Treatment of Articular Cartilage Defects in the Goat with Frozen Versus Fresh Osteochondral Allografts: Effects on Cartilage Stiffness, Zonal Composition, and Structure at Six Months

Andrea L. Pallante, MS, Simon Görtz, MD, Albert C. Chen, PhD, Robert M. Healey, BS, MBA, Derek C. Chase, MD, Scott T. Ball, MD, David Amiel, PhD, Robert L. Sah, MD, ScD, and William D. Bugbee, MD

Investigation performed at the University of California-San Diego, La Jolla, California

Background: Understanding the effectiveness of frozen as compared with fresh osteochondral allografts at six months after surgery and the resultant consequences of traditional freezing may facilitate in vivo maintenance of cartilage integrity. Our hypothesis was that the state of the allograft at implantation affects its performance after six months in vivo.

Methods: The effect of frozen as compared with fresh storage on in vivo allograft performance was determined for osteochondral allografts that were transplanted into seven recipient goats and analyzed at six months. Allograft performance was assessed by examining osteochondral structure (cartilage thickness, fill, surface location, surface degeneration, and bone-cartilage interface location), zonal cartilage composition (cellularity, matrix content), and cartilage biomechanical function (stiffness). Relationships between cartilage stiffness or cartilage composition and surface degeneration were assessed with use of linear regression.

Results: Fresh allografts maintained cartilage load-bearing function, while also maintaining zonal organization of cartilage cellularity and matrix content, compared with frozen allografts. Overall, allograft performance was similar between fresh allografts and nonoperative controls. However, cartilage stiffness was approximately 80% lower (95% confidence interval [CI], 73% to 87%) in the frozen allografts than in the nonoperative controls or fresh allografts. Concomitantly, in frozen allografts, matrix content and cellularity were approximately 55% (95% CI, 22% to 92%) and approximately 96% (95% CI, 94% to 99%) lower, respectively, than those in the nonoperative controls and fresh allografts. Cartilage stiffness correlated positively with cartilage cellularity and matrix content, and negatively with surface degeneration.

Conclusions: Maintenance of cartilage load-bearing function in allografts is associated with zonal maintenance of cartilage cellularity and matrix content. In this animal model, frozen allografts displayed signs of failure at six months, with cartilage softening, loss of cells and matrix, and/or graft subsidence, supporting the importance of maintaining cell viability during allograft storage and suggesting that outcomes at six months may be indicative of long-term (dys)function.

Clinical Relevance: Fresh versus frozen allografts represent the “best versus worst” conditions with respect to chondrocyte viability, but “difficult versus simple” with respect to acquisition and distribution. The outcomes described from these two conditions expand the current understanding of in vivo cartilage remodeling and describe structural properties (initial graft subsidence), which may have implications for impending graft failure.

The repair of articular defects with use of osteochondral allografts ideally restores the structure, function, and biology of the cartilage surface. Allograft efficacy may

be dependent on sufficient chondrocyte metabolism to preserve cartilage homeostasis and prevent degeneration. Fresh osteochondral allografts are typically implanted with high

Disclosure: One or more of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of an aspect of this work. In addition, one or more of the authors, or his or her institution, has had a financial relationship, in the thirty-six months prior to submission of this work, with an entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. Also, one or more of the authors has had another relationship, or has engaged in another activity, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

chondrocyte viability, and retrieved grafts have contained viable chondrocytes up to twenty-nine years after implantation¹⁻⁵. In contrast, frozen osteochondral allografts, used for massive oncologic joint reconstructions, have low chondrocyte viability at implantation, and grafts retrieved at approximately one to five years contain acellular and often degenerate cartilage^{6,7}. Attempts at future improvements in the application of osteochondral allografts would benefit from an understanding of how the allograft implantation state affects defect repair efficacy and the mechanisms of cartilage maintenance with the best (fresh) and worst (frozen) conditions of cartilage within allografts.

Animal models allow for a systematic and temporal evaluation of cartilage repair and remodeling. Data obtained from human allograft retrievals are skewed toward poor outcomes, since analysis is performed on “failed” cases, where revision or conversion to total joint arthroplasty was required^{5,8,9}. Clinical evaluations based on patient satisfaction and often limited by availability for long-term follow-up are not sensitive to early stage degeneration. Intradisciplinary and multiscale analyses of cartilage repair in animal models may elucidate the types of tissue changes that are indicative of early stage degeneration and may provide insight into the time course of cartilage remodeling and/or deterioration.

Results from such animal models suggest that postoperative time is an important determinant of the quality of cartilage repair. Frozen allografts appear grossly normal and similar to fresh allografts at short postoperative durations of as long as three months¹⁰⁻¹³, and then they appear discolored and roughened, often exposing subchondral bone, at eleven to twelve months¹²⁻¹⁵. In contrast, cartilage of fresh allografts remains grossly normal for as long as twelve months, with only mild degenerative changes¹⁴⁻¹⁶. Gross observations are paralleled by histopathologic changes, including reduced (for frozen) and maintained (for fresh) safranin-O staining intensity¹⁰⁻²¹. Cartilage cellularity and load-bearing function have been analyzed in relatively few studies. Qualitatively, frozen allografts appear acellular by three months, compared with fresh allografts, which contain viable cells for as long as twelve months¹²⁻¹⁶. When compared with that in the nonoperative control knees, the confined compressive stiffness of the operatively treated knees was slightly reduced in the fresh allograft areas, and markedly reduced in the frozen allograft areas with increasing postoperative duration^{12,13,15,19,20}. The reduction of cartilage load-bearing function was associated with proteoglycan content¹⁵; however, other determinants of cartilage stiffness have not been investigated. Rapid indentation testing of cartilage, which is sensitive to structural measures of cartilage degeneration²², may be useful to elucidate mechanisms of allograft deterioration or maintenance. Large-animal studies with frozen or fresh allografts at moderate time points (between three and eleven months) are needed to understand the progression of articular cartilage deterioration.

The hypothesis of this study was that the state of the allograft at implantation affects its performance after six months in vivo. The specific aim was to determine the effect of allograft

treatment (frozen or fresh) on the properties of articular cartilage from retrieved osteochondral allografts at six months in the goat model by assessing cartilage function, zonal composition, and structure. The results of this study suggest that the use of a systematic approach as discussed in this paper, including a detailed baseline analysis at six months, may aid in the evaluation of in vivo cartilage restoration.

Materials and Methods

Experimental Design

Eight adult Boer goats (age, two to three years old) underwent operative treatment of one knee in alternating left and right hindlimbs. The Institutional Animal Care and Use Committee approved all experiments and animal care procedures. All procedures were conducted in an animal facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care, and all were performed in accordance with the National Institutes of Health guidelines for care and use of laboratory animals. Each operatively treated knee received one frozen and one fresh, site-matched, osteochondral allograft implanted into alternating sites (i.e., four goats received frozen allograft in the medial femoral condyle and fresh allograft in the lateral aspect of the femoral trochlea, and four received fresh allograft in the medial femoral condyle and frozen allograft in the lateral aspect of the femoral trochlea) (Figs. 1-A and 1-B). The contralateral knee of each goat was used as the nonoperative control knee. One animal died at ten days postoperatively, of a cause unrelated to the implant. The seven remaining animals were killed six months after allograft implantation, and both the operative and the nonoperative control knee from each goat were analyzed (Fig. 1-C) to assess cartilage function, composition, and structure (Fig. 1-D).

