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ORIGINAL ARTICLE

Optimising femoral-head osteochondral allograft transplantation in a preclinical model

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Summary *Background/Objective:* Osteochondral autografting and allografting of the femoral head have been described as treatments for avascular necrosis without segmental collapse, fracture, osteochondritis dissecans, and tumours. One long-term study reported that 80% of nonsteroid-treated patients had successful outcomes. Most data are compiled from small case reports or series. Although these results are encouraging, to the authors' knowledge, there is no basic scientific evidence regarding optimal graft source or technique reported in the peer-reviewed literature. The objective of this study was to create a translational canine model to compare femoral-head osteochondral autografts and allografts with respect to safety and efficacy.

Methods: With Institutional Animal Care and Use Committee approval, skeletally mature hound-mix dogs ($n = 6$) weighing >20 kg underwent aseptic surgical implantation of osteochondral grafts using a craniolateral approach to the hip, without dislocation. Three graft options were evaluated: small auto ($n = 3$), 6-mm-diameter autograft from the trochlear ridge of the ipsilateral knee; small allo ($n = 3$), 6-mm-diameter fresh (21-day storage) allograft from a size-matched canine femoral head; or large allo ($n = 3$), 14-mm-diameter fresh (21-day storage) allograft from a size-matched canine femoral head. Small grafts were implanted into the same femoral head of three dogs, and large grafts were implanted alone in the other three dogs. The dogs were allowed unrestricted activity in their runs, and were walked on a leash for 15 minutes 5 times/wk. The outcome measures included functional, radiographic, and arthroscopic assessments at 8 weeks, and functional, chondrocyte viability, and histologic assessments at 6 months after surgery. The pre- and postoperative data were compared for

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statistically significant ($p < 0.05$) differences. Based on data from the canine study, four human patients underwent fresh (<28-day storage) osteochondral allografting using large (>30-mm diameter) size-matched femoral-head grafts. The radiographic, quality of life, and functional assessments were captured postoperatively.

Results: All grafts had >80% chondrocyte viability at the time of implantation. All grafts showed radiographic evidence for integration into host bone. Small auto and small allo showed significant ($p < 0.05$) loss in range of motion, chondrocyte viability, and articular-cartilage integrity 8 weeks after implantation, whereas large allo maintained viability and structural integrity throughout the study period. The large-allo dogs maintained full hip range of motion and hindlimb function. A similar type of large allograft (>30 mm) was performed in the four human patients. Due to the defect size, three out of the four human patients required two large allografts at the time of implantation. At the time of this manuscript's acceptance, patient follow-up ranged from 4 months to 18 months. All human patients were full weight-bearing without an assistive device, and showed no evidence of graft failure or progressive arthrosis.

Conclusion: These data provide initial translational and clinical evidence for large osteochondral allografts as a potential option for functional resurfacing of full-thickness cartilage defects of the femoral head.

Introduction

Femoral-head defects resulting from trauma or disease processes pose significant management challenges, especially when they are large and/or occur in young patients where total joint arthroplasty is not ideal and other treatment options are limited. Osteochondral allograft (OCA) transplantation has been extensively used and studied for the treatment of large articular defects of the human knee with good to excellent long-term results reported [1—3]. OCA transplantation for the treatment of femoral-head defects in human patients has also been described, but only in the form of case reports or small case series [4—7]. The largest case series in the peer-reviewed literature is from 1985, and reported that OCA transplantation of the femoral head for the treatment of post-traumatic femoral-head defects, avascular necrosis without segmental collapse, osteochondritis dissecans, and tumours to be associated with an 80% long-term success rate in patients who did not have a steroid-related aetiology [7]. However, numerous questions remain regarding optimal graft size and source and implantation technique.

The chondrocyte viability in OCAs at the time of transplantation has been reported to be critically important to the clinical success of the surgery [1,8—11]. As such, different storage methods and implantation techniques have been investigated to try to maintain chondrocyte viability in implanted OCAs above minimal acceptable levels (typically considered to be 70% viable cells) [8,12—16]. To address these factors, the Missouri Osteochondral Allograft Preservation System, a serum-free tissue-preservation method (Cook JL, Hung CT, Lima E, Stoker A, inventors. Tissue preservation system. United States patent application #US 2012/0177615 A1. 2012 Jul 12; claims pending 2015 Jun.) that has prolonged the time for maintenance of acceptable levels of chondrocyte viability in osteochondral tissues to more than twice as long the current standard-of-care based on *in vitro* and *in vivo* assessments [8,14,15], and an instrumentation system for creating tapered grafts that can be implanted such that chondrocyte viability is better preserved compared to standard cylindrical grafts [17], were developed.

