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Importance of Donor Chondrocyte Viability for Osteochondral Allografts

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Background: Osteochondral allograft (OCA) transplantation provides a biological treatment option for functional restoration of large articular cartilage defects in multiple joints. While successful outcomes after OCA transplantation have been linked to viable donor chondrocytes, the importance of donor cell viability has not been comprehensively validated.

Purpose: To use a canine model to determine the importance of donor chondrocyte viability at the time of implantation with respect to functional success of femoral condylar OCAs based on radiographic, gross, cell viability, histologic, biochemical, and biomechanical outcome measures.

Study Design: Controlled laboratory study.

Methods: After approval was obtained from the institutional animal care and use committee, adult female dogs (N = 16) were implanted with 8-mm cylindrical OCAs from male dogs in the lateral and medial femoral condyles of 1 knee. OCAs were preserved for 28 or 60 days after procurement, and chondrocyte viability was quantified before implantation. Two different storage media, temperatures, and time points were used to obtain a spectrum of percentage chondrocyte viability at the time of implantation. A successful outcome was defined as an OCA that was associated with graft integration, maintenance of hyaline cartilage, lack of associated cartilage disorder, and lack of fibrillation, fissuring, or fibrous tissue infiltration of the allograft based on subjective radiographic, gross, and histologic assessments at 6 months after implantation.

Results: Chondrocyte viability ranged from 23% to 99% at the time of implantation. All successful grafts had >70% chondrocyte viability at the time of implantation, and no graft with chondrocyte viability <70% was associated with a successful outcome. Live-dead stained sections and histologic findings with respect to cell morphological features suggested that successful grafts were consistently composed of viable chondrocytes in lacunae, while grafts that were not successful were composed of nonviable chondrocytes with infiltration of fibroblasts from the surrounding recipient tissues. In situ polymerase chain reaction (fluorescence in situ hybridization [FISH]) assays were performed in an attempt to distinguish donor (male) cells from recipient (female) cells. Unfortunately, this technique was exceptionally difficult to perform on intact articular cartilage sections, and consistent, repeatable data could not be obtained from this testing. However, the data did support histologic and live-dead data, which strongly suggested that successful grafts retained viable donor (male) chondrocytes and unsuccessful grafts degraded and were replaced by fibrous tissue populated with recipient (female) fibroblasts.

Conclusion: Viable chondrocytes in OCAs at the time of transplantation are primarily responsible for maintenance of donor articular cartilage health in the long term.

Clinical Relevance: Optimizing chondrocyte viability in all aspects of OCA transplantation—including procurement, processing, storage, transportation, and surgical implantation—needs to be a primary focus for OCA clinical use.

Keywords: osteochondral allografting; articular cartilage defects; chondrocyte viability; cell viability; donor tissues

Osteochondral allograft (OCA) transplantation is a unique treatment option that has been used clinically for more than 30 years to treat articular cartilage defects resulting from traumatic injuries, osteochondrosis, or arthritis, primarily in the knee.[21] Numerous studies have reported that OCAs are associated with survivorship between 71% and 85% at 10 years and up to 74% at 15 years.[§] Overall, outcomes after OCA treatment have been good to excellent, even in the athletic population, in which 88% of patients returned to sport, including 79% returning to their preinjury level of sport.[24,32]

Although OCA transplantation has proven clinical safety and efficacy, its use is limited by availability and logistical

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[§]References 8, 13, 14, 16, 17, 19, 20, 32, 37.