Donor Preparations

Frozen and fresh (each, $n = 8$) donor osteochondral allografts were prepared from both knees of three adult Boer goats and one knee of two adult Boer goats (age, two to four years old). Hindlimbs were received on wet ice within twenty-four hours after the animal was killed. Under aseptic conditions, each knee was harvested and divided into condylar and trochlear fragments. Fragments were thoroughly rinsed with phosphate-buffered saline solution supplemented with antibiotics and antimycotics (PSF [100 U/mL of penicillin, 100 µg/mL of streptomycin, and 0.25 µg/mL of Fungizone (amphotericin B)]). Some fragments were stored frozen at -70°C for ten days. Other fragments were stored fresh at 4°C for three days in tissue culture medium (low-glucose Dulbecco modified Eagle medium [DMEM; GIBCO, North Andover, Massachusetts], 10% fetal bovine serum, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 25 µg/mL L-ascorbic acid, and PSF). Following implantation, chondrocyte viability near the graft site was assessed with LIVE/DEAD (Molecular Probes, Eugene, Oregon) staining and fluorescent microscopy²³, and was low (<10%) for frozen and high (>90%) for fresh grafts.

Allograft Surgery

Osteochondral allografting was performed through a medial knee arthrotomy with the animal under general anesthesia. General anesthesia was induced intravenously with diazepam (0.22 mg/kg) and ketamine (10 mg/kg), and maintained with isoflurane inhalation (0.4% to 3%). In addition, cefazolin (1 g) was given intravenously prior to incision and just after closure. The knee joint was exposed through a medial parapatellar incision and lateral patellar dislocation. An osteochondral defect (7.5 mm in diameter and 5 mm in height) was created at the medial femoral condyle and the lateral aspect of the femoral trochlea, using a guide pin and surgical reamer (Arthrex, Naples, Florida) under continuous saline-solution irrigation (Fig. 2-A). An orthotopic cylindrical osteochondral plug (8 mm in diameter and 5 mm in height) was harvested from donor tissue with use of a coring reamer (Arthrex, Naples, Florida), thoroughly cleansed with saline solution, and inserted carefully into the defect with impaction with use of a graft tamp and mallet (mean plus standard deviation, 35 ± 15

Fig. 1 Schematic diagrams and time line for the experimental design, including donor preparations (A), allograft surgery (B), retrieval groups (C), and analysis for biomechanical function, cartilage composition, and osteochondral structure (D). MFC = medial femoral condyle, LT = lateral aspect of the femoral trochlea, Rx = treatment, $\varnothing$ = diameter, h = height, Non-OP = nonoperative control knees, OP = operatively treated knees, and μ CT = microcomputed tomography.

taps, each with a force of 161 ± 46 N for a duration of 2.93 ± 3.96 ms and with an impulse of 0.22 ± 0.27 Ns²⁴ until the graft was flush with the surrounding articular surface (Fig. 2-B). Following closure, the operatively treated knees were placed in a modified Schroeder-Thomas splint for thirteen days to limit weight-bearing.

Animals were monitored postoperatively for pain and lameness throughout the study. One day after the operation, the animals received intramuscular injections of long-acting antibiotic (900,000 U penicillin G [Combi-Pen-48; Bimeda, Oakbrook Terrace, Illinois]), pain medication and narcotic (0.6 mg buprenorphine), and nonsteroidal anti-inflammatory drug (150 mg ketoprofen). Pain management also included a fentanyl patch (10.2 mg released over the course of seventy-two hours). Joint inflammation and lameness were monitored until animals walked normally. After six months, animals were killed with potassium chloride (1 to 2 mmol/kg) intravenously while they were under stage-III anesthesia. One of the animals that had fresh allograft implanted in the lateral aspect of the femoral trochlea and frozen allograft implanted in the medial femoral condyle died of a suspected upper respiratory infection, unrelated to the implant, ten days after the operation, and data from analysis of the knees of that animal were not included in the study.

Repair Site Analysis

Intact knee joints were received on wet ice within twenty-four hours of sacrifice. The distal part of the femurs was harvested, photographed, and examined grossly for cartilage fill and integration. Joints were tested biomechanically, and then samples were divided, as detailed in the Appendix, and analyzed for chondrocyte biosynthesis, cellularity, matrix content, and osteochondral structure.

Biomechanical Function

Cartilage load-bearing function was assessed with use of indentation testing at the center of each osteochondral core. With use of a benchtop mechanical tester (v500cs, BioSyntech Canada, Laval, Quebec, Canada), samples were compressed rapidly, by 100 μ m, at three sites that were 0.5 mm apart (proximal to distal)²⁵. The peak load was divided by the indentation depth, normalized to cartilage thickness and indenter tip area, and three points averaged to determine the indentation material stiffness, expressed in units of MPa.

Cartilage Composition

Cellularity was assessed according to depth from the articular surface. Cells were labeled fluorescently, and full-thickness cartilage was imaged with use of confocal microscopy along a vertical profile to a depth of 45 μ m at 15- μ m intervals. Image stacks were processed with a custom routine to localize and count cells²³. Cellularity was calculated as the number of cells divided by

Fig. 2

Photographs of the allograft surgical procedure, demonstrating (A) the osteochondral defects at the medial femoral condyle (MFC) and the lateral aspect of the femoral trochlea (LT), and (B) implanted allografts at time of implantation. PROX = proximal, and DIST = distal.

cartilage volume (cells/cm³) for superficial, middle, and deep zones of cartilage, defined as 0% to 15%, 15% to 50%, and 50% to 100% of the cartilage thickness.

Matrix fixed-charge density in cartilage was assessed with use of Hexabrix-enhanced microcomputed tomography (HE- μ CT). Hexabrix (Guerbet, Bloomington, Indiana), an ionic contrast agent, distributes inversely to the fixed charge density in soft tissues, and is therefore a sensitive (inverse) indicator of proteoglycan content²⁶. HE- μ CT gray value (i.e., x-ray attenuation) was calculated, as described in the Appendix, in the superficial, middle, and deep zones, which were defined as the top 90 μ m, the next 35%, and the remaining approximately 50% of the cartilage thickness, respectively. The spatial variation in matrix fixed-charge density was illustrated with color maps representing 5% to 95% HE- μ CT gray value within the cartilage.

Osteochondral Structure

Cartilage and bone structure were assessed with use of μ CT, which was used to quantify cartilage thickness, fill, surface location, and bone-cartilage interface location (evaluated within a 2.5-mm radius of the repair center [see Appendix]). The repair center was determined as the midpoint between visually identified discontinuities in the bone, or the corresponding site in the nonoperative control knee.

