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Why Do Osteochondral Allografts Survive?: Comparative Analysis of Cartilage Biochemical Properties Unveils a Molecular Basis for Durability

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Abstract

Background—Transplantation of osteochondral allografts (OCAs) freshly preserved for 30 days has proven to be a reliable technique for cartilage resurfacing. However, the prolonged storage of allografts comes at the expense of chondrocyte viability, which declines precipitously after 14 days under refrigeration. Despite this, radiographic data indicate that most allograft cartilage remains stable for years after implantation. The apparent durability of partially devitalized cartilage begs the question of how the extracellular matrix is maintained.

Hypothesis—Compared with patients' defect cartilage, replacement OCAs freshly preserved for 36 days on average contain significantly lower levels of cartilage matrix-destructive metalloproteinases, which may contribute to the long-term stability of implanted grafts.

Study Design—Descriptive laboratory study.

Methods—Chondrocyte density was determined by the cell yield from digested cartilage and by double-strand DNA content quantified with PicoGreen assay. Chondrocyte viability was estimated by staining enzymatically isolated chondrocytes with calcein AM and ethidium homodimer-2. Cartilage proteoglycan (PG) content was analyzed with dimethylmethylene blue assay. The in vitro 48-hour release of PG-depleting metalloproteinases including matrix metalloproteinase (MMP)-1, -3, -13, and ADAMTS-5 from cartilage was examined with Western blotting. The data were compared between diseased cartilage from patients and samples from matched grafts. The relative amount of MMP-3 to its endogenous inhibitor, tissue inhibitor of MMP-1 (TIMP-1), was also determined with Western blotting.

Results—Chondrocyte density decreased linearly with allograft storage time and declined by an average of 43%. PG content decreased while the percentage of nonviable chondrocytes increased

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with storage time, with the former showing less linearity. However, PG content remained in the normal range and was significantly higher than that in patients' defect cartilage. Correspondingly, significantly less PG-depleting metalloproteinases and a much lower MMP-3/TIMP-1 ratio were detected in allograft cartilage than in patients' diseased cartilage.

Conclusion—These findings indicated that, at the time of implantation, fresh-preserved OCAs contained significantly lower levels of PG-depleting metalloproteinases compared with patients' defect cartilage, which might contribute to their long-term stability in vivo.

Clinical Relevance—The comparatively low expression of cartilage-dissolving metalloproteinases in human OCAs freshly preserved over 30 days offers support to the long-term durability of implanted grafts. Based on study data that showed similarity in the response to inflammatory cytokines between patients' cartilage and OCA cartilage, strategies that can alleviate inflammation may provide extra benefit for the survival of implanted grafts. In terms of the practice of graft preservation, agents that can keep balance between the ATP supply and demand or stabilize the cell membrane or inhibit the activation of metalloproteinases may significantly improve cell viability in fresh-preserved OCAs with a storage time longer than 5 weeks.

Keywords

osteochondral allografts; cartilage; matrix-dissolving metalloproteinases; proteoglycan; chondrocyte; cartilage repair

Sports- and activity-related knee injuries are common in the young and active population. Arthroscopic studies revealed that over 60% of these patients had chondral defects, which may increase the risk of the early onset of osteoarthritis in the injured knee.^{1,3,4,6,9,15,27} In terms of repairing chondral defects larger than 2 cm², osteochondral allograft (OCA) transplantation (OAT) as a single- stage technique has proven to be highly effective in restoring function in traumatized knee joints in young patients.²¹ Currently, fresh-preserved donor OCAs are commercially available through tissue banks, but the tissue must be maintained at 4°C until the completion of a series of microbiological and immunological tests.²⁵

Because of this screening process, which takes at least 14 days, the OCA storage time by the time of implantation usually ranges from 25 to 45 days, although recent studies suggested that the optimal storage time for OCAs was less than 28 days from procurement because of a significant decline of cellularity in fresh-preserved OCAs between days 14 and 28 in storage.^{13,30}

