombitrim (KELA Laboratoria NV, Hoogstraten, Belgium) for 3 days after the operation, both of which additionally act as broad-spectrum antibiotics for both gram-positive and gram-negative bacteria.

Harvesting and Gross Examination of the Regenerated Tissues From Defective Areas

At 6 months after implantation, the rabbits' knee joints were surgically removed and examined. For gross

evaluation, all specimens were examined under direct light microscopy by 2 independent observers, both experienced orthopaedic surgeons, who were blinded to the sample groups. The observers were asked to examine and grade samples according to the Brittberg scoring system.[13] Upon completion of the scoring, the specimens were halved using a mechanical bone saw (Fein MultiMaster Accu, C & E Fein Gmbh, Stuttgart, Germany). This was done carefully to avoid any alteration or destruction of the tissue specimen according to the technique previously established by Kamarul et al.[29,30] Half of each specimen was fixed in 10% phosphate-buffered formalin (4% formaldehyde) for histology and immunostaining, while the other half was utilized for analysis of the glycosaminoglycan (GAG) content.

Histologic Examination and Immunohistochemical Staining

Specimens that were fixed in 4% buffered formalin were decalcified. Fixed and decalcified tissues were subsequently dehydrated in ethanol in a stepwise manner from 70% up to 100%, transferred to xylene, and embedded in paraffin. At the center of each sample, 5-μm paraffin sections were prepared and placed on glass slides, dried overnight, and stored at 4°C. The samples were stained with hematoxylin and eosin for general morphologic evaluation and safranin O–fast green to detect proteoglycan. Immunohistochemical staining was performed using DAKO EnVision + System peroxidase kit (DAKO, Glostrup, Denmark). The specimen section slides were incubated in primary antibody solution (anti–collagen type II, Santa Cruz Biotechnology, Santa Cruz, California) followed by secondary antibody and substrate-chromogen solution in accordance with the manufacturer's instructions. The O'Driscoll cartilage scoring was used for histologic and histochemical assessment of the repaired cartilage specimens. The scoring was performed by 2 histologists blinded to the group status.

Biochemical Assay for GAG

The Blyscan GAG assay kit (Biocolor Ltd, Antrim, United Kingdom) was used to evaluate GAG content of the regenerated cartilage tissues in both treatment groups. Specimens were dissected into small pieces using a scalpel and digested using RIPA buffer (Merck & Co, Whitehouse Station, New Jersey) supplemented with protease inhibitors (Sigma) for 1 hour. Aliquots of each sample were mixed with DMMB (dimethylmethylene blue) dye and reagents (Biocolor) according to the manufacturer's instructions. The absorbance at 656 nm was measured using the spectrophotometer and compared with a standard plot of chondroitin sulphate provided by the manufacturer for quantitative determination of the GAG content.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 17.0, SPSS Inc, an IBM company, Chicago, Illinois). The values of Brittberg and O'Driscoll scores, as well as GAG concentrations for all tissue samples, were presented as mean ± standard deviation. Comparisons of variables between the 2 treatment groups were analyzed using the parametric 2-sided independent *t* test. Differences were considered statistically significant at $P < .05$.

RESULTS

Gross Appearance of Defect

Figure 1 illustrates an example of the gross macroscopic analysis of the representative tissues from the alloMSC, autoC, and control groups. Macroscopic examinations at 6 months after implantation revealed that defects treated with both alloMSCs and autoC showed good filling, with the surface appearing flush and smooth (Figures 1D and 1E). In contrast, none of the untreated defects in the left knees (control) (Figure 1F) showed complete filling as compared with the treated knees.

Figure 2 shows the results of Brittberg scores reflecting quantitative macroscopic evaluation of the tissue regenerate quality between the alloMSC and autoC treatment groups together with their respective controls. Both the alloMSC and autoC implantations showed superior cartilage regeneration quality as compared with the untreated left knees, as evidenced by the significantly higher Brittberg scores in the treatment groups (8.8 ± 0.8 vs 3.0 ± 0.8 for the alloMSC-control pair, and 6.6 ± 0.8 vs 2.3 ± 0.6 for the autoC-control pair; both pairs were associated with a *P* value of .001). The difference in Brittberg scores between alloMSC and autoC was significant ($P = .04$) from the 2-sided independent *t* test. No significant differences in the Brittberg scores were found between the untreated left knees (control) in both alloMSC and autoC groups ($P = .31$).

