Effects of Autogenous Bone Marrow Aspirate Concentrate on Radiographic Integration of Femoral Condylar Osteochondral Allografts

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Background: Transplantation of fresh osteochondral allografts (OCAs) is an attractive treatment option for symptomatic articular cartilage lesions in young, healthy patients. Because the lack of OCA bone integration can be a cause of treatment failure, methods for speeding and enhancing OCA bone integration to mitigate this potential complication are highly desirable.

Purpose: To determine if autogenous bone marrow aspirate concentrate (BMC) treatment of large femoral condylar OCAs would be associated with superior radiographic OCA bone integration compared with nontreated allografts during the critical first 6 months after surgery.

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

Methods: A review of patients enrolled in a prospective registry who were treated with transplantation of large OCAs to one or both femoral condyles at our institution from March 12, 2013 to March 14, 2016 was performed. Patients were stratified into 2 groups based on BMC treatment versus no BMC treatment; the treatment was nonrandomized and was rooted in a shift in practice and a continuing effort to optimize OCA transplantation at our institution. Patients were excluded if they did not have orthogonal view radiographs performed at 6 weeks, 3 months, and 6 months postoperatively. Each condyle undergoing OCA transplantation was assessed individually by an independent musculoskeletal radiologist, who was blinded to the treatment group and time point. OCAs were assessed with respect to graft integration (0%-100%; 0 = no integration, 100 = complete integration) and degree of sclerosis (0-3; 0 = normal, 1 = mild sclerosis, 2 = moderate sclerosis, and 3 = severe sclerosis) of the graft at each time point.

Results: This study identified 17 condyles in 15 patients who underwent OCA transplantation without BMC and 29 condyles in 22 patients who underwent OCA transplantation with BMC. The BMC group had significantly (P = .033) higher graft integration scores at 6 weeks, 3 months, and 6 months after surgery. Graft sclerosis was significantly (P = .017) less in the BMC group at 6 weeks and 3 months, with no significant difference at 6 months after surgery. When combining the groups to examine the influence of smoking on graft integration, nonsmokers had significantly (P = .007) higher graft integration scores at 6 months.

Conclusion: Large femoral condylar OCAs treated with autogenous BMC before implantation showed superior radiographic integration to bone and less sclerosis during the initial 6-month postoperative period. BMC treatment of OCAs may mitigate the failure of OCA bone healing.

Keywords: osteochondral allograft; bone marrow aspirate concentrate; radiographic integration; cartilage; bone healing

Articular cartilage lesions are identified in up to 66% of patients undergoing knee arthroscopic surgery, with approximately 20% of these lesions being considered high grade.[22,45] Symptomatic chondral lesions of the femoral condyles are a common problem in athletes and are associated with significant pain and loss of function.[20] Cartilage has a limited potential for spontaneous repair[3,43]; therefore, surgical intervention often offers the greatest potential for therapeutic benefit. For large (>2.5 cm^2) lesions, transplantation of fresh osteochondral allografts (OCAs) has become an increasingly popular treatment choice because of its success and the reported limitations of debridement, microfracture, and osteochondral autograft

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transfer.[12,17-19,33,41,46] Improvements in implantation techniques and selection criteria have led to a reported 88% rate of return to sport[29] and a greater than 75% 10-year survival rate after OCA transplantation.[1,13,31]

OCAs comprising size-matched donor tissue with live chondrocytes, mature hyaline cartilage, and underlying subchondral bone are transplanted in a single stage to address osteochondral or articular surface defects.[33] A low rate of graft survival typically results from the loss of cartilage integrity or inadequate integration of graft bone into the patient. Articular cartilage survival is dependent on chondrocyte viability,[4,7,9,36] while OCA bone integration occurs via the long and arduous process of creeping substitution.[32,35] Hence, techniques that expedite and enhance OCA bone integration to mitigate the risk of failure are highly desirable.