However, in many clinical studies, despite the time to implantation being greater than the suggested 28 days, the success of OCAs was still very favorable. Several follow-up studies reported that OCAs refrigerated over 24 days effectively repaired chondral or osteochondral defects located in femoral condyles in young patients.^{5,17,29} McCulloch and colleagues¹⁷ evaluated the outcomes of OAT in 25 patients (mean age, 35 years; range, 17-49 years), 35 months on average after surgery, by using objective and radiographic assessments and several subjective scoring systems. They discovered that 88% of the grafts freshly preserved for 24 days on average before implantation were successfully integrated with native tissue, and in 79% of patients, knee function was restored.¹⁷ In another study, conducted by

Williams et al²⁹ in 19 patients (mean age, 34 years), 25 months after OAT, 95% of implanted grafts with a mean storage time of 30 days at 4°C showed cartilage thickness and signal properties comparable with normal tissue when examined with magnetic resonance imaging. Furthermore, significant improvements in validated outcome instruments, including the Activities of Daily Living Scale/Function subscale of the Knee injury and Osteoarthritis Outcome Score (KOOS) and the Short Form-36, were observed 48 months after OAT. Similar observations were made by Davidson and colleagues⁵ in their 40-month follow-up study on 67 patients whose distal femur was implanted with OCAs that were hypothermically stored for 36 days.

Therefore, the question arises: why do osteochondral grafts survive clinically despite reduced cellularity due to long-term preservation? Because the long-term durability of implanted OCAs heavily relies on the integrity of the extracellular matrix (ECM) of OCA cartilage, the only viable part of the graft,²² we questioned if there is possibly a molecular basis explaining why refrigerated OCAs function as a durable cartilage-repairing material. Structurally, articular cartilage is composed of the ECM and chondrocytes, which are responsible for maintaining ECM homeostasis by balancing the production of matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of MMP (TIMPs).²⁴ Numerous studies have demonstrated that the overwhelming expression of MMP-3/stromelysin-1, MMP-13/collagenase-3, MMP-1/ collagenase-1, and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)-5/aggreacanase-2 over TIMP-1 forms a major chemical force in driving the breakdown of cartilage through dissolving proteoglycan (PG) and type II collagen fibers, 2 major protein ingredients in the ECM to form the backbone structure of articular cartilage.^{7,16,31} Proinflammatory cytokines, typically interleukin (IL)-1 β and tumor necrosis factor (TNF)- α , and ECM degradation products represented by fibronectin fragments have been proven to cause such a broken balance between MMPs and TIMPs in favor of MMPs.^{10,11}

To better understand how prolonged hypothermic conditions affect cartilage biochemical properties and to find evidence supporting fresh-preserved OCAs as durable replacements for cartilage lesions caused by knee trauma, we aimed to determine (1) the correlation between OCA storage time (25 days) and PG content, chondrocyte density, and chondrocyte viability; (2) whether PG content in fresh-preserved OCA cartilage was significantly higher than patients' pathological cartilage removed during OAT; and (3) whether the levels of PG-depleting metalloproteinases in fresh-preserved OCA cartilage were significantly lower than those in patients' pathological cartilage removed during OAT.

Methods

Acquisition of Patients' Pathological Cartilage and OCA Samples

Eight patients (mean [$\pm$ SD] age, 23.6 $\pm$ 7.3 years; median, 22 years) who sustained knee injuries with cartilage defects larger than 2.0 cm² were treated with OAT between September 2013 and October 2014 (the OAT technique is illustrated in Figure 1). Cartilage lesions, 5 in medial femoral hemicondyles and 3 in lateral femoral hemicondyles, were replaced with fresh-preserved OCAs (RTI Biologics). Those OCAs were stored at 4°C in nutrient media until the time of implantation. The mean ($\pm$ SD) storage time was 36.4 $\pm$ 6.1 days (Table 1).