Dogs were selected for this large animal model based on their extensive use in cartilage-repair research, the similar anatomy, pathologic conditions, treatment options in dogs' hips in comparison to humans, and successful use of OCAs in dogs [8,18—20]. In addition, dogs are one of the large animal species designated by the Food and Drug Administration and American Society for Testing and Materials guidelines as acceptable for preclinical studies designed to test the safety and efficacy of cartilage-repair techniques for clinical use [21,22].

The purpose of this study was to use a preclinical canine model to determine the effects of graft size and source and implantation technique on outcomes for femoral-head osteochondral transplantation with respect to safety and efficacy for clinical application in human patients.

Materials and methods

All procedures were approved by the University of Missouri's Animal Care and Use Committee. Six skeletally mature (age range 2—4 years) hound-mix (mean body weight = 28.2 kg; range 26.7—31.4 kg) purpose-bred research dogs (Marshall Farms BioResources, North Rose, NY, USA; US Department of Agriculture #21-A-008) were used. Complete orthopaedic examination and radiographs of both hips and both stifles (knees) were performed to ensure no musculoskeletal pathology was evident in any dog prior to enrolment in the study.

Clinical assessments

Orthopaedic assessments by a veterinary orthopaedic surgeon were performed on each dog at each time point (preoperatively, and 8 weeks and 6 months after surgery). The comfortable range of motion (CROM) of each hip was measured using a goniometer. With the dog standing, one limb of the goniometer was placed along the lateral axis of the femur and the other arm placed along the lateral axis of the pelvis from the centre of the iliac wing to the ischial tuberosity with the hinge point centred over the greater trochanter. The hip was then manually extended to the highest angle the dog tolerated without showing resistance or pain. The extension angle (degrees) noted on the goniometer at this point was recorded. The hip was then manually flexed to the most acute angle the dog tolerated without showing resistance or pain. The flexion angle (degrees) noted on the goniometer at this point was recorded. The flexion angle was subtracted from the extension angle to determine the CROM for each hip. Clinical lameness scores were determined for each dog at each time point (preoperatively, and 8 weeks and 6 months after surgery) based on a visual examination of gait by a veterinary orthopaedic surgeon using a 10-cm visual analogue scale and a validated grading system [8]: "0" for no observable lameness; "1" for intermittent, mild weight-bearing lameness with little, if any, change in gait; "2" for moderate weight-bearing lameness (obvious lameness with noticeable gait change); "3" for severe weight-bearing lameness ("toe touching" only); and "4" for nonweight bearing.

Surgical model

With approval from the University of Missouri's Animal Care and Use Committee, the femoral heads of purpose-bred adult (2—6 years old) mongrel dogs ($n = 10$) were aseptically harvested after humane euthanasia was performed for reasons unrelated to this study. The femoral heads were judged to be normal based on gross inspection prior to use. The femoral heads were preserved at room temperature (~25 °C) using the Missouri Osteochondral Allograft Preservation System for 21 days prior to implantation [8].

On the day of the surgery, the recipient dogs were premedicated with dexmedetomidine [5—10 μg/kg intravenous (IV)] and morphine (0.5 mg/kg IV). Anaesthesia was then induced 30 minutes following premedication using propofol (4—8 mg/kg IV). The right hindlimb of each dog was prepared for aseptic surgery. After draping, a standard craniolateral approach to the right hip (without osteotomy or dislocation) was performed. Each dog was randomly assigned to treatment of one hip with $n = 3$ in each treatment group. The graft-treatment options included: (1) small osteochondral autograft (small auto): 6-mm-diameter × 6-mm-depth cylindrical autograft aseptically obtained from the trochlear ridge of the ipsilateral knee (1 graft/knee) during the same surgical episode; (2) small OCA (small allo): 6-mm-diameter × 6-mm-depth cylindrical allograft aseptically obtained from a size-matched canine femoral head (1 graft/femoral head) of purpose-bred adult (2—6 years old) mongrel dogs and prepared as described as follows at the time of surgical implantation; and (3) large OCA (large allo): 14-mm-diameter × 6-mm-depth tapered allograft procured from a size-matched canine femoral head (one graft per femoral head) of purpose-bred adult (2—6 years old) mongrel dogs and prepared as described as follows at the time of surgical implantation.