issues involving graft procurement, disease testing, and storage before implantation. These issues depend on the relatively short time for which sufficient chondrocyte viability in the grafts can be maintained by use of current tissue storage protocols. Chondrocyte viability has been reported to be critically important for maintaining the biochemical and biomechanical properties of OCAs, which correlate directly to the clinical success of the surgery.[1,19,26,39] Several storage methods have been investigated in attempts to optimize chondrocyte viability, with each showing noticeable declines in chondrocyte viability after day 14, falling below acceptable levels (typically considered to be 70% viable cells) by 28 days after procurement.[‖] Mandatory disease testing procedures require 14 days before tissues can be released from the tissue bank to the surgeon for implantation. As such, a narrow window of time (eg, 14 days) for size matching, scheduling surgery, and transporting tissues is available to allow for optimal use of donor tissues. We wanted to find a way to preserve OCA tissue in a manner that maintains chondrocyte viability at acceptable levels for a longer time period than current tissue bank protocols permit. To this end, we developed the Missouri Osteochondral Allograft Preservation System (MOPS),[10] a serum-free tissue preservation method that has prolonged the time for maintenance of acceptable levels of chondrocyte viability in osteochondral tissues to more than twice as long as the current standard of care (SOC) based on in vitro and in vivo assessments.[12,15,33] While successful outcomes after OCA implantation have been linked to viable donor chondrocytes, the importance of donor cell viability has not been comprehensively validated. The purpose of this study was to use a canine model to determine the importance of donor chondrocyte viability at the time of implantation with respect to functional success of femoral condylar OCAs based on radiographic, gross, cell viability, histologic, biochemical, and biomechanical outcome measures.

METHODS

With approval from our institution's animal care and use committee, the stifles (knees) of purpose-bred adult (2-6 years old) male mongrel dogs were aseptically harvested after humane euthanasia was performed for reasons unrelated to this study. All soft tissues were removed in the operating room, and the knees were fully inspected to ensure that no disease was present. The procured femoral condyles were aseptically stored in either standard tissue bank media at 4°C (n = 24) or in MOPS media at room temperature (~25°C) (n = 24) for either 28 or 60 days after procurement. For the purposes of the present study, the different storage protocols were used to provide different time-zero graft viability characteristics based on our previously published data examining the effects of the storage protocols on chondrocyte viability during storage.[12]

For samples assigned to the SOC groups, the distal femurs were placed in standard storage media and stored in a dedicated refrigerator at 4°C for 28 days or 60 days. For samples assigned to the MOPS group, the distal femurs were placed in defined media within custom closed containers and stored in a dedicated clean room at room temperature (~25°C) for 28 days or 60 days.

Surgery and Aftercare

Fourteen adult mongrel female dogs were enrolled in the study (2-5 years old; 25-35 kg body weight; obtained from Marshall Farms BioResources; USDA #21-A-008). They were judged free of osteoarthritis in all joints based on physical and orthopaedic examination by 2 veterinary orthopaedic surgeons and on radiographs of the hips, elbows, and knees. At 28 days or 60 days after graft procurement, the dogs were premedicated with xylazine, morphine, and atropine; anesthetized with propofol; and maintained on isoflurane in oxygen inhaled through endotracheal intubation. The dogs were prepared for aseptic surgery of the right knee. A lateral parapatellar approach with arthrotomy was performed in the right knee, and 8-mm cylindrical focal defects were created in the weightbearing areas of the lateral and medial femoral condyles by use of commercially available allograft instrumentation. These defects were filled with site-matched OCAs, 8 mm in diameter × 8 mm in depth, obtained from the adult male cadaveric distal femurs preserved by either SOC or MOPS and implanted by use of the calibrated press-fit method of the allograft system (Figure 1). At each time point (28 days or 60 days after graft procurement), each knee received 1 SOC graft and 1 MOPS osteochondral allograft, which were alternated between medial and lateral femoral condyles.

Soft-padded, full-limb bandages were placed on the surgically treated limb of each dog. The dogs were recovered, and analgesics (morphine followed by oral analgesics) were provided postoperatively for 48 hours and then as needed based on physical parameters indicating the presence of pain. The bandages were maintained for 1 week to limit flexion and extension of the knees during the initial healing period. The dogs were restricted to their kennels and were allowed monitored daily out-of-kennel exercise in a confined area for the entire study period.

Radiographic Assessments

Craniocaudal (anteroposterior) and mediolateral digital radiographic views of surgically treated knees were obtained

[‖]References 2, 4-7, 25, 28, 29, 34, 35, 36, 39.