Histologic and Immunohistochemical Appearance of the Regenerated Cartilage Tissues

Figure 3 shows the results of the microscopic examination of the regenerate tissues in 4 groups (ie, the alloMSC-treated group, the autoC-treated group, the untreated group [negative control] and a representative specimen of a normal NZW rabbit's knee articular cartilage tissue [positive control]). The microscopic appearance of the tissue using various staining methods revealed cartilage regeneration within the treated sites although some areas appeared to be infiltrated by a small amount of fibrous tissues. In contrast, the untreated defects did not appear to undergo complete healing, but were merely filled with soft tissue instead (Figures 3C, 3G, and 3K). In the hematoxylin and eosin–stained sections, cartilage from the alloMSC-treated group (Figure 3A) showed substantial thickening of the cartilage tissue compared with the untreated defect (Figure 3C), with the chondrocytes arranged in clusters. Similar cartilage tissue thickening was also observed in the autoC-treated group (Figure 3B); however, the chondrocytes were mostly found in columnar formations. The regenerated tissues from both treatment groups showed a continuous surface with a mixture of hyaline and fibrocartilage. Immunohistochemical staining for type II

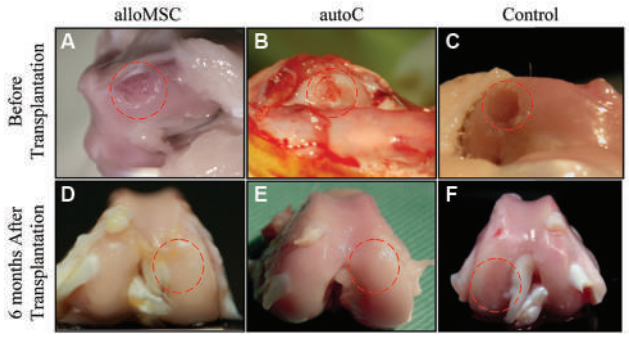

Figure 1. Macroscopic evaluation of the defective areas on articular cartilage from the alloMSC, autoC, and control groups before treatment (A, B, and C, respectively) and 6 months after transplantation (D, alloMSC; E, autoC; and F, control). There was incomplete filling of the control knee, while the defect appeared completely filled by both alloMSC and autoC transplantation. alloMSC, allogenic undifferentiated mesenchymal stem cells; autoC, autologous chondrocytes.

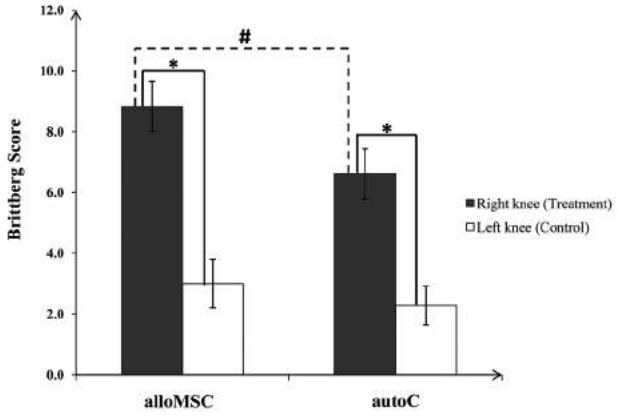

Figure 2. Brittberg scores of articular cartilage regeneration in alloMSC and autoC treatment groups were compared with their respective control on the contralateral side 6 months after transplantation. The Brittberg score was significantly higher in alloMSC compared with autoC. Both treatments were associated with significantly higher scores compared with their respective control groups. alloMSC, allogenic undifferentiated mesenchymal stem cells; autoC, autologous chondrocytes. *Significant difference from untreated, negative control ($P < .01$). #Significant difference between alloMSC and autoC group ($P < .05$).

collagen further confirmed that both the alloMSC- and autoC-treated tissue sections showed a relatively higher expression of cartilaginous collagen (Figures 3E and 3F) compared with that of the untreated defects (Figure 3G). The alloMSC- and autoC-treated tissue sections also showed relatively heavier staining with safranin O (Figures 3I and 3J) compared with the untreated defect (Figure 3K), suggesting a higher concentration of proteoglycans, one of the major components of cartilage tissue.