With the hip adducted and externally rotated, grafts were placed into the cranio(anterio)dorsal, primary load-bearing aspect of the femoral head.

Small grafts were implanted into the same femoral head (alternated between anterior and posterior) of three dogs using commercially available press-fit cylindrical OCA instrumentation (Arthrex, Inc., Naples, FL, USA) to create each graft and each recipient socket (Figure 1).

Large grafts were implanted alone in the other three dogs using press-fit tapered OCA instrumentation (Comparative Orthopaedic Laboratory, University of Missouri, Columbia, MO, USA) to create each graft and each recipient socket (Figure 1).

The surgical wounds were closed routinely. Each dog received implants in only one hip, and the contralateral hips served as unoperated controls.

After the allografts were harvested and implanted, chondrocyte viability in the remaining portions of the femoral head was assessed as described as follows to determine the chondrocyte viability at the time of implantation.

The postoperative recovery was monitored, and analgesics (morphine 0.5 mg/kg intramuscular followed by tramadol 2—4 mg/kg postoperative) were administered to the dogs for 3 days following surgery. The dogs were allowed unrestricted activity in their individual kennels, and were walked on a leash for 15 minutes 5 times/wk.

Postoperative assessments

Eight weeks and 6 months after surgery, an orthopaedic examination to assess the hip CROM and the clinical lameness and function was performed on each dog as described previously. At each of these time points, the dogs were premedicated and anaesthetised as described previously for radiographic and arthroscopic assessments. Radiographic assessments were performed by one veterinary radiologist. The ventrodorsal and lateral views of the hips were evaluated for graft incorporation and radiographic criteria for osteoarthritis [23,24]. An arthroscopic assessment of the operated hips was performed using standard portals and technique [23]. The hips were assessed for appearance and integrity of the grafts and the surrounding and apposing articular cartilage, as well as the degree of synovitis present.

Postmortem assessments

After humane euthanasia was performed under anaesthesia, the operated hip from each dog was carefully dissected and disarticulated for tissue processing by a veterinary pathologist, who was blinded to treatment groups and clinical findings. Sections from each femoral head were immediately prepared for the determination of chondrocyte viability. The chondrocyte viability in constructs was assessed using two stains to detect live and dead cells (Molecular Probes; Thermo Fisher Scientific,