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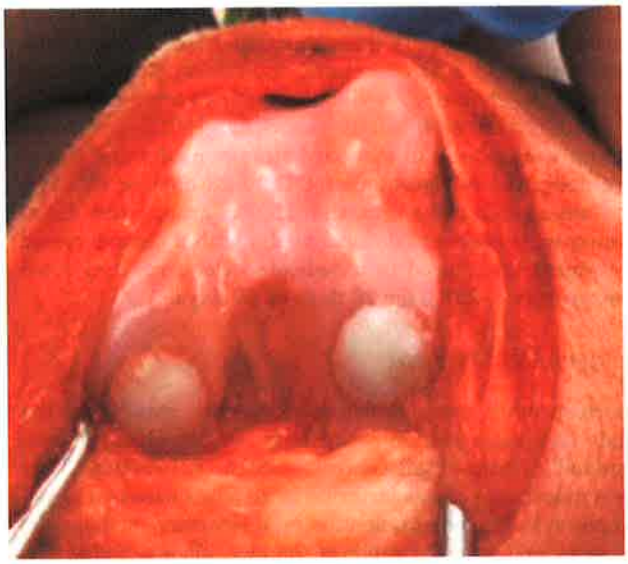

Figure 1. Intraoperative image showing 8-mm osteochondral allografts implanted in the medial and lateral femoral condyles of a dog in this study. (Reprinted with permission from Cook et al.[12])

in a standardized fashion the day before surgery, immediately postoperatively, and at 12 weeks and 6 months after surgery. The radiographs were evaluated by 1 board-certified veterinary radiologist (C.R.C.) who was blinded to dog number, group, and clinical signs by use of an established subjective system.[31] Subjective findings related to allograft integrity and positioning also were described.

Gross Examination

Dogs were humanely euthanized 6 months after surgery by intravenous overdose of pentobarbital-phenytoin. Both knees of each dog were carefully disarticulated. The articular surfaces of the patella, femur, and tibia were photographed and subjectively assessed. Each graft site was harvested individually with its surrounding cartilage and bone and divided in half to allow for cell viability and histologic assessments (half of each sample) and biochemical and biomechanical assessments (half of each sample) to be performed on every sample. Matched sections from the contralateral untreated limb were harvested and assessed in the same fashion to serve as normal (native tissue) controls.

Cell Viability

Chondrocyte viability in each implanted graft was assessed through the full thickness of the portions designated for viability-histologic testing by use of 2 stains to detect live and dead cells (Molecular Probes; Life Technologies) as per the manufacturer's suggested protocol, where live cells are stained green with calcein acetoxymethyl ester and dead cells are stained blue with SYTOX blue (Life Technologies). The percentage chondrocyte viability for each OCA was quantified by use of digital image analysis. The area of the cartilage tissue was measured, and the viable chondrocyte density was determined by dividing the number of live chondrocytes by the area of cartilage evaluated. The viable chondrocyte density for each graft was reported as the mean viable chondrocyte density (cells/μm^2) for all images analyzed from that graft.