During each surgery, full-thickness cartilage slices from lesions (mean [$\pm$ SD] wet weight, 1.3 ± 0.7 g) and from intercondylar notches (mean [$\pm$ SD] wet weight, 0.2 ± 0.1 g), minor loaded areas, were biopsied with institutional review board approval. Immediately after surgery, these cartilage slices along with the remaining OCAs were transported in sterile normal saline to the laboratory for analysis.

Process and In Vitro Culturing of Cartilage Slices

Upon acquisition, full-thickness cartilage was shaved aseptically from the remaining OCAs, which yielded a mean ($\pm$ SD) of 4.3 ± 1.3 g wet cartilage per OCA. Around 70% of OCA cartilage slices were allocated for chondrocyte isolation, and the remaining 30%, along with patient samples, were dispensed into a 24-well plate to achieve approximately 0.15 g wet cartilage per well. As a negative control, some cartilage was freeze-thawed 3 times to deliberately kill all chondrocytes.¹³ All slices were cultured in Dulbecco's Modified Eagle's medium (DMEM)/F12 supplemented with 50 U/mL penicillin, 50 mg/L streptomycin, and 2.5 mg/L amphotericin B inside a humidified 37°C incubator supplied with 5% CO₂ and 5% O₂ for 24 hours to reach equilibrium.

Cytokine Treatment and Sample Harvest

Immediately after pre-equilibration, culture media were collected and labeled as “Pre-” (pre-cytokine stimulation) for the examination of the baseline release of metalloproteinases with Western blotting. Cartilage slices were either left untreated (untreated control [UC]) or subjected to the treatment of inflammatory cytokines composed of 10 ng rhIL-1 β and 100 ng/mL rhTNF- α (R&D Systems). After another 24 hours, culture media were collected again and labeled as “Post-” (post-cytokine stimulation) for the examination of metalloproteinase release. Cartilage slices were collected for the quantitation of contents of PG and dsDNA. The experimental protocol is illustrated in Appendix Figure 1A (available online at <http://ajsm.sagepub.com/supplemental>).

Quantitation of Contents of PG and dsDNA in Cartilage

Saved cartilage slices were first weighed and then digested in 0.5 mg/mL papain digestion buffer containing 5.0 mM L-cysteine for 3 hours at 65°C. Papain digests were analyzed for PG content with dimethylmethylene blue assay, and the values were normalized to the wet weight of each cartilage slice. dsDNA content was quantitated with a Quan-iT PicoGreen dsDNA Quantitation kit (Invitrogen), and the values were adjusted to the wet weight of each cartilage slice. In each case, except case 4, contents of PG and dsDNA in patients' pathological cartilage were averaged either from 4 slices in cases 1, 5, and 6 (1 slice each in UC fresh or freeze-thawed group, 1 slice each in cytokine-treated fresh or freeze-thawed group) or from 6 slices in cases 2, 3, and 7 (for cases 2 and 3: 2 slices each in UC fresh or cytokine-treated group, 1 slice each in UC or cytokine-treated freeze-thawed group; for case 7: 3 slices each in UC or cytokine-treated fresh group). In each case, contents of PG and dsDNA in OCA cartilage were averaged from 6 slices (for cases 1-3 and 5 and 6: 2 slices each in UC fresh or cytokine-treated group, 1 slice each in UC or cytokine-treated freeze-thawed group).

Determination of Release of Metalloproteinases or TIMP-1 From Cartilage

To examine the daily release of metalloproteinases or TIMP-1 from cultured cartilage slices over a 48-hour period, medium samples were first dialyzed against deionized water for 48 to 72 hours and then concentrated with a speed vacuum. Each concentrated sample was denatured with an equal volume of 2X sample buffer and was reduced with 0.05 M DTT. Loading was normalized to 3.8 mg wet cartilage, and the samples were resolved by a 10% SDS-acrylamide/bis gel. Proteins were blotted onto nitrocellulose membranes, followed by incubation with 1% BSA/ TBST containing anti-MMP-1 antibody, anti-MMP-13 antibody (Abcam), or anti-ADAMTS-5 antibody (Novus Biologicals). Some blots were split into 2 halves using the 38-kDa protein marker: the top half incubated with anti-MMP-3 antibody (Abcam) and the bottom one with anti-TIMP-1 antibody (Novus Biologicals). An HRP-conjugated anti-rabbit IgG antibody (Sigma-Aldrich) was employed to retrieve the signals of MMPs, ADAMTS-5, or TIMP-1.