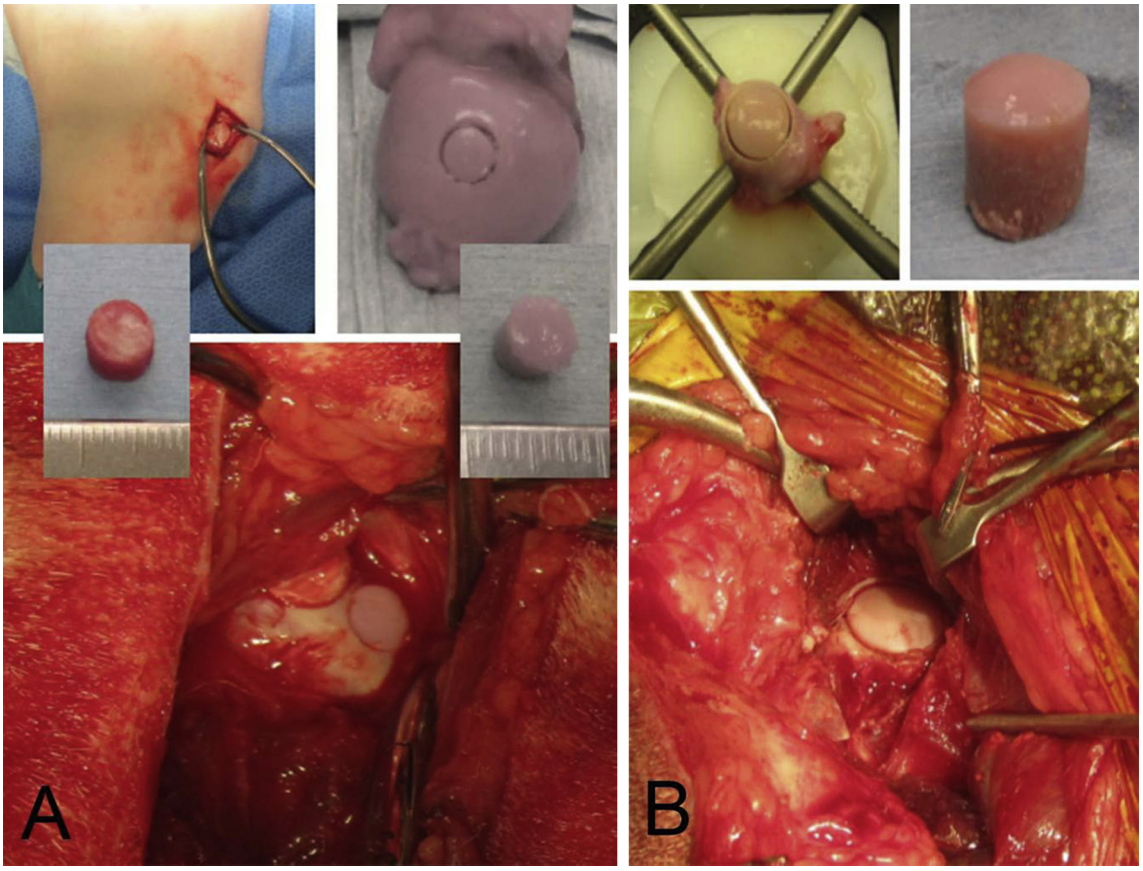

Figure 1 Clinical photo of canine (A) small-autograft (top left) and small-allograft harvest (top right) and implantation, and (B) large-allograft harvest and implantation.

Waltham, MA, USA), as per the manufacturer's suggested protocol, where live cells are stained green with calcein AM and dead cells are stained with SYTOX Blue (Thermo Fisher Scientific). The percent chondrocyte viability for each graft was quantified using digital image analysis [8].

The remaining portion of each femoral head was placed in 10% neutral buffered formalin fixative. After fixation, tissues were decalcified using 10% EDTA in phosphate-buffered saline until the end point of decalcification was reached as indicated by the ammonium-oxalate test (i.e., absence of detectable calcium in the decalcifying fluid). After decalcification, the tissues were dehydrated, paraffin embedded, and cut on a microtome into 5-μm sections for histologic examination. The specimens were deparaffinised; rehydrated; and stained with haematoxylin and eosin to determine the cell distribution and tissue morphology, with toluidine blue to assess the proteoglycan distribution, and with picrosirius red to determine the collagen integrity [25]. The osteochondral sections were evaluated by two veterinary pathologists who were blinded to the treatment group and clinical findings, and were scored based on the criteria described in the Osteoarthritis Research Society International histologic assessment system for dogs [26].

Statistical analyses

All statistical analyses were performed using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). Comparisons between the three treatment groups were performed using one-way analysis of variance and the Holm—Šidák method for multiple pairwise comparisons for continuous data, and Kruskal—Wallis one-way analysis of variance on ranks and Tukey test for multiple pairwise comparison procedures for categorical data. Differences with $p \leq 0.05$ were considered statistically significant.

Human patients

Based on data from the canine study, four human patients underwent fresh (<28-day storage) osteochondral allografting using large (>30-mm diameter) size-matched femoral-head grafts. Three patients had post-traumatic avascular necrosis (acetabular fracture dislocation, femoral neck fracture, and slipped capital femoral epiphysis), and the last patient had an acute traumatic femoral-head defect. The technique used for the human procedures included a surgical hip dislocation as described by Ganz et al [27]. The fresh grafts were obtained from certified tissue banks and were stored using standard tissue-bank protocols (4 °C) for <28 days from harvest. They were harvested, and the recipient site was prepared using the Arthrex graft system. The grafts were implanted using manual pressure (no mallet impaction) for a press-fit technique without hardware (Figure 2). Quality of life and functional assessments were captured postoperatively. A similar type of large allograft (>30 mm) was performed in the four patients.

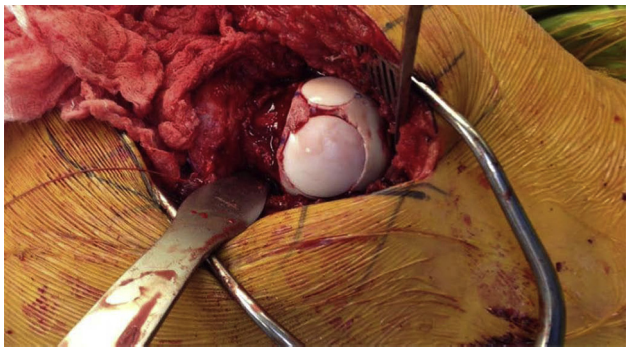

Figure 2 Clinical photo of implantation of two osteochondral allografts through a surgical dislocation in a 16-year-old male.