Cell Tracking

In situ polymerase chain reaction (PCR) (fluorescence in situ hybridization [FISH]) assays were performed. On day 1, osteochondral sections on Trubond 380 adhesive microscope slides (Tru Scientific) were deparaffinized with 3 successive washes of xylene (5 minutes each) followed by a 5-minute wash with absolute ethanol. Tissue sections were successively washed in the following solutions at room temperature unless otherwise indicated: 0.2 N HCl for 20 minutes as a pretreatment to increase hybridization signal, 2× saline-sodium citrate (SSC) buffer for 5 minutes, 1.0 M thiocyanate at 80°C for 45 minutes to break the protein cross-links formed by formalin fixation, 2× SSC buffer for 5 minutes, 100 μg/mL RNAse at 37°C for 15 minutes, 2× SSC buffer for 5 minutes each (2 washes), 0.4% pepsin in 0.02 N HCl at 37°C for 20 minutes to digest protein and enhance primer penetration, Dulbecco's phosphate-buffered saline 1× (Gibco; Life Technologies Co) for 5 minutes each (3 washes), and alcohol dehydration series (50%, 70%, 95%, 100%, and 100%) for 3 minutes each. A PCR mixture containing Y chromosome–specific primers (X, SRY, Y, and 650), 10× PCR buffer, $MgCl_2$, biotin dNTP mix, and Platinum Taq DNA polymerase (Invitrogen; Life Technologies Co) was placed over heated and prewetted (PCR buffer) tissue sections. A Frame-seal incubation chamber (Pro-Rad Laboratories) was added to each slide to keep the PCR mixture in direct contact with the osteochondral sections with minimal spillage. Control assays composed of no primers present (negative control) and of male-only or female-only soft tissues (skeletal muscle and liver) were performed. A coverslip was added to each slide to prevent evaporation. Slides were then placed in the Lab-Tek II chamber incubation tray (Simport Scientific Inc). By use of an Integrated Separation Systems programmable PCR oven, amplification was performed for 30 cycles composed of 1 minute at 94°C, 1 minute at 56°C, and 1 minute at 72°C. Slides were stored at 4°C overnight. On day 2, coverslips were removed and Frame-seal incubation chambers replaced, and osteochondral sections were washed with the following solutions at room temperature unless otherwise indicated: 2× SSC/0.1% Tween 20 for 5 minutes each (2 washes), 2× SSC/0.1% Tween 20 at 45°C for 5 minutes each (2 washes), 2× SSC/0.1% Tween 20 at 60°C for 5 minutes each (2 washes), and 1× Tris-buffered saline (TBS)/0.05% Tween 20 for 5 minutes. In the dark room, tetramethylrhodamine (TRITC)-streptavidin conjugate (Dako North America) in TBS (1:25) was added to tissue sections for 1.5 hours at 37°C and then rinsed twice with 2× SSC for 5 minutes each. Frame-seal incubation chambers were removed, and osteochondral sections were coverslipped with diluted

(1:1) Vectashield HardSet mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc). Slides were examined with an Olympus BX51 microscope with fluorescence capability (EXFO, X-cite series 120 fluorescence illumination system) by use of Texas Red and DAPI fluorescence filters for detection of signal and nuclei, respectively.

Histologic Assessment

Each OCA was placed in 10% neutral-buffered formalin fixative until processed for histologic examination. After fixation, tissues were processed as previously described.[12] Osteochondral sections through the full thickness of the portions designated for viability-histologic testing were evaluated by 2 board-certified veterinary pathologists (K.K., C.C.B.) who were blinded to dog number, group, and clinical findings; sections were scored for structural disorders, chondrocyte disorders, proteoglycan loss, collagen integrity, tidemark integrity, and subchondral bone plate changes based on criteria described in the Osteoarthritis Research Society International (OARSI) histologic assessment system for dogs.[11]

Biomechanical Assessment

Previously reported methods were used to determine the material properties of the cartilage in the graft in the femoral condyles and compare them with cartilage in the site-matched region of the contralateral femoral condyles.[15] In brief, biomechanical testing (3.9-mm diameter indenter and 4.0-mm diameter sample well) was performed with a computer-controlled load frame (Instron 8821s) and optical position tracking (Certus; Northern Digital Inc). After equilibration under a tare load (5 N), the tissue was deformed to 10% tissue strain at a ramp rate of 1 μm/s followed by dynamic loading with a sinusoidal strain (±5%) at 1 Hz. Strain measurements were performed with optical position tracking (Certus). These measurements yielded an instantaneous tissue modulus (E_Y) at the end of the stress-relaxation test and a dynamic modulus at 1 Hz (G) from the sinusoidal test.