Bone marrow aspirate concentrate (BMC), which contains osteoprogenitor cells and osteoinductive proteins, is a cost-effective adjuvant approved for clinical use in augmenting bone healing. BMC potentiates an anabolic anti-inflammatory environment that may accelerate and promote the process of OCA bone integration.[5,23] Previous animal studies and clinical trials have indicated that BMC may be effective in accelerating bone integration to treat large cartilage lesions when used as an adjuvant therapy.[14-16,25,28,38] Therefore, this study was designed to test the hypothesis that large femoral condylar OCAs augmented with BMC would be associated with superior radiographic OCA bone integration and less sclerosis compared with allografts without BMC during the critical first 6 months after surgery.

METHODS

After institutional review board approval, a retrospective review of patients enrolled in a prospective registry who were treated with transplantation of large OCAs to one or both femoral condyles at our institution from March 12, 2013 to March 14, 2016 was performed. At our institution, surgical indications for OCA transplantation in the knee included the presence of large (>2.5 cm^2), symptomatic grade IV lesions of the femoral condyles and the failure of nonsurgical or previous surgical treatments. The registry review resulted in the identification of 67 patients treated with OCA transplantation using grafts obtained from 1 of 3 accredited United States tissue banks and implanted by a single surgeon. Patients were excluded if they did not have orthogonal view radiographs (true anteroposterior and true lateral) performed at our institution at 6 weeks (±1 weeks), 3 months (±2 weeks), and 6 months (±2 weeks) postoperatively using the same digital X-ray unit (General Electric Healthcare) and technique. The application of inclusion/exclusion criteria resulted in the selection of 39 condyles in 36 patients who underwent OCA transplantation. Clinical notes were reviewed to characterize initial presentations and demographic information. Procedural and follow-up notes were used to determine diagnostic and surgical details and postoperative complications.

Patients were stratified into 2 groups based on treatment with or without autogenous BMC. The treatment was nonrandomized and was determined by a shift in practice based on a continuing effort to optimize OCA transplantation at our institution and the emerging evidence.[34] In addition to the body of evidence supporting the use of BMC to augment bone healing,[4,14-16,25,28,34,38] an institutional review board–approved study conducted at our institution validated the technique and system used in the present study for producing autogenous BMC containing osteoprogenitor cells in platelet-rich plasma (PRP) (Cook JL, et al, unpublished data, 2016). Bone marrow aspirated from the anterior iliac crest, distal femoral metaphysis, and proximal tibial metaphysis of patients undergoing procedures requiring bone grafts was analyzed for red blood cell, white blood cell, and platelet counts compared with whole blood and assayed for osteoprogenitor cell content by determining colony-forming units (CFUs) in culture based on crystal violet staining. In all patients studied (n = 9), BMC from each site was composed of osteoprogenitor cells (mean, 36 CFU/mL at day 7 [range, 0-139 CFU/mL]) in PRP (mean platelet concentration, 2.0× whole blood [range, 1.2×-2.6×]) apart from 1 tibial sample, which did not produce CFUs. There were no significant differences among the harvest sites based on these data (Cook JL, et al, unpublished data, 2016).

Condyles in the no BMC group received large cylindrical grafts (n = 7; 1-3 per condyle) or shell OCAs (n = 10; 1 per condyle) according to current standard-of-care techniques, which included power irrigation of the osseous portion of the OCA with saline. Condyles in the BMC group received large cylindrical grafts (n = 8; 1-3 per condyle) or shell OCAs (n = 21; 1 per condyle) treated with BMC. For BMC treatment, bone marrow (80-120 mL) was aspirated from the ipsilateral distal femoral metaphysis undergoing OCA transplantation for patients in the BMC group. Bone marrow aspirate was obtained before OCA transplantation via lateral or medial direct metaphyseal access using a commercially available bone marrow aspiration needle (Arthrex) in three to four 30-mL syringes containing 5 mL of Anticoagulant Citrate Dextrose Solution, Solution A each and then processed in the operating theater using a commercially available system (Angel System; Arthrex). Bone marrow was obtained via slow, controlled syringe aspiration with needle movement as needed to maintain consistent flow. Once BMC was aseptically retrieved, the osseous portion of the OCA was power irrigated with saline. The osseous portion was then saturated with autogenous BMC for at least 2 minutes immediately