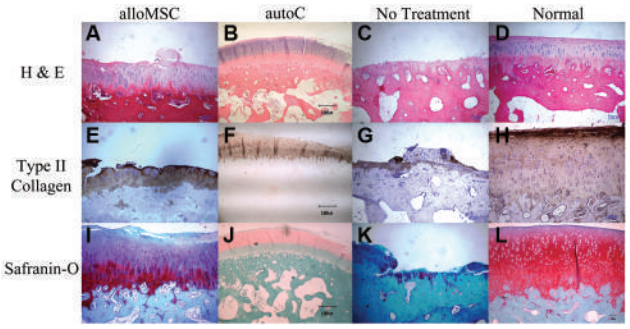

Figure 3. Immunohistochemical staining of rabbit femoral condyles from alloMSC-treated (A, E, I), autoC-treated (B, F, J), and untreated (C, G, K) tissues at 6 months after transplantation. A representative specimen of a normal cartilage tissue is shown (D, H, L) as a reference for evaluation of the normal cartilage healing. The type II collagen is stained in brown, and the safranin O (ie, a measure of proteoglycans characterizing cartilage tissue) is stained in red. alloMSC, allogenic undifferentiated mesenchymal stem cells; autoC, autologous chondrocytes. Scale bars = 100 μm.

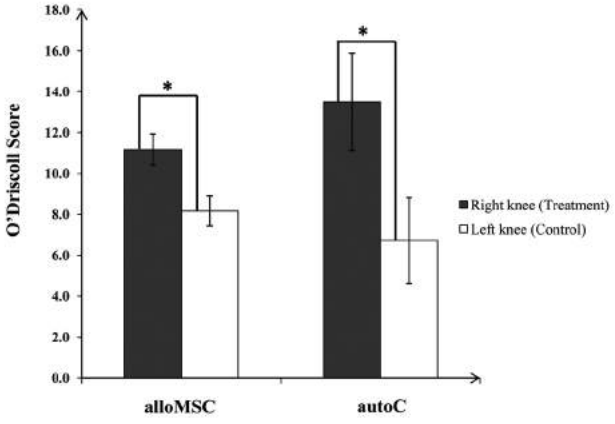

Figure 4. Quantitative histologic evaluation of the regenerated cartilage using O'Driscoll scores at 6 months after treatment with alloMSC and autoC together with their respective controls. Results showed significantly higher scores in the treatment groups compared with their controls. However, there are no significant differences in the scores between the 2 treatment groups. alloMSC, allogenic undifferentiated mesenchymal stem cells; autoC, autologous chondrocytes. *Significant difference from untreated, negative control ($P < .01$).

The average difference in the O'Driscoll score (ie, quantitative histologic grading of the tissues) between the treatment groups appeared to be very small and nonsignificant (Figure 4), with mean values of 11.2 ± 0.8 and 13.5 ± 2.4 for alloMSC and autoC groups, respectively ($P > .05$). However, both treatment groups had significantly higher O'Driscoll scores compared with their respective controls. The mean O'Driscoll score for the alloMSC-control was 8.2 ± 0.7, and that for the autoC-control was 6.7 ± 2.1.

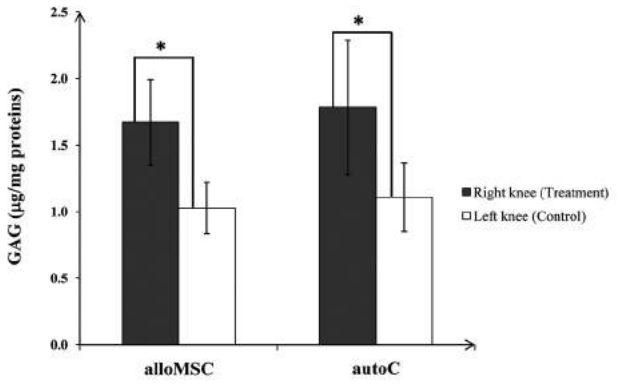

Figure 5. Comparison of the glycosaminoglycan (GAG) concentrations in the alloMSC and autoC treatment groups with their respective control groups, showing significant differences between each treatment with its respective control but no significant differences between the 2 treatment groups. alloMSC, allogenic undifferentiated mesenchymal stem cells; autoC, autologous chondrocytes. *Significant difference from untreated, negative control ($P < .05$).