Assessment of Chondrocyte Yield and Viability in OCA Cartilage

OCA cartilage slices were first weighed and then minced with a sterile size 21 surgical blade. Minced cartilage was digested in serum-free DMEM containing 0.4% protease (Sigma-Aldrich) for 1.5 hours at 37°C and subsequently incubated with 0.02% collagenase (Sigma-Aldrich) for 16 hours. Cell suspension was stained with 1 µg/mL calcein AM and 1 µM ethidium homodimer-2 (Life Technologies) for 5 to 10 minutes at 37°C before being examined on a hemocytometer with an Olympus BX60 fluorescence microscope. Total cell counts, including red and green cells, were averaged from 5 squares and adjusted to wet weight cartilage to obtain the chondrocyte yield from OCA cartilage of each case. Chondrocyte viability was calculated as the percentage of green cells of total cell counts.

Statistical Analysis

The mean ($\pm$ SD) contents of PG and dsDNA were calculated from the data derived from 7 cases. The difference of data sets between patient and OCA cartilage samples was determined with the Student *t* test. To quantify the expression of MMP-3, ADAMTS-5, MMP-1 and -13, and TIMP-1, the mean brightness of each pixel and the number of pixels of each band in an inverted blot were measured with Adobe Photoshop CC. Next, the absolute intensity (AI) of each band was computed by multiplying the mean brightness by the number of pixels. In each case, the relative cumulative release of each examined protein over a 48-hour period was calculated by dividing the summed AI of 2 bands from patient samples by that from corresponding OCA ones. The response to cytokines manifested by MMP-3 upregulation was calculated as a ratio of the AI of the posttreatment band to pretreatment band. The mean ($\pm$ SD) of the ratios from 6 patient and OCA samples was compared with the Student *t* test. Significance was set at the .05 level.

Results

Chondrocyte yield and dsDNA content declined with OCA storage time. The chondrocyte yield dropped by 4.9% per day in a linear fashion ($R^2 = 0.8$), while dsDNA content decreased by 4.7% per day with slightly less linearity ($R^2 = 0.6$) (Figure 2A). Furthermore,

the percentage of dead cells in yielded chondrocytes increased with storage time ($R^2 = 0.6$), while PG content decreased in a less linear fashion ($R^2 = 0.2$) (Figure 2B).

Although PG content in OCA cartilage declined with storage time, it was still remarkably higher than that in patients' diseased cartilage at the time of implantation. Except for case 3, the OCAs contained significantly higher PG than did the patients' diseased cartilage (Figure 3A). Of all 7 cases, OCAs contained a mean ($\pm$ SD) of $48.5 \pm 9.8 \mu\text{g PG/mg wet cartilage}$, which was significantly higher than that of patients' diseased cartilage ($24.7 \pm 16.8 \mu\text{g PG/mg wet cartilage}$) ($P = .007$) (Figure 3B). By contrast, the mean ($\pm$ SD) chondrocyte density, reflected by dsDNA content, was lower in OCAs ($57.2 \pm 25.5 \text{ ng/mg wet cartilage}$) than in patients' cartilage ($107.5 \pm 88.3 \text{ ng/mg wet cartilage}$). However, the difference was not statistically different ($P = .173$) (Figure 3C). Over a 48-hour period, untreated patients' defect cartilage sampled from the lesions released 10.4-fold (95% CI, ± 10.1) more MMP-3 than did OCA cartilage (Figure 4, A and B). Moreover, cartilage sampled from the intercondylar notch of patients' injured knees in 3 of 5 examined cases responded more strongly to inflammatory cytokines by upregulating MMP-3 expression than did cartilage either from the lesions or the matched OCAs. Overall, cytokines upregulated MMP-3 expression in patients' notch cartilage by 3.3-fold (SD, ± 1.2), in OCA cartilage by 2.8-fold (SD, ± 2.7), and in patients' lesion cartilage by 2.0-fold (SD, ± 0.6). However, this difference in the response to cytokines was not statistically significant (patients' notch vs OCA, $P = .767$; patients' notch vs lesion, $P = .076$) (Figure 4, A and C).