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TABLE 1
Demographic Information of Patients Who Underwent OCA Transplantation[a]

Parameter	No BMC	BMC	*P* Value
Sex, male/female	6/9	6/16	.30
Age, mean ± SD, y	33.4 ± 10.3	38.2 ± 11.9	.21
Body mass index, mean ± SD, kg/m^2	33.2 ± 6.3	29.1 ± 5.1	.10
Smoking	6	5	.30
Diabetes mellitus	1	1	.70
ASA physical status, mean ± SD	1.8 ± 0.6	1.7 ± 0.5	.50
Femoral condyle, lateral/medial	8/9	11/18	.80
OCA size, mean ± SD, cm^2	3.5 ± 1.6	3.3 ± 1.3	.70
No. of grafts, mean (range)	1.4 (1-3)	1.3 (1-3)	.27
Concomitant procedures (no BMC: n = 8; BMC: n = 9)			
ACL reconstruction	1	1	.70
Femoral osteotomy	0	1	.50
Meniscectomy	0	1	.50
Meniscus transplant	6	8	.45
Tibial osteotomy	4	2	.25
PCL reconstruction	1	0	.47
PLC reconstruction	1	0	.47

[a]Values are presented as n unless otherwise indicated. ACL, anterior cruciate ligament; ASA, American Society of Anesthesiologists; BMC, bone marrow aspirate concentrate; OCA, osteochondral allograft; PCL, posterior cruciate ligament; PLC, posterolateral corner.

before implantation. All other aspects of the intraoperative procedure and postoperative care were identical for each group.

Each condyle undergoing OCA transplantation was assessed individually by an independent musculoskeletal radiologist, who was blinded to the treatment group and time point. Both radiographic views from each patient at each time point were evaluated with respect to radiographic graft integration and graft sclerosis. With regard to graft integration, OCAs were assigned a score between 0% and 100% that corresponded with the estimated level of integration (0 = no integration, 100 = complete integration) into the patient's bone based on discreteness of the graft-host border, trabecular architecture of the grafted area of the condyle, and anatomic contour and geometry of the condyle.[6,8,34] Sclerosis was assessed based on relative radiopacity of the graft bone in comparison to normal femoral condylar bone and graded as 0 = normal, 1 = mild sclerosis, 2 = moderate sclerosis, or 3 = severe sclerosis at each time point.[8,26]

Patient demographics and radiographic findings were assessed for statistically significant differences within the groups over time using repeated-measures analysis of variance and between the groups using unpaired *t* tests, rank-sum tests, and Fisher exact tests. A *P* value <.05 was deemed to be statistically significant for all analyses.

RESULTS

A review of the registry and the application of inclusion/exclusion criteria identified 17 condyles in 15 patients who underwent OCA transplantation without BMC and 29 condyles in 22 patients who underwent OCA transplantation with BMC. There were 6 male and 16 female patients in the BMC group, with a mean age of 38.2 years (range, 18-56 years), compared with 6 male and 9 female patients in the no BMC group, with a mean age of 33.4 years (range, 15-47 years). The mean body mass index (BMI) in the BMC group was 29.1 kg/m^2 (range, 17-37 kg/m^2), while patients in the no BMC group had a mean BMI of 33.2 kg/m^2 (range, 20-43 kg/m^2). Five patients in the BMC group and 6 patients in the no BMC group self-identified as smokers. The mean size of the grafts was 3.3 ± 1.3 cm^2 for the BMC group and 3.5 ± 1.6 cm^2 for the no BMC group. For both groups, the graft depth ranged from 6 to 10 mm. Eight patients in the no BMC group and 9 patients in the BMC group underwent concomitant procedures at the time of surgery. There were no statistically significant differences between the groups for patient age, sex, BMI, American Society of Anesthesiologists (ASA) physical status classification system, and OCA size or proportions of lateral versus medial condyles, patients with diabetes, and smokers (Table 1).