Cartilage histopathology was assessed with the modified Mankin score²⁷ with the aid of safranin-O and hematoxylin-and-eosin staining, as osteochondral allograft repair resembles normal hyaline cartilage structure at the time of implant. The Mankin scale is sensitive to early stage degeneration and was previously found to correlate with indentation stiffness²². The histopathology score was graded on a scale of 0 to 15, with high scores corresponding to degeneration. Graft-host bone integration was assessed with binary histological evaluation of immune response (cellular infiltrate and/or pannus), fibrous marrow, subchondral cysts, and bone-healing.

Statistical Analysis

Data are presented as the mean plus the standard deviation of the mean. The effect of allograft treatment (i.e., no treatment, frozen allograft, or fresh allograft) and site (i.e., medial femoral condyle or lateral aspect of the femoral trochlea) on cartilage cellularity, stiffness, thickness, HE- μ CT gray value, and structural parameters was determined with use of two-way analysis of variance of the mean (ANOVA). For cellularity and HE- μ CT gray-value data, zone (superficial, middle, or deep) was considered a repeated measure, and a three-way repeated-measures ANOVA was performed. At each site, Tukey post hoc comparisons were performed to compare treatments with significant differences ($p < 0.05$). For nonparametric data (i.e., histopathology scores), samples were analyzed analogously with use of the Friedman test and the Dunn post hoc analysis. Power analysis ($\alpha = 0.05$ and $1 - \beta = 80\%$) was performed with cartilage indentation stiffness as the primary end point, and four samples per treatment group were determined to be the amount needed to detect a difference of 150% of the standard deviation of the mean, which would be an effect size sufficient to distinguish microfracture repair in the cartilage of treated knees from the normal hyaline cartilage of untreated knees at six months²⁸. For secondary end points for which effect size may be smaller, power may not be sufficient to detect significant differences.

Linear regression analysis was performed to assess how the performance of the allograft was related to cartilage composition, surface irregularity, and cartilage degeneration. Biomechanical function (stiffness) was correlated with cartilage cellularity, and relative matrix fixed-charge density. Biomechanical function was also correlated with histopathology and surface irregularity with use of the nonparametric Spearman rank method²⁹. Coefficients of determination (R^2 [parametric] and ρ^2 [nonparametric]) were reported for significant relationships ($p < 0.05$).

Source of Funding

This work was supported by grants from the National Institutes of Health and Howard Hughes Medical Institute Professors Program.

Results

Biomechanical Function

Cartilage material stiffness varied with allograft treatment ($p < 0.001$), but not with site ($p = 0.3$), whereas cartilage thickness varied with site ($p < 0.001$) but was not affected by treatment ($p = 0.3$). Cartilage stiffness in the nonoperative control specimens was similar to that in the fresh allograft specimens ($p > 0.9$). Stiffness was approximately 80% lower in the frozen allograft specimens than in the nonoperative control specimens (95% confidence interval [CI], 79% to 84%) or fresh allograft specimens (95% CI, 73% to 87%) and was 11.5 ± 4.4 MPa in the specimens from the medial femoral condyle and 14.2 ± 5.3 in the specimens from the lateral aspect of the femoral trochlea in the nonoperative control specimens. Stiffness in frozen allograft specimens was only 2.0 ± 0.8 MPa in the specimens from the medial femoral condyle ($p < 0.05$ versus the nonoperative control specimen, and $p = 0.1$ versus the fresh allograft specimen, Fig. 3-A) and 2.7 ± 0.5 MPa in the specimens from the lateral aspect of the femoral trochlea ($p < 0.01$ versus the nonoperative control specimen, and $p < 0.05$ versus the fresh allograft specimens, Fig. 3-B). The mean cartilage thickness was approximately 60% lower (95% CI, 52% to 68%) in specimens from the lateral aspect of the femoral trochlea (0.86 ± 0.23 mm) than it was in specimens from the medial femoral condyle (1.44 ± 0.34 mm, Figs. 3-C and 3-D).

Cartilage Composition

Chondrocyte cellularity varied with allograft treatment ($p < 0.001$), but not with site ($p = 0.2$). Cellularity was approximately 96%

Fig. 3

Effect of in vivo allograft treatment on cartilage stiffness (A and B) and cartilage thickness (C and D) in retrieved osteochondral allografts at the medial femoral condyle (MFC) (A and C) and the lateral aspect of the femoral trochlea (LT) (B and D) after six months. The colored bars indicate the mean, and the error bars indicate the standard deviation of the mean. The asterisk indicates $p < 0.05$, and the double asterisk indicates $p < 0.01$. Rx = treatment, n = number of specimens, Non-OP = nonoperative control specimens, and OP = operatively treated specimens.

Fig. 4
Effect of in vivo allograft treatment on cartilage cellularity in retrieved osteochondral allografts at the medial femoral condyle (MFC) (A) and the lateral aspect of the femoral trochlea (LT) (B) after six months. The colored bars indicate the mean, and the error bars indicate the standard deviation of the mean. The asterisk indicates $p < 0.05$, the double asterisk indicates $p < 0.01$, and the triple asterisk indicates $p < 0.001$. Rx = treatment, n = number of specimens, S = superficial, M = middle, D = deep, Non-OP = nonoperative control specimens, and OP = operatively treated specimens.

lower in frozen allograft specimens than it was in nonoperative control specimens (95% CI, 94% to 98%) and fresh allograft specimens (95% CI, 94% to 99%) from the medial femoral condyle (each, $p < 0.001$, Fig. 4-A) and the lateral aspect of the femoral trochlea (each, $p < 0.01$, Fig. 4-B). Throughout the cartilage depth, cellularity varied by zone ($p < 0.001$), with significant interactions between zone and treatment ($p < 0.05$) and zone and site ($p < 0.05$). At the medial femoral condyle, cellularity in the nonoperative control specimens decreased

with depth from the articular surface and was a mean of $38.6 \pm 19.6 \times 10^6$ cells/cm³ in the superficial zone, $18.4 \pm 6.9 \times 10^6$ cells/cm³ in the middle zone, and $13.6 \pm 6.0 \times 10^6$ cells/cm³ in the deep zone, and was similar to the cellularity in the fresh allograft specimens ($p > 0.5$). At the lateral aspect of the femoral trochlea, cellularity in the nonoperative control specimens did not vary significantly with depth ($p = 0.1$), and was $39.0 \pm 7.6 \times 10^6$, $31.4 \pm 13.8 \times 10^6$, and $28.6 \pm 13.3 \times 10^6$ cells/cm³ in the superficial, middle, and deep zones, respectively, and was

Fig. 5
Representative (A through F) color maps of Hexabrix-enhanced microcomputed tomography (HE-μCT) analysis and corresponding safranin-O (Saf-O) histology (G through L) at the medial femoral condyle (MFC) (A, B, C, G, H, and I) and the lateral aspect of the femoral trochlea (LT) (D, E, F, J, K, and L) of nonoperative control (Non-OP) (A, D, G, and J), frozen (B, E, H, and K), and fresh (C, F, I, and L) retrieved osteochondral allografts after six months in vivo. PROX = proximal, and DIST = distal.