Biochemical Assessment

After biomechanical testing, the biochemical content from the entire articular cartilage sections of each portion of the OCAs designated for biomechanical-biochemical evaluation was assessed by first measuring the sample's wet weight, lyophilizing overnight, and then measuring dry weight. Gross water content was determined from the difference. Once dry, the samples were digested with papain overnight at 60°C. Aliquots of the digest were analyzed for glycosaminoglycan (GAG) content by use of the 1,9-dimethylmethylene blue dye-binding assay. An additional aliquot was assessed for collagen content by use of a colorimetric assay to detect orthohydroxyproline (OHP) content. OHP content was converted to total collagen content with the conversion of 1:10 ratio of OHP to collagen. Each biochemical constituent (GAG and collagen) was normalized to the tissue dry weight.

Definition of a Successful Outcome

For the dogs in this study, a successful outcome was defined as an OCA that was associated with graft integration, maintenance of hyaline cartilage, lack of associated cartilage disorder, and lack of fibrillation, fissuring, or fibrous tissue infiltration of the allograft based on subjective radiographic, arthroscopic, gross, and histologic assessments at 6 months after implantation.

Statistical Analyses

All statistical analyses were performed with a computer software program (Sigma Plot). Data from each group at each time were combined, and means ± SDs were determined. A Pearson product moment test was used to assess strength and significance of correlations. A t test was performed to determine differences between groups with respect to chondrocyte viability. A 1-way analysis of variance was performed to determine differences among groups with respect to each outcome measure of continuous data (chondrocyte viability, GAG, collagen, and biomechanical assessments) at each time. Rank sum test was performed to determine differences among groups with respect to each outcome measure of categorical data (histologic scores) at each time. Significance was set at $P < .05$. Odds ratios were calculated to determine effect size for proportions of grafts from each group with less than 70% chondrocyte viability.

RESULTS

Functional outcomes for the dogs in this study in association with preservation systems have been previously reported.[12]

Comparison of Osteochondral Allografts After 28 Days or 60 Days

Chondrocyte viability ranged from 22.9% to 99% on day 28 and from 24.7% to 99% on day 60 (Figure 2). Seven grafts with less than 70% chondrocyte viability were implanted in recipient dogs' knees.

Assessment of Allograft Integrity and Positioning at 12 Weeks and 6 Months

Radiographic assessments performed at 12 weeks and 6 months after implantation showed evidence for progressive osseous integration of SOC and MOPS grafts with variable degrees of associated sclerosis. No radiographic evidence of failure of osseous integration was found for any graft. All knees showed radiographic evidence of effusion or synovial proliferation, ranging from mild to moderate. No subjective differences were noted between groups with respect to radiographic assessments.

Figure 2. Live-dead images showing the range in chondrocyte viability seen in grafts at time of implantation: (A) 23% chondrocyte viability, (B) 71% chondrocyte viability, (C) 99% chondrocyte viability (calcein acetoxymethyl ester/Sytox blue stain; original magnification ×4).

TABLE 1
Summary of Chondrocyte Viability and Histologic Scores for Grafts With Successful Versus Unsuccessful Outcomes[a]

OCA Preservation Duration	6-Month Outcome	Chondrocyte Viability, %	Histologic Score
28 days	Successful	89.9 ± 9.6	5.7 ± 4.7
	Unsuccessful	57.2 ± 11.6	11.3 ± 3.9
60 days	Successful	82.9 ± 9.4	7.4 ± 3.2
	Unsuccessful	33.8 ± 19.9	19.1 ± 3.6

[a]Values are reported as mean ± SD. The outcome was considered "successful" in grafts that were associated with graft integration, maintenance of hyaline cartilage, lack of associated cartilage disease, and lack of fibrillation, fissuring, or fibrous tissue infiltration of the allograft based on subjective radiographic, gross, and histologic assessments 6 months after implantation. OCA, osteochondral allograft.

Assessment of Gross, Cell Viability, Histologic, Biochemical, and Biomechanical Characteristics at 6 Months

Grafts with greater than 70% chondrocyte viability at the time of implantation were subjectively superior to grafts with less than 70% chondrocyte viability at the time of implantation with respect to gross appearance when assessed 6 months after implantation. For both preservation periods, chondrocyte viability at the time of implantation appeared to correspond well to subjective gross and histologic assessments in that grafts with viability less than 70% at the time of implantation were all considered to have failed (Tables 1 and 2).