A side-by-side and case-by-case comparison of the expression of ADAMTS-5, a metalloproteinase specifically dissolving the protein component of PG, was also made between patients' and OCA cartilage. When examined with Western blotting, there were 4 major bands (102, 76, 60, and 52 kDa) observed. Consistent with the MMP-3 results, those bands were in higher intensities on the side of the blot loaded with samples from patients' cartilage than from OCA cartilage (Figure 5, lanes 1-8 vs 9-14). Over a 48-hour period, the total release of ADAMTS-5 from untreated patients' diseased cartilage was a mean ($\pm$ SEM) 152.2 ± 85.2 -fold over that from an equal mass of OCA cartilage. In the group treated with cytokines, the mean ($\pm$ SEM) fold increase was 60.1 ± 27.2 for fresh slices and 73.2 ± 30.4 for freeze-thawed slices. Moreover, cytokines evoked a mean ($\pm$ SEM) 14.3 ± 6.0 -fold more release of ADAMTS-5 from cartilage sampled from the intercondylar notch of patients' injured knees than from OCA cartilage (Table 2). Interestingly, in 3 of 5 cases, more ADAMTS-5 release was observed in freeze-thawed patients' lesion cartilage than in unprocessed fresh tissue (Table 2, cases 1, 3, and 5). However, unlike the effect on MMP-3 expression, inflammatory cytokines did not upregulate ADAMTS-5 release from either patients' or OCA cartilage (Figure 5 and Table 2). Because collagenases deplete PG in cartilage via digesting the type II collagen network that normally holds PG in, a comparison of the expression of 2 main collagenases was also made between patients' and OCA cartilage. Similar to MMP-3 or ADAMTS-5, the expression of MMP-13 and -1, which are 2 main collagenases involved in cartilage degeneration, was much higher in patients' lesion or notch cartilage than in OCA cartilage. In patients' cartilage, the expression of both pro-MMP-13 and active MMP-13 was upregulated upon cytokine stimulation. However, only the expression of pro-MMP-13 (65 kDa) was elevated in OCA cartilage by the same cytokine treatment (Figure 6A, lanes 4 and 8 vs lane 12). When compared case by case, over a 48-

hour period, the fold increase of total MMP-13 release from untreated patients' lesion cartilage relative to that from corresponding OCA cartilage ranged from 3.0 to infinity. In the group treated with cytokines, the fold increase ranged from 1.4 to 1340.8 for unprocessed fresh cartilage and ranged from 2.8 to 29.3 for freeze- thawed cartilage. Furthermore, the release of MMP-13 from the intercondylar notch of patients' injured knees was also much greater than that from OCA cartilage, with a fold increase ranging from 2.5 to 26.0 upon the stimulation of cytokines (Table 3). Consistent with MMP-13 results, the expression of another collagenase, MMP-1, was evidently higher in patients' cartilage than in OCA cartilage. When examined with Western blotting, 2 forms of MMP-1, the dimer (102 kDa) and the monomer (54 kDa), were detected in 5 of 6 patients' cartilage, while only the MMP-1 dimer was detected in OCAs. The total release of MMP-1 over a 48-hour period from untreated patients' lesion or notch cartilage was a mean ($\pm$ SEM) 5.5 ± 1.6 -fold and 2.9-fold over that from OCA cartilage, respectively. In the group treated with cytokines, the mean ($\pm$ SEM) relative fold increase changed to 5.0 ± 1.8 and 5.2 ± 1.9 , respectively (Figure 6, B and C).