Fig. 6
Effect of in vivo allograft treatment on Hexabrix-enhanced microcomputed tomography (HE-μCT) gray value in cartilage of retrieved osteochondral allografts at the medial femoral condyle (MFC) (A) and the lateral aspect of the femoral trochlea (LT) (B) after six months. The colored bars indicate the mean, and the error bars indicate the standard deviation of the mean. The asterisk indicates $p < 0.05$, and the double asterisk indicates $p < 0.01$. Rx = treatment, n = number of specimens, S = superficial, M = middle, D = deep, Non-OP = nonoperative control specimens, and OP = operatively treated specimens.

similar to the cellularity in the fresh allograft specimens for each zone (each, $p = 1.0$).

Collectively, HE-μCT analysis and safranin-O histology indicated that matrix fixed-charge density was similar between the nonoperative control specimens and the fresh allograft specimens and was lower in the frozen allograft specimens. HE-μCT gray value for the nonoperative control specimens and the fresh allograft specimens demonstrated the normal spatial variation of matrix fixed charge, which increases with depth from the articular surface. High HE-μCT gray value (shown in red and yellow), which represents low matrix fixed charge, was present throughout the depth of the frozen allograft specimens (Figs. 5-B and 5-E), and only at the articular surface of the nonoperative control specimens (Figs. 5-A and 5-D) and fresh allograft specimens (Figs. 5-C and 5-F). Low HE-μCT gray value (shown in blue), which represents high matrix fixed charge, was absent in the frozen allograft specimens and was present in the deep zone of the nonoperative control specimens and the fresh allograft specimens. Representative safranin-O histology confirmed the extensive loss of proteoglycans in the frozen allograft specimens versus the nonoperative control specimens and the fresh allograft specimens (Figs. 5-G through 5-L). The safranin-O score in the nonoperative control specimens was similar to that in the fresh allograft specimens ($p > 0.3$). The safranin-O score was 3.0 ± 0.0 , indicating absence of staining, in the frozen allograft specimens, which was higher than that in the nonoperative control specimens and the fresh allograft specimens from the medial femoral condyle (each, $p < 0.05$) or the lateral aspect of the femoral trochlea (each, $p < 0.001$, Table I).

Quantitatively, in addition to facilitating the visualization of overall qualitative patterns, HE-μCT delineated the spatial variation of matrix fixed charge throughout the cartilage depth. HE-μCT gray value varied with cartilage zone ($p < 0.001$) but not with allograft treatment ($p = 0.4$), with a significant interaction between zone and treatment ($p < 0.05$). HE-μCT gray value in the nonoperative control specimens was similar to that in the fresh allograft specimens from the medial femoral

Fig. 7
Effect of in vivo allograft treatment on cartilage fill (A and B), cartilage surface location (C and D), and bone-cartilage interface location (E and F) at the medial femoral condyle (MFC) (A, C, and E) and the lateral aspect of the femoral trochlea (LT) (B, D, and F) after six months. The colored bars indicate the mean, and the error bars indicate the standard deviation of the mean. The asterisk indicates $p < 0.05$. Rx = treatment, and n = number of specimens.

condyle and the lateral aspect of the femoral trochlea (each, $p > 0.5$). In the deep zone, matrix fixed-charge density was lower in the frozen allograft specimens than it was in the nonoperative control specimens or the fresh allograft specimens from the medial femoral condyle (each, $p < 0.01$, Fig. 6-A) or the lateral aspect of the femoral trochlea ($p < 0.05$ versus the nonoperative control specimens, and $p = 0.06$ versus the fresh allograft specimens (Fig. 6-B), as indicated by the approximately 55% increase in HE- μ CT gray value as compared with the gray value in the nonoperative control specimens (95% CI, 22% to 67%) and fresh allograft specimens (95% CI, 39% to 92%).

Osteochondral Structure

Cartilage and bone structure (measured according to cartilage fill, cartilage surface location, and bone-cartilage interface location) and bone-cartilage interface location varied according to the type of allograft treatment used ($p < 0.001$). In specimens from the medial femoral condyle, cartilage fill was approximately 55% lower (95% CI, 34% to 100%) in the frozen allograft specimens than it was in the fresh allograft specimens ($p < 0.05$, Fig. 7-A), in association with -1.12 ± 0.94 mm and -1.46 ± 0.88 mm depression of the cartilage surface and bone-cartilage interface, respectively (each, $p <$

0.05, Figs. 7-C and 7-E-, respectively). In specimens from the lateral aspect of the femoral trochlea, cartilage fill tended to be lower by approximately 20% (95% CI, 3% to 55%) in the frozen allograft specimens than in the fresh allograft specimens ($p = 0.1$, Fig. 7-B); the cartilage surface tended to be depressed by -0.30 ± 0.07 mm ($p = 0.1$, Fig. 7-D), and the bone-cartilage interface was depressed by -0.48 ± 0.29 mm ($p < 0.05$, Fig. 7-F).

Gross macroscopic observations of allografts were visually consistent with the quantified structural parameters. Overall, the joints appeared macroscopically normal, with no osteophyte formation or extensive degeneration (Figs. 8-A and 8-B). Implanted allografts remained clearly demarcated from the surrounding host cartilage at the time of retrieval. At the medial femoral condyle, the cartilage surface of two of the three frozen allografts appeared macroscopically sunken with respect to the surrounding host cartilage (Figs. 8-C-2 and 8-C-3), whereas the cartilage surface of two of the four fresh allografts appeared macroscopically smooth (Figs. 8-D-4 and 8-D-5) or flush with the surrounding host cartilage with minimal uneven surfaces (Figs. 8-D-6 and 8-D-7). At the lateral aspect of the femoral trochlea, the cartilage surface of all four frozen allografts appeared macroscopically flush with the surrounding host cartilage, with visible fibrous tissue or degeneration at the graft margins (Figs. 8-E-2, 8-E-4, 8-E-5, and 8-E-7), whereas

Fig. 8

Gross macroscopic images of representative knee joints (A and B) after six months in vivo, and individual retrieved FROZEN (blue) and FRESH (green) osteochondral allografts at the medial femoral condyle (MFC) (C and D) and the lateral aspect of the femoral trochlea (LT) (E and F). Numbers in lower right corner indicate the recipient animal number. Arrowheads indicate borders of the allograft and host cartilage. PROX = proximal, DIST = distal, and Rx = treatment.