Glycosaminoglycan Concentrations in the Regenerated Tissues

Quantitative determination of GAG levels in the tissue samples revealed a dramatic and significant increase in GAG concentrations in both the alloMSC- and autoC-treated knees as compared with the untreated knees ($P = .001$). However, there was no significant difference in GAG levels between the 2 treatment groups ($P = .74$). The mean GAG/protein concentrations in the treated knees were 1.7 ± 0.3 and 1.8 ± 0.5 μg GAGs/mg proteins for the alloMSC and autoC groups, respectively. For the control knees, the mean GAG/protein concentrations for the alloMSC and autoC groups were 1.0 ± 0.2 and 1.1 ± 0.3 μg GAGs/mg proteins, respectively (Figure 5). No significant differences for both knees were observed between these groups ($P > .05$).

DISCUSSION

The use of both autoC and alloMSCs produced comparable cartilage repair outcome, and was significantly better than those left untreated. Although the superior outcome of using cell-based therapy is not unexpected, it is anticipated that differences between the use of both cell sources should have been evident in the study. In particular, alloMSC is postulated to result in superior tissue repair as compared with autoC in view of the numerous advantages potentially conferred by MSCs in cartilage regeneration, as further discussed in this section. In the current study, the morphologic, histologic, and biochemical profiles of the regenerated cartilage tissue using the two cell sources were compared. This was envisaged to provide more objective evidence and further insight into the effectiveness of the repair process following either alloMSC or autoC treatment. To the best of our knowledge, this study is the first to report the results of direct parallel comparison between autoC and alloMSC for repair of focal cartilage defects in an animal model in vivo.

The complementing results observed from the various parameters examined in this study support the findings that cell therapies induce cartilage regeneration after injury.[13,15,20,22,30,50] Histologic observations revealed several notable features that may be of interest. In the alloMSC-treated group, chondrocytes in the histologic sections appeared to be clustered in groups, while in the autoC-treated group, cells were in columnar arrangements. However, the difference in the histologic appearance between the 2 treatment groups did not appear to affect the O'Driscoll histologic score. It is unclear whether the different structural arrangements and orientation of cells within the repair site result in any significant alterations in the cartilage biomechanical function. Further studies investigating potential correlation between the different histologic structures of the regenerated cartilage and its biomechanical integrity would be of substantial value.

The outcomes of the present study appear to support the clinical findings published by Nejadnik et al,[38] who concluded that transplanted autologous bone marrow–derived MSCs in patients is as effective as ACT. An apparent limitation noted in the referred study is that there was an absence of standardization of variables such as the size and the depth of cartilage defects as well as patient age. These factors might affect the healing process and therefore could potentially confound the results of the study. It was previously demonstrated that increased passages and donor age contributed toward reducing the rate of MSC differentiation, which is critical to cartilage healing.[7,9,45,46] Thus, we attempted to overcome this issue by conducting an animal study in a more controlled environment, which included ensuring the uniformity of defect size, homogeneous cartilage tissue, similar animal age, and using an animal from a smaller genetic pool. Apart from using animals within a narrow margin of age group, parallel comparison between the treated and untreated groups was conducted within the same animal in the present study. The use of cells embedded in alginates for transplantation was necessary to ensure that transplantation could be conducted more conveniently in a standardized manner. In addition, MSCs maintained in a 3-dimensional environment such as that provided by the alginate scaffold promotes chondrogenic differentiation and produces selective upregulation of cartilage-specific genes.[17,26,52] Previous studies have also shown that chondrocytes in alginate beads remain viable up to 8 months in culture and produce cell-associated matrix similar to hyaline articular cartilage.[21,23-25]