Discussion

By using comparative analysis, we potentially revealed a molecular basis for the apparent stability of OCAs freshly preserved for 36 days on average. Our data indicated that, at the time of implantation, fresh-preserved OCAs contained significantly higher PG content than did patients' pathological cartilage. This was consistent with the observation that a significantly lower expression of PG- depleting metalloproteinases, including MMP-1, -3, -13, and ADAMTS-5, was detected in OCA cartilage than in patients' diseased cartilage. Furthermore, our data showed a much lower MMP-3 to TIMP-1 ratio in OCA cartilage than in patients' cartilage, indicating a more favorable MMP-3/TIMP-1 balance existing in OCA cartilage than in patients' cartilage. TIMP-1 binds to MMP-3 in 1:1 stoichiometry to inhibit the proteolytic activity of MMP-3.⁸ The higher an MMP-3 to TIMP-1 ratio is in the cartilage matrix, the more degenerative the tissue will be. For the 4 cases examined in our study, the mean ($\pm$ SD) MMP-3 to TIMP-1 ratio in OCA cartilage was 121.1 ± 86.5 , which was 65% lower than that in patients' lesion cartilage or 75% lower than that in patients' notch cartilage (see Appendix Figure A2, parts A and B, available online). This result provided another layer of supporting evidence for the higher PG content observed in OCA cartilage than in patients' diseased cartilage.

To our knowledge, we are also the first to report the linear correlation between chondrocyte density in fresh- preserved human OCAs and storage time. Hypothermic storage conditions of fresh preservation gradually cause a mismatch between the ATP supply and demand in chondrocytes, which eventually results in necrotic cell death.²³ According to the work by Williams et al,³⁰ chondrocyte viability was well maintained above 97% when human OCAs were stored at 4°C for up to 14 days but fell to merely 70% at 28 days in storage. In another study conducted by Pearsall and colleagues,¹⁹ the mean chondrocyte viability was 67% in 9 human OCAs freshly preserved for 34 days on average. However, neither study dissected the dynamic relationship between OCA storage time and chondrocyte density, an index similarly important to cell viability in terms of the survival of implanted grafts. Our study not only showed comparable cell viability results with those of Pearsall et al¹⁹ (mean storage time, 36

days; mean viability, 71%) but also revealed a linear and inverse correlation between chondrocyte viability and storage time. More importantly, we employed 2 different approaches to examine the effect of the prolonged preservation of OCAs on chondrocyte density and obtained highly consistent results suggesting a linear decline of chondrocyte density with storage time. This linear relationship was also demonstrated in fresh-preserved sheep cartilage.²⁸

The rising level of chondrocyte death in fresh-preserved OCAs might lead to the upregulation of matrix-degrading proteases, including metalloproteinases, which leaked from ruptured lysosomes of necrotic chondrocytes.² With Western blotting, we could detect 4 major cartilage-dissolving metalloproteinases released from 3.8 mg wet OCA cartilage. However, compared with patients' diseased cartilage, OCA cartilage released significantly lower amounts of those degrading metalloproteinases, especially MMP-3, which was 10.4-fold lower. This remarkable difference in metalloproteinase levels between OCA cartilage and patients' diseased cartilage may be caused by any or all of the following: (1) intrinsically low expression of cartilage-damaging metalloproteinases in OCAs from young and healthy donors^{12,14} and (2) upregulated expression of metalloproteinases (MMP-3 in particular) in traumatized knee cartilage.²⁶

Because of those relatively low levels of cartilage-degrading metalloproteinases in OCAs freshly preserved for 36 days on average, PG content in those grafts was well preserved in the normal range (25-62 µg PG/mg wet cartilage)²⁰ and was significantly higher than that in patients' pathological cartilage replaced at the time of implantation. Although our data showed that the chondrocyte density in those fresh-preserved OCAs reflected by dsDNA content declined to only 57% of the normal value (~100 ng dsDNA/mg wet cartilage),¹⁸ this density was not statistically different from that in patients' diseased cartilage, suggesting that a similar number of chondrocytes in OCA cartilage maintained a significantly higher PG level than did patients' diseased cartilage.