TABLE I Histopathology and Cartilage Surface Degeneration Scores*

	MFC			LT		
	Non-OP	Frozen	Fresh	Non-OP	Frozen	Fresh
Number of specimens	7	3	4	7	4	3
Histopathology score† (avg rank)‡	2.1 ± 1.9§ (13.9)	8.3 ± 1.5 (25.8)	2.5 ± 1.0 (16.9)	0.4 ± 0.5# (7.5)	7.5 ± 1.0 (24.4)	0.0 ± 0.0** (4.5)
Safranin-O score (avg rank)‡	0.6 ± 0.8§ (11.9)	3.0 ± 0.0 (25.0)	0.5 ± 0.6§ (12.0)	0.4 ± 0.5** (11.3)	3.0 ± 0.0 (25.0)	0.0 ± 0.0** (7.0)
Surface irregularity score (avg rank)‡	0.7 ± 0.8 (15.8)	1.0 ± 0.0 (20.5)	1.0 ± 0.0 (20.5)	0.0 ± 0.0** (7.0)	1.0 ± 0.0 (20.5)	0.0 ± 0.0** (7.0)

*MFC = medial femoral condyle, LT = lateral aspect of the femoral trochlea, Non-OP = nonoperative control specimens, Frozen = frozen allograft specimens, and Fresh = fresh allograft specimens. †Histopathology score (0-15) was determined by assigning a score of 0-2 or 0-3 (with 0 representing normal, and 2 or 3 representing severe degeneration) for the following histologic characteristics: surface irregularity (0-2), vertical clefts into the transitional or radial zone (0-2), transverse clefts (0-2), cloning (0-3), hypocellularity (0-3), and safranin-O staining (0-3)²⁸. ‡Average rank was calculated for the nonparametric Friedman test, in which the ordinal value for a sample is replaced by its rank (with ties, from 1-28) when the data are sorted from low to high. §p < 0.05. #p < 0.001. **p < 0.001 versus frozen.

the cartilage surface of two of the three fresh allografts was smooth (Figs. 8-F-3 and 8-F-6).

Histopathology and surface irregularity scores varied according to type of allograft treatment (each, $p < 0.001$), site (each, $p < 0.01$), and with significant interaction between treatment and site (each, $p < 0.05$). The histopathology score was similar between the nonoperative control specimens and the fresh allograft specimens, and was higher, corresponding to more degeneration, for the frozen allograft specimens (Table I). For the specimens from the medial femoral condyle, the histopathology score for the nonoperative control specimens was 2.1 ± 1.9 , which was lower than that for the frozen allograft

specimens ($p < 0.05$), and similar to that for the fresh allograft specimens ($p = 0.8$), but the surface irregularity score did not vary among treatments ($p > 0.6$), and was 0.9 ± 0.5 for all of the specimens from the medial femoral condyle. For the specimens from the lateral aspect of the femoral trochlea, the histopathology score for the nonoperative control specimens was 0.4 ± 0.5 , which was lower than that for the frozen allograft specimens ($p < 0.001$) and similar to that for the fresh allograft specimens ($p = 0.3$). Concomitantly, surface irregularity scores were indistinguishable between the nonoperative control specimens and the fresh allograft specimens ($p = 1.0$, Table I) but higher for the frozen allograft specimens than for the

Fig. 9

Representative hematoxylin and eosin bone histology for nonoperative control (Non-OP) (A through D), FROZEN (E through H), and FRESH (I through L) allografts at the medial femoral condyle (MFC) (A, B, E, F, I, and J) and the lateral aspect of the femoral trochlea (LT) (C, D, G, H, K, and L). Insets show areas of enlargement. **Figs. 9-A through 9-D** Bone marrow contained primarily fat cells. **Figs. 9-E through 9-L** Bone marrow was fibrotic, containing spindle-shaped purple fibroblasts in addition to fat cells. **Figs. 9-E and 9-I** Bone cysts are indicated by arrows; bone cysts were visually confirmed by gross evaluation (either as void spaces in bone or containing gelatinous-like material). **Figs. 9-F and 9-J** Cellular infiltrate was noted as clusters of small, round pink eosinophils.

Fig. 10

Parametric regression analysis (A and B) and Spearman rank correlation of cartilage stiffness (C and D) versus cellularity (A), Hexabrix-enhanced microcomputed tomography (HE-μCT) gray value (Gray Val) in the deep zone (B), histopathology score (C), and surface irregularity score (D) for medial femoral condyle (MFC) (filled symbols) and lateral aspect of the femoral trochlea (LT) (open symbols). Data points correspond to individual nonoperative control (Non-OP) (red circles), frozen (blue squares), and fresh (green triangles) allograft retrievals. Significance (p) and correlation coefficients (R² for A and B, ρ² for C and D) were determined. Lines represent the linear regression fits of the data and are shown only to indicate trends.

nonoperative control specimens or the fresh allograft specimens (each, $p < 0.001$).

All frozen and fresh allografts exhibited osseous union at the graft-host junction but were variably accompanied by structural abnormalities of bone-healing (Fig. 9). At the medial femoral condyle, fibrosis was present in the bone marrow of two of the three frozen allograft specimens and in two of the four fresh allograft specimens and cellular infiltrates were present in two of the three frozen allograft specimens and in one of the four fresh allograft specimens. Bone-marrow histology was similar in the specimens from the lateral aspect of the femoral trochlea; the marrow was fibrotic in three of the four frozen allograft specimens and in two of the three fresh allograft specimens and was accompanied by occasional cellular infiltrates (one allograft specimen each in the fresh and frozen allograft groups). In contrast, subchondral cysts were widespread (i.e., in two of the three frozen specimens and all four of the fresh specimens) at the medial femoral condyle but were less common

(i.e., in one of the three fresh allografts and one of the four frozen allografts) at the lateral aspect of the femoral trochlea.

Determinants of Allograft Performance

Biomechanical properties correlated significantly with cartilage cellularity, matrix fixed-charge density, and surface degeneration. The combined results of the specimens at both the medial femoral condyle and the lateral aspect of the femoral trochlea showed that cartilage stiffness correlated positively with overall cellularity ($R^2 = 0.46$, $p < 0.001$, Fig. 10-A), and negatively with deep-zone HE-μCT gray value ($R^2 = 0.23$, $p < 0.05$, Fig. 10-B), histopathology score ($\rho^2 = 0.75$, $p < 0.001$, Fig. 10-C), and surface irregularity score ($\rho^2 = 0.52$, $p < 0.01$, Fig. 10-D). The separate results from the medial femoral condyle and the lateral aspect of the femoral trochlea also exhibited trends that were similar to the combined results, except with regard to cartilage stiffness versus surface irregularity for the specimens at the medial femoral condyle ($\rho^2 = 0.25$, $p = 0.4$, Fig. 10-D).

Discussion

These results demonstrate marked differences in articular cartilage zonal composition, structure, and function of osteochondral allografts depending on their fresh versus frozen treatment, as early as six months after the repair procedure. The cartilage of frozen osteochondral allografts was mechanically soft (Fig. 3), acellular (Fig. 4), depleted of matrix fixed charge and proteoglycans (Figs. 5-B, 5-E, 5-H, 5-K, and 6), and visibly deteriorated (Fig. 8), suggesting that storage conditions that eliminate chondrocyte viability at implantation also reduce allograft performance *in vivo*. In contrast, the cartilage of fresh osteochondral allografts had similar mechanical properties (Fig. 3) along with similar cellular (Fig. 4) and matrix (Figs. 5-C, 5-F, 5-I, 5-L, and 6) zonal organization as the site-matched nonoperative control cartilage (Figs. 3, 4, 5-A, 5-D, 5-G, 5-J, and 6), and appeared smooth and flush to the surrounding host cartilage (Fig. 8). Maintenance of cartilage load-bearing function was associated with zonal maintenance of chondrocyte cellularity and matrix fixed charge. Thus, the *in vivo* performance of osteochondral allografts at six months paralleled the chondrocyte viability within the allograft at the time of implantation.