Results

All dogs had full CROM in both hips with no apparent hindlimb lameness or dysfunction prior to surgery. All allografts had >80% chondrocyte viability at the time of implantation based on live—dead staining of the remaining portions of the femoral-head grafts. All grafts were successfully implanted into each hip, and all dogs survived surgery and recovered without complication. No evidence for infection, graft rejection, or other untoward responses was noted.

All grafts in each group showed radiographic evidence for integration into the host bone by 8 weeks postoperatively (Figure 3). However, only dogs in the large-allo group were continued to the 6-month time point, as small-auto and small-allo hips showed significant ($p < 0.05$) lameness, dysfunction, and loss of CROM (Table 1), and arthroscopic and radiographic evidence for loss of articular-cartilage integrity with associated osteoarthritis at the 8-week assessment point. Therefore, these dogs underwent humane euthanasia under anaesthesia at this time point to characterise further the pathology by assessing the chondrocyte viability and histology as intended.

Hips in the large-allo group showed no significant loss of function or CROM when compared to the contralateral control hips at 8 weeks and 6 months after surgery (Table 1). The radiographic assessment of the large-allo hips showed graft integration into the host bone at 8 weeks postoperatively with absence of any radiographic evidence for osteoarthritis for all dogs throughout the 6-month study period (Figure 3). The arthroscopic assessment of the large-allo hips revealed maintenance of integrity of all grafts with lack of articular-cartilage pathology of grafts, surrounding femoral-head cartilage, or apposing acetabular cartilage (Figure 4). Mild synovitis was noted in each hip.

The percent chondrocyte viability was maintained at levels documented to be associated with long-term graft function for 6 months after implantation in large-allo grafts [8]. At 8 weeks after implantation, the percent chondrocyte viability in small-allo and small-auto grafts was significantly ($p < 0.05$) lower than in the large-allo grafts, and well below the levels associated with long-term success (Table 2, Figures 4 and 5).

Based on the histologic assessments, the small-allo and small-auto grafts had significantly ($p < 0.05$) more severe (higher score) pathology at 8 weeks after implantation when compared to the large-allo grafts at 6 months after implantation (Table 2). The small-auto grafts were associated with the most severe pathology. In the small grafts, histopathologic findings consisted of articular surface fibrillation and fissuring, loss of proteoglycan staining,

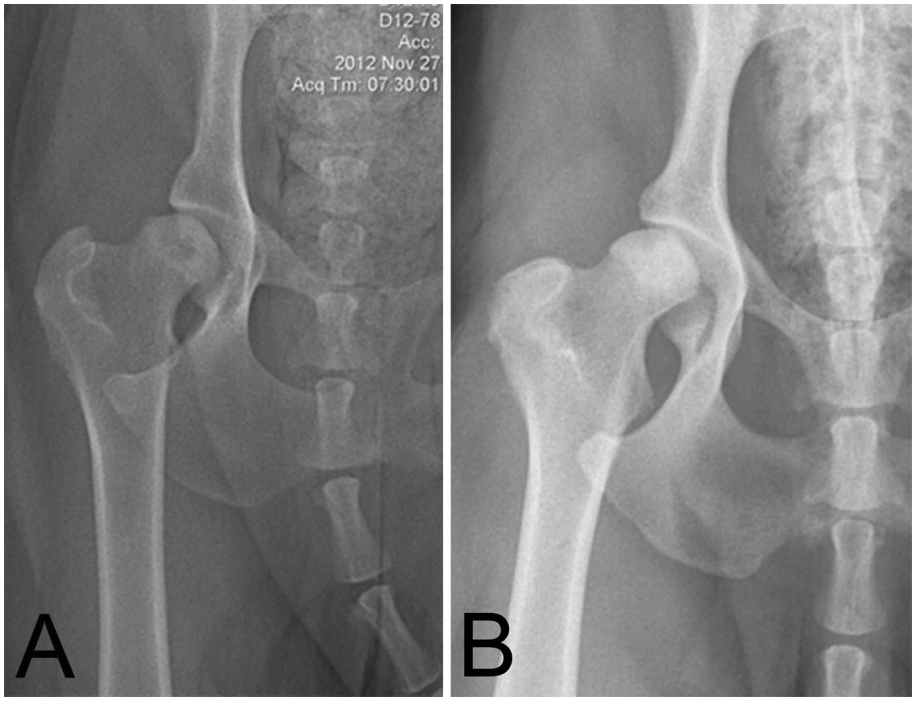

Figure 3 Anteroposterior canine hip radiograph of (A) small auto- and allograft at 8 weeks, and (B) large allograft at 6 months.