The use of MSCs as a treatment approach for articular cartilage injury should be considered since it is associated with a greater number of advantages and has comparable efficacy to that of ACI. One of the established limitations of ACI is the insufficiency in cell supply. Autologous chondrocyte implantation is commonly associated with limited ability of the harvested chondrocytes to proliferate and undergo differentiation.[35] In contrast, MSCs have a higher capacity of self-replication and differentiation both in vitro and in vivo.[44] Mesenchymal stem cells are also versatile

and can be expanded for a prolonged period of time without transforming into other cell phenotypes.[32,54] On the contrary, chondrocytes often lose their cartilaginous phenotype during expansion in monolayer culture.[5,6] Mesenchymal stem cells can facilitate homing and engraftment of other stem cells, which may facilitate tissue healing. They can potentially be mobilized into the circulation in response to injury signals, and exert their reparative effects at the site of injury.[31] However, despite all of these advantages, it should be appreciated that the entire process of MSC harvest and implantation is rather complex and should be well understood before its application in the clinical field. The use of allogenic MSCs (or alloMSCs) in clinical applications has yet to be ascertained, although several studies using animal models have shown promising results.[3,15,37,49,51,53] While there may be concerns of graft rejection and poor tissue healing because of immune-incompatibility between alloMSCs and the host tissues, in vitro studies suggest that human leukocyte antigen (HLA)–mismatched MSCs do not provoke an immune response in the host and are even able to suppress allogenic lymphocyte proliferation.[39] However, alloMSC transplantation in humans needs to be further investigated in a rigorous manner to ensure that this potential therapeutic strategy remains free from any considerable problems.

Our study presents several limitations that are worth noting. Because of constrained financial resources, the tissue analyses could not be further extended into biochemical evaluation and/or gene expression analysis. Financial restriction also prohibited functional assessment of the repaired site (eg, biomechanical testing). Thus, it was difficult to ascertain whether the observed difference in histologic appearance of the regenerated cartilage between alloMSCs and autoC affects the biomechanical function of the tissues. Nevertheless, we consider that this study still provides valuable findings since the conclusions were drawn using well-accepted experimental techniques utilized in several other previous studies.[27,38,53] Introduction of another treatment arm that utilizes a standard treatment approach for cartilage repair, such as subchondral drilling or microfracture, would have led to a more comprehensive conclusion. However, since the study was not designed to validate alloMSC or autoC against a standard treatment procedure, as well as the limitation in resources, this action was not pursued. Improvement could also be made to the study by using autologous MSCs instead of allogenic sources. This, however, can only be performed in larger animal models, given that extracting MSCs from rabbit marrow may prove to be too challenging. The issue of cell viability following cell culture in alginate between the 2 treatment groups was not specifically addressed in the current study, which could be further incorporated in future studies.

In conclusion, chondral defects treated with allogenic MSCs or autoC produced hyaline-like repair tissue that had significantly better quality than that of the control untreated knees. However, both alloMSC and autoC demonstrated comparable tissue regenerative profiles. In view of the additional advantages conferred by MSCs, such as self-renewal ability ensuring sufficient cell supply and lesser donor-site morbidity, the use of MSCs to treat full thickness articular cartilage defects remains a viable and attractive therapeutic option. Nevertheless, caution should be exercised when translating these findings into clinical applications owing to the intrinsically rapid healing potential of rabbits compared with human cartilage. A better understanding of the complex molecular events that are involved in chondrogenesis induced by MSCs and the in vivo integration of cell constructs with the native articular cartilage is required before widespread use of MSCs could be strongly advocated in the clinical field.