On the basis of our comparative analysis of cartilage biochemical properties, we speculate that the replacement of metalloproteinase-enriched defect cartilage with metalloproteinase-scarce fresh-preserved OCA cartilage provides a relatively "clean" environment that may attenuate the vicious cycle composed of metalloproteinases, matrix fragments, and inflammatory cytokines and in turn protect the remaining native cartilage and the graft from being dissolved away. This may form a molecular basis for the long-term durability of OCAs that have been freshly preserved for a significant period of time (see Appendix Figure A1, part B).

Nonetheless, among 6 cases examined, OCA cartilage of case 7 was the only one showing a similar expression level of MMP-3 to that observed in patients' diseased cartilage. To clarify whether this relatively high MMP-3 expression in OCA cartilage affects allograft stability, future studies will focus on the correlation of MMP expression in OCA cartilage at the time of implantation with the clinical outcomes of implanted OCAs. Data collected from our study reported here and in the future will guide us in improving the management of knees implanted with OCAs and in improving the preservation of OCAs.

Overall, we think that the take-home message derived from our study is that, based on our data, which showed similarity in the response to inflammatory cytokines between patients' cartilage and OCA cartilage, strategies that can alleviate inflammation may provide an extra benefit for the survival of implanted grafts. In terms of the practice of graft preservation, agents that can keep balance between the ATP supply and demand or stabilize the cell membrane or inhibit the activation of metalloproteinases may significantly improve cell viability in fresh-preserved OCAs with a storage time longer than 5 weeks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Illustration of the osteochondral allograft transplantation technique.

Figure 2.

(A) The effect of OCA storage time on chondrocyte density. In each case, chondrocyte density was determined by the number of chondrocytes isolated from 1.0 mg of wet OCA cartilage and the mass of dsDNA per milligram of wet OCA cartilage. For the latter, the values were averaged from 4 or 6 slices of OCA cartilage. Bars represent the SD. (B) The effect of OCA storage time on chondrocyte mortality and PG content. In each case, chondrocyte mortality was computed as the percentage of ethidium homodimer-2-positive

(dead) cells in total isolated chondrocytes from 1.0 mg of wet OCA cartilage. PG content was averaged from 4 or 6 slices of OCA cartilage. Bars represent the SD.

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Figure 3.

(A) Difference in PG content between patients' diseased cartilage removed from lesions and matched donor OCA cartilage in each case. Except for case 4, after 48-hour in vitro culturing, patients' lesion cartilage (4 or 6 slices in each case) or OCA cartilage (6 slices per case) was first subjected to papain digestion and then analyzed for PG content with dimethylmethylene blue assay. For each case, the mean PG content is plotted. Error bars represent the SD. For case 4, the in vitro culturing step was skipped. Patients' diseased cartilage or OCA cartilage (1 slice each) was directly analyzed for PG content. The overall differences in contents of (B) PG and (C) dsDNA between patients' diseased cartilage from lesions and OCA cartilage. To obtain the mean level of PG or dsDNA in each type of cartilage samples of all 7 cases, the mean content of PG or dsDNA of each case (case 4 not included) was first computed from analyzing 4 or 6 slices of each type of cartilage samples. Next, the mean content of PG or dsDNA of each case (cases 1-3 and 5-7) and the corresponding value from case 4 were further averaged to yield the final mean level of PG or dsDNA in patients' diseased cartilage or OCA cartilage. Error bars represent the SD.

Figure 4.