For both groups, OCA bone incorporation increased at each subsequent time point ($P < .001$). Six weeks after surgery, the mean graft incorporation was 43.1% ± 22.8% in the BMC group compared with 25.6% ± 25.0% in the no BMC group ($P = .03$). By 3 months after surgery, graft incorporation increased to a mean 67.2% ± 17.9% in the BMC group compared with 50.6% ± 28.9% in the no BMC group ($P = .033$). At the 6-month time point, the mean graft incorporation was 84.1% ± 8.4% in the BMC group, while the mean graft incorporation was 74.4% ± 15.9% in the no BMC group ($P = .017$) (Table 2 and Figure 1). With respect to graft sclerosis, the BMC group displayed a significantly lower mean degree of sclerosis than the no BMC group at 6 weeks (1.4 ± 0.6 vs 1.9 ± 0.6, respectively; $P = .016$) and 3 months (1.2 ± 0.5 vs 1.7 ± 0.7, respectively; $P = .017$), while the difference at 6 months was not significant (0.9 ± 0.5 vs 1.2 ± 0.5, respectively; $P = .20$) (Table 2).

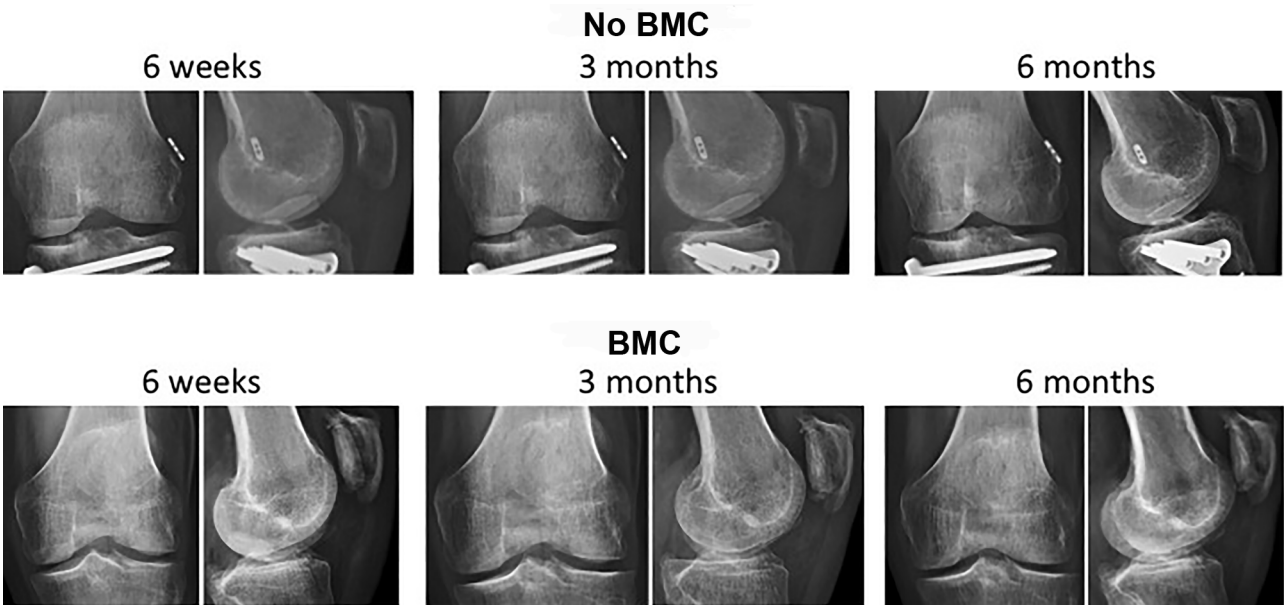

Figure 1. Representative radiographs displaying transplanted osteochondral allografts at 6 weeks, 3 months, and 6 months. BMC, bone marrow aspirate concentrate.