Table 1 Functional outcome measures.

Group	2 mo			6 mo		
	Lameness	Function	CROM	Lameness	Function	CROM
Normal	0 ± 0	10 ± 0	141 ± 3	0 ± 0	10 ± 0	142 ± 4
Small	2.3 ± 0.6*	7.5 ± 0.5*	108 ± 3*			
Large	0.7 ± 0.6	9 ± 0.2	135 ± 4	0.3 ± 0.6	9.8 ± 0.4	139 ± 5

* Indicates statistically significant ($p < 0.05$, rank-sum test) difference from others in the column.
CROM = comfortable range of motion.

fibrous tissue and fibrocartilage infiltration, and/or chondrocyte necrosis and/or apoptosis at 8 weeks after implantation (Figure 4). Histologically, the large-allo grafts showed maintenance of hyaline cartilage structure and integrity with only focal, minor decreased proteoglycan staining and chondrocyte necrosis and/or apoptosis at the periphery of grafts (Figure 5).

Human patients

Using large allografts, three out of the four patients required two grafts at the time of implantation due to the defect size. At the time of this abstract, patient follow up ranged from 4 months to 18 months. All patients are full weight bearing without an assistive device, and show no evidence of graft failure or progressive arthrosis. No patient was requiring narcotic pain medication or any regular anti-inflammatories. One patient was occasionally using tramadol, but rated her pain 0 out of 10 at her 18-month visit, but could reach up to 4 out of 10 with activities.

Table 2 Chondrocyte viability and histologic scores.

Group	% CV at implantation	% CV at sacrifice	Histology score
Small auto	NA	19 ± 13 [a]	48 ± 10 [c]
Small allo	92 ± 9	22 ± 23 [a]	29 ± 3 [d]
Large allo	90 ± 11	84 ± 14 [b]	14 ± 4 [e]

Different letters indicate statistically significant ($p < 0.05$, rank-sum test) difference from others in the column.
% CV = % chondrocyte viability; NA = not applicable.

Discussion

The results of this translational canine model study suggest that OCAs created and implanted using tapered OCA

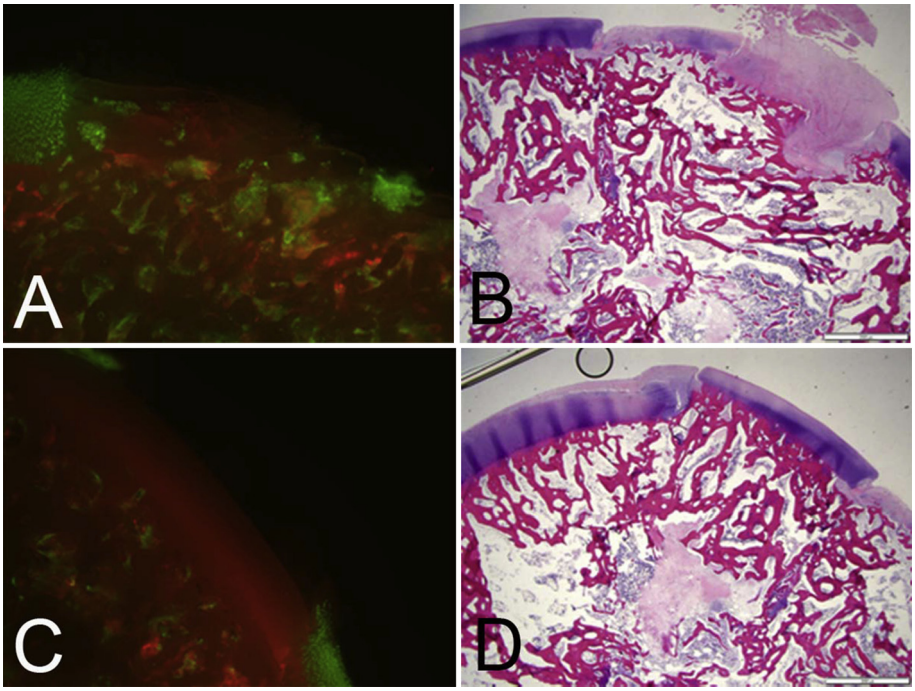

Figure 4 (A) Chondrocyte viability for small autograft showing the majority of graft chondrocytes dead (red stain), and (B) histological evaluation showing mostly fibrous tissue at the graft site. The small allograft evaluation showed a similar amount of chondrocytes dead on (C) viability staining, but some peripheral graft incorporation and remaining cartilage on (D) histological evaluation.