Live-dead stained sections and histologic findings with respect to cell morphologic features suggested that successful grafts were consistently composed of viable chondrocytes in lacunae, while grafts that were not successful were composed of nonviable chondrocytes with infiltration of fibroblasts from the surrounding recipient tissues (Figure 3). In situ PCR (FISH) assays were performed in an attempt to distinguish donor (male) cells from recipient (female) cells. Unfortunately, this technique was exceptionally difficult to perform on intact articular cartilage sections, and consistent, repeatable data could not be obtained from this testing. However, the data did support histologic and live-dead data, which strongly suggested that successful grafts retained viable donor (male) chondrocytes and unsuccessful grafts degraded and were replaced by fibrous tissue populated with recipient (female) fibroblasts (Figure 4).

Histologic Assessment at 6 Months

For 28-day and 60-day storage, significantly ($P < .05$) higher OARSI scores (ie, indicating more severe pathologic abnormalities) were noted for grafts with less than 70% chondrocyte viability at the time of implantation. Subjectively, morphologic features of hyaline cartilage and proteoglycan staining were better preserved in grafts with greater than 70% chondrocyte viability. Chondrocyte loss, clustering, and phenotypic changes were more frequently observed in grafts with less than 70% chondrocyte viability at the time of implantation. Similarly, fibrillation, fissuring, and fibrous tissue infiltration were observed in OCAs with less than 70% chondrocyte viability at the time of implantation (Figure 5).

Biochemical Assessments

GAG content was greater ($P < .009$) in native articular cartilage compared with cartilage from 28-day and 60-day grafts when assessed 6 months after implantation. GAG content was not different in native articular cartilage compared with cartilage from 60-day grafts that had greater than 70% chondrocyte viability at the time of implantation when

TABLE 2
Chondrocyte Viability Data for Individual Dogs Based on Preservation Method and Duration[a]

OCA Preservation Duration and Method	Animal No.	Chondrocyte Viability, %	Viable Chondrocyte Density, cells/μm^2	Outcome
28 days				
SOC	1	88.6	0.00085	Successful
	2	75.0	0.0008	Successful
	3	40.4	0.00052	Unsuccessful
	4	80.1	0.00082	Successful
	5	63.6	0.00058	Unsuccessful
	6	57.5	0.00059	Unsuccessful
	7	78.6	0.00081	Successful
MOPS	1	98.8	0.00094	Successful
	2	95.1	0.0009	Successful
	3	66.9	0.00073	Unsuccessful
	4	96.3	0.00089	Successful
	5	93.3	0.00091	Successful
	6	88	0.00084	Successful
	7	91.2	0.00086	Successful
60 days				
SOC	8	24.7	0.00042	Unsuccessful
	9	37.5	0.00045	Unsuccessful
	10	25.5	0.00044	Unsuccessful
	11	41.3	0.00049	Unsuccessful
	12	31.4	0.00043	Unsuccessful
	13	33.8	0.00048	Unsuccessful
	14	28.8	0.00041	Unsuccessful
MOPS	8	99.2	0.001	Successful
	9	79.5	0.00083	Successful
	10	75.1	0.0008	Successful
	11	79.4	0.00084	Successful
	12	81.6	0.00087	Successful
	13	48.8	0.0006	Unsuccessful
	14	82.8	0.00083	Successful

[a]MOPS, Missouri Osteochondral Allograft Preservation System; OCA, osteochondral allograft; SOC, standard of care.