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REFERENCES

1. Ab-Rahim S, Selvaratnam L, Kamarul T. The effect of TGF-beta1 and beta-estradiol on glycosaminoglycan and type II collagen distribution in articular chondrocyte cultures. *Cell Biol Int*. 2008;32(7):841-847.
2. Adachi N, Ochi M, Deie M, Ito Y. Transplant of mesenchymal stem cells and hydroxyapatite ceramics to treat severe osteochondral damage after septic arthritis of the knee. *J Rheumatol*. 2005;32(8):1615-1618.
3. Anraku Y, Mizuta H, Sei A, et al. Analyses of early events during chondrogenic repair in rat full-thickness articular cartilage defects. *J Bone Miner Metab*. 2009;27(3):272-286.
4. Bartlett W, Skinner JA, Gooding CR, et al. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. *J Bone Joint Surg Br*. 2005;87(5):640-645.
5. Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell*. 1978;15(4):1313-1321.
6. Benya PD, Padilla SR, Nimni ME. The progeny of rabbit articular chondrocytes synthesize collagen types I and III and type I trimer, but not type II: verifications by cyanogen bromide peptide analysis. *Biochemistry*. 1977;16(5):865-872.
7. Bos PK, Verhaar JA, van Osch GJ. Age-related differences in articular cartilage wound healing: a potential role for transforming growth factor beta1 in adult cartilage repair. *Adv Exp Med Biol*. 2006;585:297-309.
8. Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. *Biotechnol Bioeng*. 2006;93(6):1152-1163.
9. Braun J, Hack A, Weis-Klemm M, et al. Evaluation of the osteogenic and chondrogenic differentiation capacities of equine adipose tissue-derived mesenchymal stem cells. *Am J Vet Res*. 2010;71(10):1228-1236.
10. Brittberg M. Autologous chondrocyte transplantation. *Clin Orthop Relat Res*. 1999;367(Suppl):S147-S155.
11. Brittberg M. Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced autologous chondrocyte implantation procedure. *Am J Sports Med*. 2010;38(6):1259-1271.
12. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. 1994;331(14):889-895.