The use of the goat model to study cartilage restoration by osteochondral allografting involved the consideration of a number of issues. Two experimental sites (the medial femoral condyle and the lateral aspect of the femoral trochlea) within each joint doubled the examined sample number while also providing similar overall joint environments for frozen or fresh treatment groups. Alternating treatments between sites avoided possible effects specific to one site. Even with the limited sample numbers and some variation in gross observations within treatment groups, there were clear differences in some parameters (i.e., stiffness, cellularity, and matrix fixed-charge density) irrespective of site, and differences in other parameters (i.e., osteochondral structure) that were site dependent.

While the general consensus from the literature suggests that fresh grafts perform “better” than frozen grafts, this study expands on such generalizations and introduces three new key findings in this animal model. First, by six months after the repair procedure, the cartilage of frozen osteochondral allografts exhibited failure based on softening and associated deterioration of structural indices, particularly cellularity, deep-zone fixed charge, and surface irregularity. Second, the cartilage of fresh osteochondral allografts maintained its zonal distribution of cellular and matrix components and load-bearing function in a manner similar to those of articular cartilage from the nonoperative control specimens. Third, allografts failed differently at the medial femoral condyle and the lateral aspect of the femoral trochlea, with graft subsidence at the medial femoral condyle but not at the lateral aspect of the femoral trochlea, suggesting that repair efficacy is site dependent and possibly load dependent.

At six months, frozen allografts already exhibited clear progression toward failure, with loss of chondrocytes, reduced proteoglycan content and cartilage stiffness, and associated surface and/or bone collapse at the medial femoral condyle. Reduced cellularity (Fig. 4) and proteoglycan synthesis rates

(see Appendix) suggest inadequate biological remodeling capacity and the inability to sustain long-term function. End-stage failure of frozen allografts, accompanied here by loss of zonal architecture, increased surface irregularity and reduced stiffness and proteoglycan content (Figs. 3 through 8), which was consistent with the results from previous long-term animal studies¹²⁻¹⁵ and with grafts retrieved after limb-salvage oncologic reconstructive surgery^{6,7}. Hence, six months was a sufficient time for grafts to fail at the medial femoral condyle and for grafts to exhibit progressive deterioration toward failure at the lateral aspect of the femoral trochlea.

In contrast, fresh allografts had preserved depth-dependent tissue properties that were similar to those in nonoperative control cartilage, and they also maintained their capacity for biological homeostasis. Even in mechanically demanding environments, fresh allografts mimicked “normal” zonal organization of the cellular and matrix components present in nonoperative control articular cartilage (Figs. 4 through 6). Previous allograft animal studies did not analyze depth-related variations in chondrocyte cellularity^{14,15,18} and matrix content^{13,14,17-20}, which are important because chondrocytes exhibit zone-specific functions³⁰. Appropriate zonal chondrocyte organization and proteoglycan synthesis rates in fresh allografts provided biological remodeling that served to preserve the articular surface, sustain bulk tissue properties (including cartilage fill, matrix content, and load-bearing function), maintain the bone-cartilage interface and underlying bone structure, and prevent progressive deterioration to graft collapse (Fig. 3 and Figs. 5 through 9). Usage of such fresh allografts has resulted in clinical success rates of >75%³¹⁻³⁷. However, the relative importance of cartilage zone(s) to preserve depth-dependent tissue homeostasis and prevent failure progression remains to be determined.

The different effects of allograft treatment on osteochondral structure at the medial femoral condyle and the lateral aspect of the femoral trochlea suggest that repair efficacy at mechanically demanding sites is more sensitive to decreased implant viability. In a goat model, spontaneous repair of cartilage defects at the lateral aspect of the femoral trochlea had better histological scores and gross visual appearance than those seen at the medial femoral condyle after six and twelve weeks *in vivo*³⁸. In the present six-month study, frozen allografts had reduced cartilage fill and increased surface degeneration at the medial femoral condyle but not at the lateral aspect of the femoral trochlea, which may be due to inherent differences between anatomic sites, with cartilage thickness and loading environment likely contributing to the variation. Thus, higher chondrocyte viability at the time of implant may be needed to sustain biological remodeling at mechanically demanding sites, such as the medial femoral condyle.

Allograft performance may also be related to effective osseous integration. Fresh allograft retrievals often demonstrated that graft failure was associated with limited osseous incorporation, graft collapse, osteonecrosis, and pannus formation⁵. Such osseous integration, influenced, in part, by the immunogenicity of allogeneic bone, has been improved by reducing surface antigens (by freezing)^{39,40} or matching leukocyte-antigens, with

accompanying improvement in clinical outcomes^{41,42}. While subchondral bone appeared generally maintained, there was histopathological evidence of bone abnormalities for both frozen and fresh allografts in the present study. Antigen matching was not attempted, in accordance with current clinical practice, but may be a contributing factor. Further investigation of the relationship between osseous remodeling and cartilage maintenance is needed.

This systematic approach, which was used to evaluate cartilage restoration with use of frozen and fresh allografts, could also be applied to the efficacy of other cartilage repair therapies, tissue-engineered cartilaginous and/or osteocartilaginous grafts, and stored osteochondral grafts. Chondrocytes, in grafts stored routinely at 4°C to accommodate regulatory screening for infectious diseases⁴³, are susceptible, with increasing storage duration, to cell death especially at the articular surface⁴⁴⁻⁵². Usage of allografts stored at 4°C leads to some short-term clinical improvement as compared with preoperative patient function⁵³⁻⁵⁶. The long-term in vivo performance of allografts that have been stored at 4°C, which are used after prolonged storage durations (range, ten to forty-three days)⁵³⁻⁵⁶ and represent an intermediate condition of cellularity, are unknown. Further analysis of zonal chondrocyte organization following cartilage repair treatments may help to elucidate the critical number of functional chondrocytes that are needed in the appropriate cartilage zone(s) to maintain the surrounding matrix and ensure the subsequent biomechanical function of the allograft in vivo.

Appendix

eA An appendix describing the methods used to analyze tissue division for repair-site analysis, cartilage metabolism, cartilage matrix fixed-charge density, microcomputed tomographic assessment of cartilage and bone structure, histological processing, and the results with regard to cartilage metabolism

is available with the online version of this article as a data supplement at jbjs.org. ■

NOTE: This work was supported by grants from the National Institutes of Health (AR055637 [and -03S1] and AR044058) and an award to the University of California at San Diego from the Howard Hughes Medical Institute (HHMI) through the HHMI Professors Program (for R.L.S.). We thank Karen D. Bowden for technical histology assistance, Frederick L. Harwood for radiolabeling studies, and Professor Koichi Masuda and Doshisha University for use of a Leica confocal microscope.