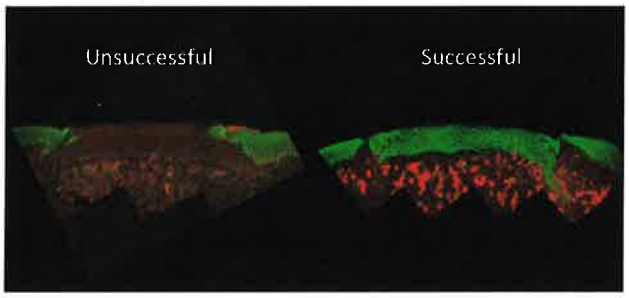

Figure 3. Live-dead images of femoral condyles of dogs in this study showing cell morphologic characteristics of an unsuccessful graft composed of nonviable chondrocytes with infiltration of fibroblasts from the surrounding recipient tissues, and a successful graft composed of viable chondrocytes in lacunae (calcein acetoxymethyl ester/Sytox blue stain; original magnification ×4).

assessed at 6 months ($P = .34$). No differences in collagen content were found in native cartilage compared with cartilage from 28-day or 60-day grafts at 6 months ($P > .2$).

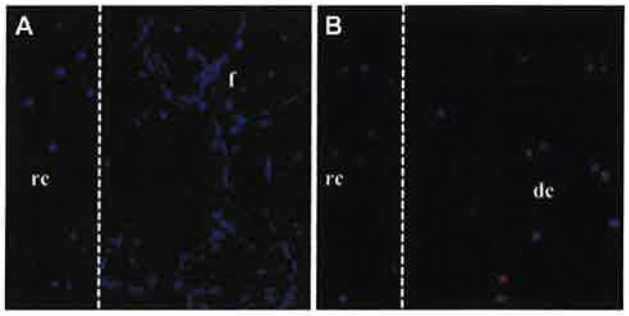

Figure 4. Photomicrographs from in situ PCR for cell gender identification. Viable cells in grafts that had the appearance and architecture of reparative fibrous tissue and fibrocartilage (A) were female cells (no red staining), indicating that these cells were fibroblasts (f) from the recipient that had migrated into the graft site. Viable cells in grafts that maintained articular cartilage appearance and architecture (B) were male cells (red staining), indicating that these cells were donor chondrocytes (dc) that had maintained viability and phenotype in the transplanted cartilage for at least 6 months after implantation into dogs in this study. rc, recipient chondrocytes in articular cartilage adjacent to the grafts (original magnification ×40).

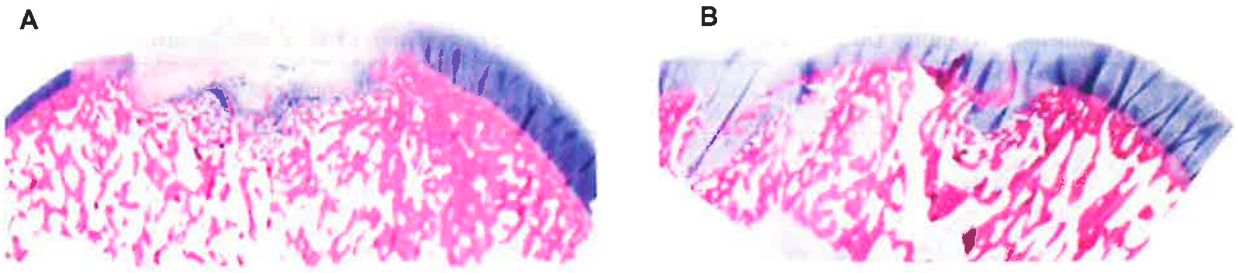

Figure 5. Photomicrographs showing (A) chondrocyte loss, clustering, and phenotypic changes as well as fibrillation, fissuring, and fibrous tissue infiltration in a graft with less than 70% chondrocyte viability at the time of implantation. (B) Morphologic features of intact hyaline cartilage and robust proteoglycan staining in a graft with greater than 70% chondrocyte viability at the time of implantation (Toluidine blue stain; original magnification ×2).

Biomechanical Assessment

Instantaneous tissue modulus and dynamic modulus were not different in native articular cartilage compared with cartilage from 28-day or 60-day grafts with greater than 70% chondrocyte viability at the time of implantation when assessed at 6 months ($P > .12$).