TABLE 2
Graft Integration in Patients Who Underwent OCA Transplantation With or Without BMC[a]

Time Point	Graft Integration, %	*P* Value	Graft Sclerosis	*P* Value
6 weeks		**.03**		**.016**
BMC	43.1 ± 22.8		1.4 ± 0.6	
No BMC	25.6 ± 25.0		1.9 ± 0.6	
3 months		**.033**		**.017**
BMC	67.2 ± 17.9		1.2 ± 0.5	
No BMC	50.6 ± 28.9		1.7 ± 0.7	
6 months		**.017**		.20
BMC	84.1 ± 8.4		0.9 ± 0.5	
No BMC	74.4 ± 15.9		1.2 ± 0.5	

[a]Values are presented as mean ± SD. Bolded values are statistically significant. BMC, bone marrow aspirate concentrate; OCA, osteochondral allograft.

The OCA groups were combined to examine the influence of smoking on graft integration. There were no significant differences in graft integration between smokers and nonsmokers at the 6-week and 3-month time points. However, by 6 months, nonsmokers had significantly higher mean graft integration than smokers (83.7% ± 8.2% vs 72.1% ± 17.5%, respectively; $P = .007$) (Table 3). When considering only those grafts treated with BMC, there were no statistically significant differences in graft integration between smokers and nonsmokers ($P > .36$) during the study period. For patients in this study, 1 (6% non-BMC) bone healing complication (symptomatic failure of allograft bone integration requiring surgical revision with bone grafting at 7 months postoperatively) occurred in a smoker in the no BMC group, while no bone healing complications occurred in the BMC group during the study period. No other complications related to the OCA transplantation procedure were noted in any patient in either group during the study period. Clinical outcomes and graft survival are followed in all patients included in the registry and will be reported for ongoing studies.

TABLE 3
Graft Integration in Smokers Versus Nonsmokers[a]

Time Point	Graft Integration, %	*P* Value
6 weeks		.90
Smokers	36.7 ± 27.7	
Nonsmokers	35.6 ± 24.2	
3 months		.40
Smokers	55.0 ± 33.9	
Nonsmokers	62.8 ± 18.6	
6 months		**.007**
Smokers	72.1 ± 17.5	
Nonsmokers	83.7 ± 8.2	

[a]Values are presented as mean ± SD. Bolded values are statistically significant.

DISCUSSION

To the authors' knowledge, this is the first clinical study to directly examine the effects of BMC for expediting OCA bone incorporation. The data showed that large femoral condylar OCAs treated with BMC were associated with better radiographic graft integration scores at 6 weeks, 3 months, and 6 months after transplantation. Graft sclerosis was also significantly less (more normal) in BMC-treated OCAs during the early healing period. Taken together, the results of this study suggest that BMC treatment of OCAs may mitigate the failure of donor bone healing based on more rapid integration into the patient, with less sclerotic change, during the critical period of healing after surgery.

For more than 30 years, OCA transplantation has gained traction as a treatment modality for large cartilage defects of the knee as well as the ankle, hip, and shoulder. Previous reports validated the safety, efficacy, and survivorship of this surgical procedure.[1,13,31] While numerous studies have focused on the importance of chondrocyte viability,[4,7,9,36] there has been relatively little focus on the osseous integration of these grafts after transplantation. OCAs integrate into host bone through the plodding process of creeping substitution,[32,35] which requires complete cellular repopulation, revascularization, and matrix replacement and remodeling. Unfortunately, the prolonged timeline for completion of this complicated process elevates the risk for graft failure when integration cannot keep pace with biomechanical and biological demands on the graft. Impending or definitive failure of allograft bone integration is diagnosed based on clinical symptoms in conjunction with radiographic findings of radiolucency at the graft-host border, graft subsidence, contour irregularities, and/or loss of normal trabecular architecture of the grafted condyle, including subchondral cysts and/or moderate to severe sclerosis at more than 6 months after transplantation.[6,8,34] As such, techniques aimed at enhancing and accelerating the process of OCA bone integration during this critical 6-month window are vital to improving patient outcomes. To date, the primary method for mitigating this potential problem has been creating OCAs with the minimal amount of bone required for stable implantation. Recommendations for graft thickness of 6 to 9 mm (3-7 mm of bone) are generally followed.