Andrea L. Pallante, MS
Albert C. Chen, PhD
Robert L. Sah, MD, ScD
Department of Bioengineering,
University of California at San Diego,
9500 Gilman Drive, MC 0412,
La Jolla, CA 92093-0412

Simon Görtz, MD
Derek C. Chase, MD
Scott T. Ball, MD
Department of Orthopaedic Surgery,
University of California at San Diego,
200 West Arbor Drive, MC 8894,
San Diego, CA 92103-8894

Robert M. Healey, BS, MBA
David Amiel, PhD
Department of Orthopaedic Surgery,
University of California at San Diego,
9500 Gilman Drive, MC 0863,
La Jolla, CA 92093-0863

William D. Bugbee, MD
Division of Orthopaedic Surgery, Scripps Clinic,
10666 North Torrey Pines Road, MS 116,
La Jolla, CA 92037.
E-mail address: Bugbee.William@scrippshealth.org

References

- Convery FR, Akeson WH, Amiel D, Meyers MH, Monosov A. Long-term survival of chondrocytes in an osteochondral articular cartilage allograft. A case report. *J Bone Joint Surg Am*. 1996;78:1082-8.
- Jamali AA, Hatcher SL, You Z. Donor cell survival in a fresh osteochondral allograft at twenty-nine years. A case report. *J Bone Joint Surg Am*. 2007;89:166-9.
- Maury AC, Safir O, Heras FL, Pritzker KP, Gross AE. Twenty-five-year chondrocyte viability in fresh osteochondral allograft. A case report. *J Bone Joint Surg Am*. 2007;89:159-65.
- McGoveran BM, Pritzker KP, Shasha N, Price J, Gross AE. Long-term chondrocyte viability in a fresh osteochondral allograft. *J Knee Surg*. 2002;15:97-100.
- Williams SK, Amiel D, Ball ST, Allen RT, Tontz WL Jr, Emmerson BC, Badlani NM, Emery SC, Haghighi P, Bugbee WD. Analysis of cartilage tissue on a cellular level in fresh osteochondral allograft retrievals. *Am J Sports Med*. 2007;35:2022-32.
- Enneking WF, Campanacci DA. Retrieved human allografts: a clinicopathological study. *J Bone Joint Surg Am*. 2001;83:971-86.
- Enneking WF, Mindell ER. Observations on massive retrieved human allografts. *J Bone Joint Surg Am*. 1991;73:1123-42.
- Oakeshott RD, Farine I, Pritzker KP, Langer F, Gross AE. A clinical and histologic analysis of failed fresh osteochondral allografts. *Clin Orthop Relat Res*. 1988;233:283-94.
- Kandel RA, Gross AE, Ganel A, McDermott AG, Langer F, Pritzker KP. Histopathology of failed osteoarticular shell allografts. *Clin Orthop Relat Res*. 1985;197:103-10.
- Frenkel SR, Kubiak EN, Truncala KG. The repair response to osteochondral implant types in a rabbit model. *Cell Tissue Bank*. 2006;7:29-37.
- Marco F, Leon C, Lopez-Oliva F, Perez AJ, Sanchez-Barba A, Lopez-Duran Stern L. Intact articular cartilage cryopreservation. In vivo evaluation. *Clin Orthop Relat Res*. 1992;283:11-20.
- Schachar NS, Novak K, Hurtig M, Muldrew K, McPherson R, Wohl G, Zernicke RF, McGann LE. Transplantation of cryopreserved osteochondral dowel allografts for repair of focal articular defects in an ovine model. *J Orthop Res*. 1999;17:909-19.
- Hurtig MB, Novak K, McPherson R, McFadden S, McGann LE, Muldrew K, Schachar NS. Osteochondral dowel transplantation for repair of focal defects in the knee: an outcome study using an ovine model. *Vet Surg*. 1998;27:5-16.
- Stevenson S, Dannucci GA, Sharkey NA, Pool RR. The fate of articular cartilage after transplantation of fresh and cryopreserved tissue-antigen-matched and mismatched osteochondral allografts in dogs. *J Bone Joint Surg Am*. 1989;71:1297-307.
- Ranawat AS, Vidal AF, Chen CT, Zelken JA, Turner AS, Williams RJ 3rd. Material properties of fresh cold-stored allografts for osteochondral defects at 1 year. *Clin Orthop Relat Res*. 2008;466:1826-36.
- Pearce SG, Hurtig MB, Boure LP, Radcliffe RM, Richardson DW. Cylindrical press-fit osteochondral allografts for resurfacing the equine metatarsophalangeal joint. *Vet Surg*. 2003;32:220-30.
- Bae JY, Matsumura K, Wakitani S, Kawaguchi A, Tsutsumi S, Hyon SH. Beneficial storage effects of epigallocatechin-3-o-gallate on the articular cartilage of rabbit osteochondral allografts. *Cell Transplant*. 2009;18:505-12.
- Malinin T, Temple HT, Buck BE. Transplantation of osteochondral allografts after cold storage. *J Bone Joint Surg Am*. 2006;88:762-70.
- Oates KM, Chen AC, Young EP, Kwan MK, Amiel D, Convery FR. Effect of tissue culture storage on the in vivo survival of canine osteochondral allografts. *J Orthop Res*. 1995;13:562-9.