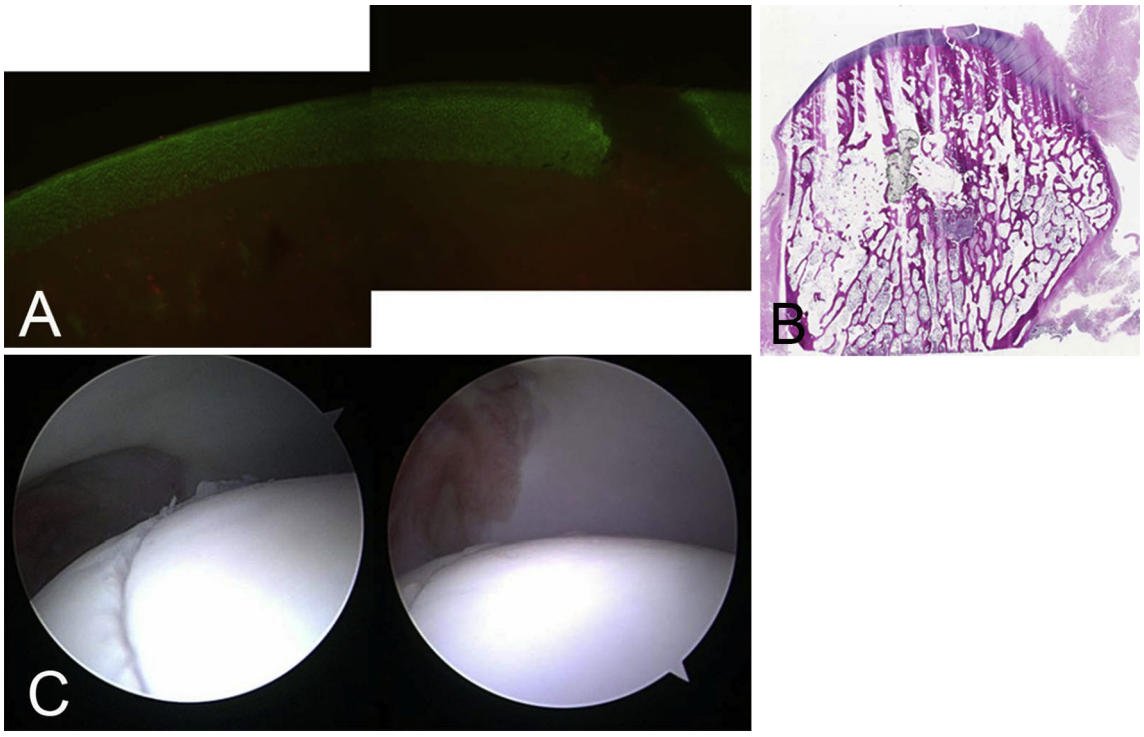

Figure 5 Evaluation of the large allograft: (A) chondrocyte viability showing the majority of chondrocytes to be viable (green) except for at the graft seam; (B) histological evaluation showing good graft incorporation and chondrocytes; and (C) arthroscopic evaluation at 6 months showing good graft articular surface without irregularity except at graft seam.

instrumentation to cover a large surface area of the femoral head are superior to smaller cylindrical allografts and autografts. The large allografts were significantly better for all clinical and histological evaluations. Although all grafts showed evidence for incorporation into the host bone radiographically, the large allografts retained chondrocyte viability and function at 6 months, whereas the small grafts required early sacrifice at 8 weeks due to poor clinical function.

Mosaicplasty, the use of small autologous osteochondral plugs, has been used more commonly in the knee, but has been described in the hip as well [28,29]. Typically, this technique is used for cartilage lesions <2 cm^2 for the femoral head [29] and 2.5 cm^2 for the femoral condyle [28]. Although the technique is beneficial in smaller lesions, use for larger lesions is cautioned when multiple grafts are required due to donor-site morbidity, peripheral graft chondrocyte death during graft harvest [30], chondrocyte death during impaction of the graft, graft-height mismatch [31], and the long-term effect of fibrocartilage fill between grafts [28]. However, using large allografts leads to fewer grafts harvested (fewer chondrocytes die), manual pressure used for press-fit technique (fewer chondrocytes die), and fewer articular surface irregularities—fewer seams and opportunities for graft-height mismatch.