The present study examined the clinical results of a novel method for improving OCA integration. By saturating the osseous portion of the OCA with autogenous BMC immediately before implantation, radiographic integration of femoral condylar OCAs was improved. Based on previous data,[39] the benefits associated with BMC treatment of OCAs likely result from the combination of osteoprogenitor cells and osteoinductive and vascularization-enhancing proteins known to be present. There is some direct evidence for this with respect to OCAs from an in vitro study showing significantly higher levels of osteoprogenitor cells and more potentially beneficial protein profiles associated with BMC treatment of OCAs in tissue culture compared with those treated with PRP or the standard of care of saline irrigation.[39] Interestingly, there is evidence to suggest that the osteoprogenitor cells also have immunomodulatory mechanisms of action,[27] which could also redirect the allogenic-based process of creeping substitution to a more efficient and effective graft integration process. This possibility is supported by the superior radiographic integration data from this study as well as the finding that BMC-treated OCAs were associated with less sclerosis during integration.

Smoking is a well-established risk factor for bone healing complications such as nonunion, malunion, and fractures.[30,37,42,44] However, there are relatively little data on the effect of cigarette smoking on OCA integration. It is plausible to assume that the bone component of an OCA would be impacted by the same factors influencing fracture remodeling. Yet, in one study of preoperative variables affecting outcomes after OCAs in the knee, Nuelle et al[33] found that smoking did not have a significant effect on patient outcomes. In the present study, smoking did not significantly affect graft integration at the 6-week and 3-month time points, but at 6 months, nonsmokers displayed significantly better radiographic graft incorporation. Further, the only bone healing complication noted in this study was in a smoker who received a non-BMC graft. Importantly, BMC may also help mitigate the detrimental effects of smoking on OCA incorporation based on the findings in this study, which showed no significant differences in graft integration between smokers and nonsmokers with BMC-treated OCAs. However, additional work must be done before any conclusions regarding this effect can be made, and smoking must be considered at least a relative risk factor for OCA transplantation based on available data.

The authors recognize that there are limitations to the present study. There were 17 and 29 femoral condyles in the no BMC group and BMC group, respectively. Moving forward, work must be done to verify these results in a larger patient population consisting of OCA transplantation to regions other than the femoral condyle. The primary outcome measures of this study were radiographic graft integration and graft sclerosis. These are both qualitative measures that were assigned numerical values by a musculoskeletal radiologist, who was blinded to the treatment group and time point. In assessing these factors, there will likely be some degree of interobserver variation, but we believe that this bias was limited by having the same radiologist view all images in a blinded fashion. The inclusion of advanced diagnostic imaging such as computed tomography or magnetic resonance imaging may provide more sensitive and specific data; however, the application of a global scoring system to radiographic imaging has been shown to produce reliable results, and it allows for valid clinical studies using a standard-of-care, longitudinal diagnostic modality.[8,26,40] Only 6-month imaging data were examined and reported for the present study. Midterm and long-term results with respect to OCA survival rates and functional outcomes are critical data to continue to follow and report, which is ongoing at our institution. The focus of the present study, however, was to specifically target one of the most common failure mechanisms for OCA transplantation, allograft bone integration, during its most critical period. While differences in the radiographic appearance in successful cases would likely be similar in the longer term, failure appears to be more

dependent on the initial time to integration,[2,10,11] which was the focus of the present study. The data reported provide initial support for this conclusion, providing the impetus for our decision to report these novel data at an early time point to inform surgeons and patients. Despite these limitations, the authors suggest that the results of this study are clinically applicable and that patients undergoing OCA transplantation in the knee may benefit from the addition of autogenous BMC treatment of donor bone.

Large femoral condylar OCAs treated with autogenous BMC immediately before implantation showed superior radiographic integration to bone and less sclerosis during the initial 6-month postoperative period. These data suggest that BMC treatment of OCAs may mitigate the failure of OCA bone healing. On the basis of the safety profile and cost-effectiveness of autogenous BMC,[21,24] the authors employ BMC treatment of the osseous portion of all large OCAs used for transplantation in their patients.

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