20. Glenn RE Jr, McCarty EC, Potter HG, Juliao SF, Gordon JD, Spindler KP. Comparison of fresh osteochondral autografts and allografts: a canine model. *Am J Sports Med.* 2006;34:1084-93.
21. Rodrigo JJ, Sakovich L, Travis C, Smith G. Osteocartilaginous allografts as compared with autografts in the treatment of knee joint osteocartilaginous defects in dogs. *Clin Orthop Relat Res.* 1978;134:342-9.
22. Bae WC, Temple MM, Amiel D, Coutts RD, Niederauer GG, Sah RL. Indentation testing of human cartilage: sensitivity to articular surface degeneration. *Arthritis Rheum.* 2003;48:3382-94.
23. Pallante AL, Bae WC, Chen AC, Görtz S, Bugbee WD, Sah RL. Chondrocyte viability is higher after prolonged storage at 37 degrees C than at 4 degrees C for osteochondral grafts. *Am J Sports Med.* 2009;37 Suppl 1:24S-32S.
24. Borazjani BH, Chen AC, Bae WC, Patil S, Sah RL, Firestein GS, Bugbee WD. Effect of impact on chondrocyte viability during insertion of human osteochondral grafts. *J Bone Joint Surg Am.* 2006;88:1934-43.
25. McCarty WJ, Pallante AL, Rone RJ, Bugbee WD, Sah RL. The proteoglycan metabolism of articular cartilage in joint-scale culture. *Tissue Eng Part A.* 2010;16:1717-27.
26. Palmer AW, Guldberg RE, Levenston ME. Analysis of cartilage matrix fixed charge density and three-dimensional morphology via contrast-enhanced micro-computed tomography. *Proc Natl Acad Sci U S A.* 2006;103:19255-60.
27. Shapiro F, Glimcher MJ. Induction of osteoarthritis in the rabbit knee joint. *Clin Orthop Relat Res.* 1980;147:287-95.
28. Lane JG, Healey RM, Chen AC, Sah RL, Amiel D. Can osteochondral grafting be augmented with microfracture in an extended-size lesion of articular cartilage? *Am J Sports Med.* 2010;38:1316-23.
29. Glantz SA. *Primer of biostatistics.* 3rd ed. San Francisco, CA: McGraw-Hill; 1992.
30. Aydelotte MB, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. I. Morphology and cartilage matrix production. *Connect Tissue Res.* 1988;18:205-22.
31. Beaver RJ, Mahomed M, Backstein D, Davis A, Zukor DJ, Gross AE. Fresh osteochondral allografts for post-traumatic defects in the knee. A survivorship analysis. *J Bone Joint Surg Br.* 1992;74:105-10.
32. Bugbee WD. Fresh osteochondral allografts. *J Knee Surg.* 2002;15:191-5.
33. Chu CR, Convery FR, Akeson WH, Meyers M, Amiel D. Articular cartilage transplantation. Clinical results in the knee. *Clin Orthop Relat Res.* 1999;360:159-68.
34. Convery FR, Meyers MH, Akeson WH. Fresh osteochondral allografting of the femoral condyle. *Clin Orthop Relat Res.* 1991;273:139-45.
35. Emmerson BC, Görtz S, Jamali AA, Chung C, Amiel D, Bugbee WD. Fresh osteochondral allografting in the treatment of osteochondritis dissecans of the femoral condyle. *Am J Sports Med.* 2007;35:907-14.
36. Garrett JC. Fresh osteochondral allografts for treatment of articular defects in osteochondritis dissecans of the lateral femoral condyle in adults. *Clin Orthop Relat Res.* 1994;303:33-7.
37. McDermott AG, Langer F, Pritzker KP, Gross AE. Fresh small-fragment osteochondral allografts. Long-term follow-up study on first 100 cases. *Clin Orthop Relat Res.* 1985;197:96-102.
38. Davissan T, Zhang R, Masini M, Coyle S, Aponte C, Long M, Hawkins M. Repair of osteochondral defects in goats is modulated by anatomic site, timepoint, and treatment. Presented at the Combined Meeting of the Orthopaedic Research Society; 2007 Oct 20-24; Honolulu, HI.
39. Friedlaender GE, Goldberg VM, editors. *Bone and cartilage allografts: biology and clinical applications.* Park Ridge, IL: American Academy of Orthopaedic Surgeons; 1991.
40. Rodrigo JJ, Thompson E, Travis C. Deep-freezing versus 4 degrees preservation of avascular osteocartilaginous shell allografts in rats. *Clin Orthop Relat Res.* 1987;218:268-75.
41. Sirlin CB, Brossmann J, Boutin RD, Pathria MN, Convery FR, Bugbee W, Deutsch R, Lebeck LK, Resnick D. Shell osteochondral allografts of the knee: comparison of MR imaging findings and immunologic responses. *Radiology.* 2001;219:35-43.
42. Friedlaender GE, Strong DM, Tomford WW, Mankin HJ. Long-term follow-up of patients with osteochondral allografts. A correlation between immunologic responses and clinical outcome. *Orthop Clin North Am.* 1999;30:583-8.
43. Guidance for industry: screening and testing of donors of human tissue intended for transplantation. U.S. Department of Health and Human Services, Food and Drug Administration Center for Biologics Evaluation and Research; July 1997. www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM188251.pdf. Accessed 2009 May 14.
44. Ball ST, Amiel D, Williams SK, Tontz W, Chen AC, Sah RL, Bugbee WD. The effects of storage on fresh human osteochondral allografts. *Clin Orthop Relat Res.* 2004;418:246-52.
45. Csöngé L, Bravo D, Newman-Gage H, Rigley T, Conrad EU, Bakay A, Strong DM, Pellet S. Banking of osteochondral allografts, Part II. Preservation of chondrocyte viability during long-term storage. *Cell Tissue Bank.* 2002;3:161-8.
46. Dontchos BN, Coyle CH, Izzo NJ, Didiano DM, Karpie JC, Logar A, Chu CR. Optimizing CO2 normalizes pH and enhances chondrocyte viability during cold storage. *J Orthop Res.* 2008;26:643-50.
47. Pearsall AW 4th, Tucker JA, Hester RB, Heitman RJ. Chondrocyte viability in refrigerated osteochondral allografts used for transplantation within the knee. *Am J Sports Med.* 2004;32:125-31.
48. Pennock AT, Wagner F, Robertson CM, Harwood FL, Bugbee WD, Amiel D. Prolonged storage of osteochondral allografts: does the addition of fetal bovine serum improve chondrocyte viability? *J Knee Surg.* 2006;19:265-72.
49. Teng MS, Yuen AS, Kim HT. Enhancing osteochondral allograft viability: effects of storage media composition. *Clin Orthop Relat Res.* 2008;466:1804-9.
50. Williams JM, Virdi AS, Pylawka TK, Edwards RB 3rd, Markel MD, Cole BJ. Prolonged-fresh preservation of intact whole canine femoral condyles for the potential use as osteochondral allografts. *J Orthop Res.* 2005;23: 831-7.
51. Williams RJ 3rd, Dreese JC, Chen CT. Chondrocyte survival and material properties of hypothermically stored cartilage: an evaluation of tissue used for osteochondral allograft transplantation. *Am J Sports Med.* 2004;32:132-9.
52. Williams SK, Amiel D, Ball ST, Allen RT, Wong VW, Chen AC, Sah RL, Bugbee WD. Prolonged storage effects on the articular cartilage of fresh human osteochondral allografts. *J Bone Joint Surg Am.* 2003;85:2111-20.
53. Williams RJ 3rd, Ranawat AS, Potter HG, Carter T, Warren RF. Fresh stored allografts for the treatment of osteochondral defects of the knee. *J Bone Joint Surg Am.* 2007;89:718-26.
54. McCulloch PC, Kang RW, Sobhy MH, Hayden JK, Cole BJ. Prospective evaluation of prolonged fresh osteochondral allograft transplantation of the femoral condyle: minimum 2-year follow-up. *Am J Sports Med.* 2007;35:411-20.
55. Davidson PA, Rivenburgh DW, Dawson PE, Rozin R. Clinical, histologic, and radiographic outcomes of distal femoral resurfacing with hypothermically stored osteoarticular allografts. *Am J Sports Med.* 2007;35:1082-90.
56. LaPrade RF, Botker J, Herzog M, Agel J. Refrigerated osteoarticular allografts to treat articular cartilage defects of the femoral condyles. A prospective outcomes study. *J Bone Joint Surg Am.* 2009;91:805-11.
57. Chan EF, Liu E, Semler E, Aberman H, Simon T, Truncala K, Chen AC, Sah RL. Association of 3-dimensional cartilage and bone structure with articular cartilage properties in and adjacent to autologous osteochondral grafts after 6 and 12 months in a goat model. *Cartilage.* 2011; in press.