13. Brittberg M, Nilsson A, Lindahl A, Ohlsson C, Peterson L. Rabbit articular cartilage defects treated with autologous cultured chondrocytes. *Clin Orthop Relat Res*. 1996;326:270-283.
14. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol*. 2007;213(2):341-347.
15. Dashtdar H, Rothan HA, Tay T, et al. A preliminary study comparing the use of allogenic chondrogenic pre-differentiated and undifferentiated mesenchymal stem cells for the repair of full thickness articular cartilage defects in rabbits. *J Orthop Res*. 2011;29(9):1336-1342.
16. Diduch DR, Jordan LC, Mierisch CM, Balian G. Marrow stromal cells embedded in alginate for repair of osteochondral defects. *Arthroscopy*. 2000;16(6):571-577.
17. Duggal S, Fronsdal KB, Szoke K, Shahdadfar A, Melvik JE, Brinchmann JE. Phenotype and gene expression of human mesenchymal stem cells in alginate scaffolds. *Tissue Eng Part A*. 2009;15(7):1763-1773.
18. Friedenstein AJ. Precursor cells of mechanocytes. *Int Rev Cytol*. 1976;47:327-359.
19. Gao J, Knaack D, Goldberg VM, Caplan AI. Osteochondral defect repair by demineralized cortical bone matrix. *Clin Orthop Relat Res*. 2004;427(Suppl):S62-S66.
20. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. *J Orthop Res*. 1989;7(2):208-218.
21. Guo JF, Jourdian GW, MacCallum DK. Culture and growth characteristics of chondrocytes encapsulated in alginate beads. *Connect Tissue Res*. 1989;19(2-4):277-297.
22. Han SH, Kim YH, Park MS, et al. Histological and biomechanical properties of regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold in vivo. *J Biomed Mater Res A*. 2008;87(4):850-861.
23. Hauselmann HJ, Aydelotte MB, Schumacher BL, Kuettner KE, Gitelis SH, Thonar EJ. Synthesis and turnover of proteoglycans by human and bovine adult articular chondrocytes cultured in alginate beads. *Matrix*. 1992;12(2):116-129.
24. Hauselmann HJ, Fernandes RJ, Mok SS, et al. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. *J Cell Sci*. 1994;107(Pt 1):17-27.
25. Hauselmann HJ, Masuda K, Hunziker EB, et al. Adult human chondrocytes cultured in alginate form a matrix similar to native human articular cartilage. *Am J Physiol*. 1996;271(3 Pt 1):C742-C752.
26. Herlofsen SR, Kuchler AM, Melvik JE, Brinchmann JE. Chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells in self-gelling alginate discs reveals novel chondrogenic signature gene clusters. *Tissue Eng Part A*. 2011;17(7-8):1003-1013.
27. Hui JH, Chen F, Thambyah A, Lee EH. Treatment of chondral lesions in advanced osteochondritis dissecans: a comparative study of the efficacy of chondrocytes, mesenchymal stem cells, periosteal graft, and mosaicplasty (osteochondral autograft) in animal models. *J Pediatr Orthop*. 2004;24(4):427-433.
28. Hunziker EB. Articular cartilage repair: basic science and clinical progress—a review of the current status and prospects. *Osteoarthritis Cartilage*. 2002;10(6):432-463.
29. Kamarul T, Ab-Rahim S, Tumin M, Selvaratnam L, Ahmad TS. A preliminary study of the effects of glucosamine sulphate and chondroitin sulphate on surgically treated and untreated focal cartilage damage. *Eur Cell Mater*. 2011;21:259-271; discussion 270-271.
30. Kamarul T, Selvaratnam L, Masjuddin T, et al. Autologous chondrocyte transplantation in the repair of full-thickness focal cartilage damage in rabbits. *J Orthop Surg (Hong Kong)*. 2008;16(2):230-236.
31. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell*. 2009;4(3):206-216.
32. Kim J, Kang JW, Park JH, et al. Biological characterization of long-term cultured human mesenchymal stem cells. *Arch Pharm Res*. 2009;32(1):117-126.
33. Ma HL, Hung SC, Lin SY, Chen YL, Lo WH. Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads. *J Biomed Mater Res A*. 2003;64(2):273-281.
34. Manfredini M, Zerbinati F, Gildone A, Faccini R. Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique. *Acta Orthop Belg*. 2007;73(2):207-218.
35. Marlovits S, Zeller P, Singer P, Resinger C, Vecsei V. Cartilage repair: generations of autologous chondrocyte transplantation. *Eur J Radiol*. 2006;57(1):24-31.
36. Minas T. Autologous chondrocyte implantation for focal chondral defects of the knee. *Clin Orthop Relat Res*. 2001;391(Suppl):S349-S361.
37. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum*. 2003;48(12):3464-3474.
38. 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(6):1110-1116.
39. Niemeyer P, Krause U, Kasten P, et al. Mesenchymal stem cell-based HLA-independent cell therapy for tissue engineering of bone and cartilage. *Curr Stem Cell Res Ther*. 2006;1(1):21-27.
40. Pelttari K, Steck E, Richter W. The use of mesenchymal stem cells for chondrogenesis. *Injury*. 2008;39(Suppl 1):S58-S65.
41. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*. 2000;374:212-234.
42. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143-147.
43. Prockop DJ, Gregory CA, Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. *Proc Natl Acad Sci U S A*. 2003;100(Suppl 1):11917-11923.
44. Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*. 2002;20(6):530-541.
45. Szeparowicz P, Popko J, Sawicki B, Wolczynski S. Is the repair of articular cartilage lesion by costal chondrocyte transplantation donor age-dependent? An experimental study in rabbits. *Folia Histochem Cytobiol*. 2006;44(3):201-206.
46. Vasara AI, Hyttinen MM, Pulliainen O, et al. Immature porcine knee cartilage lesions show good healing with or without autologous chondrocyte transplantation. *Osteoarthritis Cartilage*. 2006;14(10):1066-1074.
47. Vinatier C, Bouffi C, Merceron C, et al. Cartilage tissue engineering: towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr Stem Cell Res Ther*. 2009;4(4):318-329.
48. Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noel D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. *Trends Biotechnol*. 2009;27(5):307-314.
49. 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(4):579-592.
50. Wakitani S, Kimura T, Hirooka A, et al. Repair of rabbit articular surfaces with allografts of chondrocytes embedded in collagen gels [in Japanese]. *Nippon Seikeigeka Gakkai Zasshi*. 1989;63(5):529-538.
51. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res*. 2007;25(7):913-925.
52. Xu J, Wang W, Ludeman M, et al. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. *Tissue Eng Part A*. 2008;14(5):667-680.
53. Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy*. 2007;23(2):178-187.
54. Yoo JU, Barthel TS, Nishimura K, et al. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. *J Bone Joint Surg Am*. 1998;80(12):1745-1757.
55. Zippel N, Schulze M, Tobiasch E. Biomaterials and mesenchymal stem cells for regenerative medicine. *Recent Pat Biotechnol*. 2010;4(1):1-22.