Case-by-case comparison of MMP-3 temporal release between patient and OCA cartilage samples. (A) The levels of MMP-3 in culture media released from the equal weight of patient or OCA cartilage samples were determined with Western blotting. BM, blank media; fresh, unprocessed cartilage as opposed to freeze-thawed cartilage; F-T, freeze-thawed or negative control cartilage; Pre, pre-cytokine stimulation or first 24-hour in vitro culturing; Po, post-cytokine stimulation or second 24-hour in vitro culturing; UC, untreated control. (B) The fold increase of the cumulative release of MMP-3 over a 48-hour period from untreated patients' diseased cartilage samples over that from OCA cartilage samples. (C)

The fold increase of MMP-3 release stimulated by cytokine treatment. Bars represent the 95% CI.

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Figure 5.

Comparison of the temporal release of ADAMTS-5 between patient and OCA cartilage samples. A representative Western blot showing the differential release of ADAMTS-5 between patient and OCA cartilage samples in case 6.

Figure 6.

Comparison of the temporal release of collagenases between patient and OCA cartilage samples. (A) A representative Western blot showing the differential release of MMP-13 between patient and OCA cartilage samples in case 2. (B) A representative Western blot showing the differential release of MMP-1 between patient and OCA cartilage samples in case 5. (C) The fold increases of MMP-1 release from patients' cartilage relative to OCA cartilage in the untreated control group and cytokine-treated group, respectively. The intensities of MMP-1 bands in Western blots of 6 cases (cases 1-3 and 5-7) were measured, compared, and plotted. Error bars represent the SEM.

Table 1

Summary of case details essential to the study.

Case No.	Patient (OCA Recipient)			OCA Donor		
	Age (Yrs)	Location of Injury	Time Span between Injury and OAT (Yrs)	Age (Yrs)	OCA Origin	Storage Time at 4°C (Days)
1	18	Medial femoral hemicondyle	2.2	28	Medial femoral hemicondyle	44
2	19	Lateral femoral hemicondyle	2.0	20	Lateral femoral hemicondyle	40
3	25	Lateral femoral hemicondyle	3.7	37	Lateral femoral hemicondyle	42
4	33	Medial femoral hemicondyle	0.3	22	Medial femoral hemicondyle	32
5	14	Lateral femoral hemicondyle	1.0	45	Lateral femoral hemicondyle	36
6	19	Medial femoral hemicondyle	0.2	19	Medial femoral hemicondyle	38
7	27	Medial femoral hemicondyle	0.3	42	Medial femoral hemicondyle	25
8	34	Medial femoral hemicondyle	2.0	23	Medial femoral hemicondyle	34
Mean±S.D.	23.6±7.3		1.5±1.2	29.5±10.4		36.4±6.1

Table 2

Expression of ADAMTS-5 in patient cartilage relative to OCA cartilage.

Case No. & Stats.	Pt. Lesion vs. OCA		Pt. Notch vs. OCA	
	Fresh		F-T	
	U.C.	(+) Cyto.	(+) Cyto.	(+) Cyto.
Case No.1	29.6	51.7	98.2	N/A
Case No.2	2.8	8.5	8.0	N/A
Case No.3	18.0	18.4	176.4	N/A
Case No.5	52.9	57.6	64.1	N/A
Case No.6	516.2	190.7	19.3	N/A
Case No.7	293.5	33.6	N/A	1.1
Mean	152.2	60.1	73.2	14.3
SD	208.6	66.7	68.1	N/A
SEM	85.2	27.2	30.4	6.0

Table 3

Expression of MMP-13 in patient cartilage relative to OCA cartilage.

Case No.	Pt. Lesion vs. OCA			Pt. Notch vs. OCA		
	Fresh		F-T	Fresh		F-T
	U.C.	(+) Cyto.	(+) Cyto.	U.C.	(+) Cyto.	(+) Cyto.
Case No.1	∞	1340.8	29.3	N/A	N/A	N/A
Case No.2	3.0	8.2	3.8	N/A	N/A	26.0
Case No.3	15.7	1.4	4.7	N/A	N/A	5.2
Case No.5	32.0	27.8	15.8	N/A	N/A	6.5
Case No.6	∞	5.5	2.8	N/A	N/A	2.9
Case No.7	713.9	3.5	N/A	1.0	1.0	2.5