Previous clinical reports are few, but osteochondral grafts have been clinically successful in the femoral head. Indications for this procedure usually have few available alternatives. Avascular necrosis without segmental collapse and traumatic defects are the most common indications. Unfortunately, these problems usually occur in young patients who are not good arthroplasty candidates, and most patients would like to avoid arthrodesis. Although a proximal femoral osteotomy can be used to offload the affected area, it is technically challenging and is not a viable option if the femoral-head defect is too large [32,33]. Unfortunately, arthrosis is inevitable.

If osteochondral allografting is being considered, early detection is important because the patient should not have radiographic signs of arthrosis. The contraindications for this procedure include inflammatory arthritis, avascular necrosis with segmental collapse, and evidence of arthrosis [7].

Previous case reports have shown the technique can be successful. Evans and Providence [4] reported the success of the technique for a patient with osteochondritis dissecans after trauma with short-term follow up.

To date, three case series have been published. The first clinical series with relatively long-term follow up was reported in 1985 [7]. Meyers [7] reported his results of 25 hips in 21 patients using fresh OCAs. The procedure was considered a success if the patient had minimal pain, did not require narcotic pain management, and was ambulating without an assistive device. Follow up ranged from 9 months to 63 months. Fifty percent (5/10) of the patients with avascular necrosis and segmental collapse, or those who were taking chronic steroids failed. Most failures occurred within the 1st year, and all failures went on to total hip arthroplasty. Eighty percent (12/15) of the patients without a steroid-related aetiology were considered successful.

The procedure does appear to improve the Harris hip scores [5,6]. Kosashvili et al [6] reported on eight patients with up to 24-months follow up and showed a 26.2 average

improvement in Harris hip scores. Six out of eight (75%) patients had "good mobility" and had functional grafts at 42 months. Two patients (25%) were considered to have graft failures. Likewise, Khanna et al [5] reported the first prospective case series and showed significant improvement in Harris hip scores with average follow up of 41.6 months. Postoperative magnetic -resonance-imaging graft evaluation was performed in 10 of 17 patients. Thirteen of 17 (76.5%) patients had fair or good outcomes. The postoperative magnetic resonance imaging finding appeared to have no correlation with functional results. They also recommended not performing the procedure on patients that have a steroid-related aetiology.

Large OCA procedures for the femoral head may require larger prospective series with longer follow up to prove their effectiveness. The previous series do not report the average size of the defect grafted. Defect size may play a role in patient selection and eventual success of the procedure.

Study limitations

Although statistically significant differences were realised for all outcome measures, there are limitations to this study. With only three dogs in each group and small autografts and small allografts placed in the same hips, it is not possible to clearly delineate which variables were responsible for the significant differences noted. The failure of the small grafts may have been influenced by their relative size and associated biomechanics, the instrumentation system used, and/or their relative positioning and proximity in the femoral head. However, the study design did allow for narrowing down the causes of significant differences to graft size and/or the instrumentation system used, in that preservation was the same for all allografts, an autograft control was used for small grafts, small autografts and allografts were alternated in location, and the use of multiple small grafts together mimics the clinical standard of care. Therefore, the significantly better outcomes associated with large allografts are likely due to the larger size more optimally restoring the articular surface contour of the femoral head with improved biomechanics and/or providing more donor chondrocytes to preserve, which were better preserved with the use of the tapered instrumentation system that does not require tamping.

Lastly, the data from human patients are based on a very small single-centre series with short-term follow up, and even the published clinical series do not have follow up greater than ~5 years [5–7]. Therefore, further translational and clinical studies are required before definitive conclusions regarding the safety, efficacy, and optimal technique can be made.

These data provide initial translational and clinical evidence for large OCAs as a potential option for functional resurfacing of full-thickness cartilage defects of the femoral head.

Conflicts of interest

Cristi R. Cook, Samuel P. Franklin, James P. Stannard, and James L. Cook receive grant support, consultant fees, and/or royalties from Arthrex, Inc., which is a supplier of some of the surgical equipment used in this study.

James L. Cook and Aaron M. Stoker are inventors of the Missouri Osteochondral Allograft Preservation System used in this study. Ferris M. Pfeiffer, James L. Cook, James P. Stannard and Aaron M. Stoker are inventors of the tapered osteochondral allografting systems used in this study.

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