DISCUSSION

OCA transplantation is a successful treatment for large articular cartilage defects; however, use and success of OCAs are limited by availability of acceptable grafts, often because of loss of chondrocyte viability during storage. This preclinical animal model study verified the previously reported findings regarding the importance of donor chondrocyte viability in OCAs with respect to successful outcomes.[1,19,22,23,26,27,38,39] The findings supported the previously stated recommendation for a minimum of 70% chondrocyte viability at the time of implantation for all grafts that are to be used clinically. The data from this study suggest that donor chondrocytes contributed to maintenance of functional hyaline cartilage in the grafts based on the finding that all successful grafts had greater than 70% chondrocyte viability at the time of implantation. Grafts that failed to maintain the architecture and function of hyaline cartilage all had less than 70% chondrocyte viability at the time of implantation. Successful grafts were consistently composed of viable chondrocytes in lacunae, while grafts that were not successful were composed of nonviable chondrocytes with infiltration of fibroblasts from the surrounding recipient tissues. Together with previous studies,[¶] these data suggest that viable chondrocytes in OCAs at the time of transplantation are primarily responsible for maintenance of the long-term health of donor articular cartilage.

The limitations of this study include the use of a canine model with a relatively small number of dogs and use of a single 6-month endpoint. In addition, the inability to consistently obtain valid in situ PCR data precludes definitive differentiation of the source of all cells in each graft. However, dogs serve as a legitimate translational model based on anatomic, biomechanical, and joint physiologic characteristics and also based on the use of OCAs for successful treatment of clinical canine patients.[3,9,18] As well, the in situ PCR data that were obtained matched histologic and cell viability assessments, which showed that successful grafts retained viable round cells in lacunae (donor [male] chondrocytes) while viable cells in unsuccessful grafts were composed of infiltrating spindyloid and fusiform cells (recipient [female] fibroblasts).

In this translational animal model study, chondrocyte viability at the time of implantation corresponded well to clinical and histologic measures of success, with a relative threshold of 70% chondrocyte viability being acceptable for use. As previously reported,[12] the MOPS was better able to preserve grafts above this threshold compared with current tissue banks' SOC and could do so for up to 60 days after procurement. This is a critical advancement, because chondrocyte viability in preserved grafts has been reported to rapidly decline from the completion of mandatory disease testing (days 14-17) and to fall below acceptable levels by day 28 after procurement using current tissue bank protocols.[2,4-7,25,28,29,34-37] Similarly, Ranawat et al[30] reported superior histologic and biomechanical properties for cold-stored viable OCAs compared with acellular freeze-thawed grafts implanted in sheep for 1 year. While those investigators suggested no differences in chondrocyte viability in cold-stored grafts over the 42-day preservation period, no quantitative chondrocyte viability data were reported, and the images provided demonstrate loss of viability in day 42 grafts compared with day 1 grafts. MOPS was able to preserve 86% of grafts used in the present study for both 28 and 60 days compared with 57% and 0%, respectively, using current tissue bank protocols; as well, successful outcomes based on arthroscopic, gross, cell viability, histologic, biochemical, and biomechanical characteristics associated with MOPS-stored allografts were higher than those using the current SOC system. This is a promising development in OCA technology that potentially can improve the quantity of grafts available for use and the quality of grafts being implanted.

In summary, all successful OCAs used in this preclinical canine model had greater than 70% chondrocyte viability

[¶]References 1, 19, 22, 23, 26, 27, 30, 38, 39.

at the time of implantation, while no graft with chondrocyte viability less than 70% at the time of implantation was associated with a successful outcome. MOPS-preserved grafts had significantly higher chondrocyte viability and viable chondrocyte density, they could be preserved at acceptable levels for up to 60 days after procurement, and they were associated with more successful outcomes compared with current SOC grafts. Taken together with previous data showing donor chondrocytes remaining in grafts for up to 25 years after implantation,[19] these data strongly suggest that viable chondrocytes in OCAs at the time of transplantation are primarily responsible for long-term maintenance of donor articular cartilage health.[#] Therefore, optimizing chondrocyte viability in all aspects of OCA transplantation—including procurement, processing, storage, transportation, and surgical implantation—needs to be a primary focus for OCA clinical use, and surgeons should ascertain this component of graft quality from tissue banks supplying osteochondral allografts.

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