Enhancement of the current osteochondral allograft transplantation: Importance of graft specific characteristics and improvement of graft storage condition

PhD thesis book

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1. Introduction

Cartilage lesions are common sequelae of knee injury and pose a significant health burden to patients limiting sports practicing and routine activities. Besides affecting patients' quality of life, cartilage injuries are highly associated with the development of osteoarthritis (OA), a potentially irreversible outcome.[1,2] Cartilage has a very limited healing potential due to its and once articular cartilage is damaged, full recovery of its structure, function, and biomechanical properties is unlikely and is usually a step toward progression to OA. [3-6] Consequently, the restoration of a symptomatic cartilage lesion is essential to avoid or slow down the OA progression.[3,7-10] To delay or avoid a prosthetic arthroplasty, multiple treatment options are available to restore the injured cartilage depending on patient and lesion characteristics including marrow stimulation technique (MST), autologous matrix-assisted chondrogenesis (AMIC), osteochondral autograft transplantation (OAT), particulated juvenile allograft cartilage (PJAC), aragonite-based osteochondral scaffold, autologous chondrocyte implantation (ACI), and osteochondral allograft transplantation (OCA).[3,7,11,12]

Although choosing the proper cartilage restoration procedure is influenced by numerous factors, MST, AMIC or OAT are preferred procedures for small chondral lesions. [13-15] In contrast, large full-thickness chondral and osteochondral defects are frequently treated with ACI and OCA resulting in high patient satisfaction rates and significantly improved functional outcomes. [13,16-21] (Figure 1.)

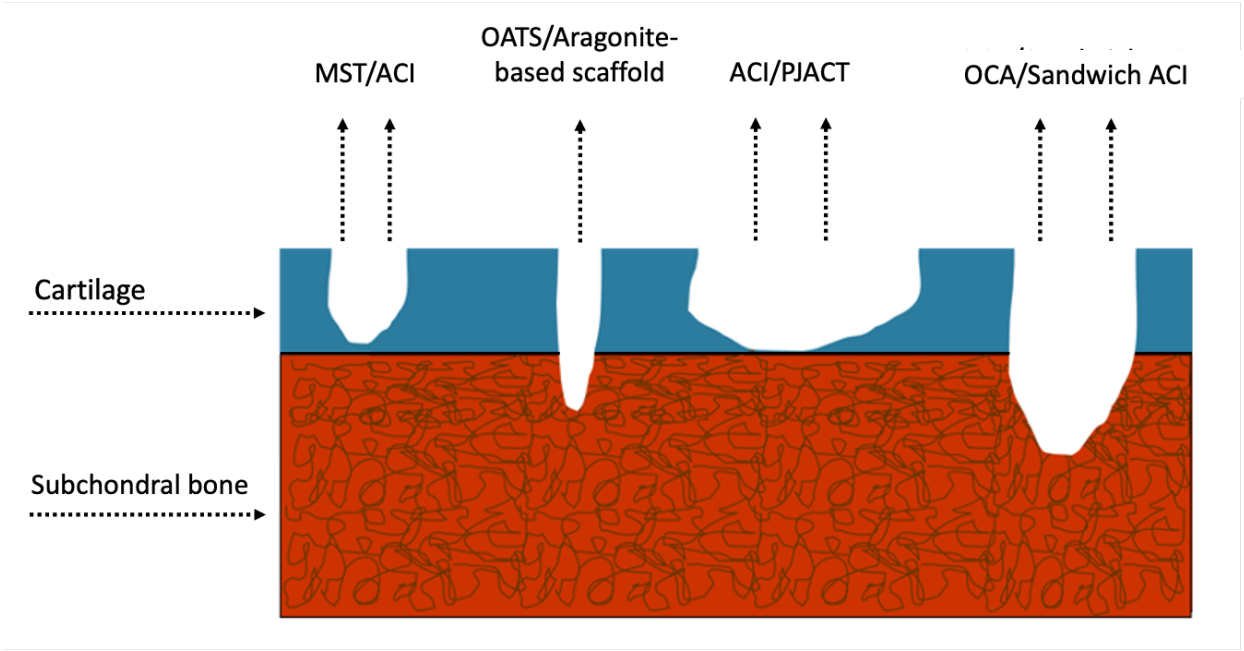

Figure 1. Cartilage repair options for different cartilage defects.[10]

OCA transplantation

OCA transplantation is a versatile single-step cartilage repair procedure that uses allograft tissue permitting the treatment of large osteochondral defects with a best-matched osteochondral explant. This procedure can restore both the damaged articular cartilage and the underlying subchondral bone and therefore enables surgeons to address the often-concomitant subchondral pathology in patients with chondral defects.[22-24]

Current indications for OCA transplantation include large focal chondral or osteochondral lesions, failure of previous cartilage repairs, osteochondritis dissecans, osteonecrosis and posttraumatic osteochondral lesions.[9,17,22-27] Even though OCA transplantation is indicated for a challenging population numerous studies have shown good clinical outcomes following OCA transplantation with success rates reported between 50-89% in 10 years.[20,23-25,28-30] With such broad reported rates of success however, numerous studies have sought to identify predictors

associated with improved graft survival following transplantation. [11,23,31-33] As our understanding of these predictors and principles develops, we have come to better appreciate how both patient and graft-related factors each contribute toward outcomes after OCA transplantation surgery.

Patient or recipient related factors and their effect on OCA survival has been more thoroughly studied and is therefore currently better understood. Factors including increased BMI and advanced age are patient-related risk factors known to affect OCA transplantation outcomes, and now represent valuable considerations when indicating patients for OCA transplantation.[20,34] Increasing insights into various patient characteristics, surgical techniques, as well as biological and immunological factors are being incorporated into our treatment algorithms for cartilage repair and OCA transplantation.[17, 18, 20, 27] Yet our understanding of graft-specific factors for failure remains limited, and has become of increased interest amongst researchers and clinicians seeking to optimize rates of success following OCA transplantation.[33,35-37]

The current OCA storage condition

Currently, OCAs are stored at 4°C for 14 days while being screened for pathogens before transplantation. Here in the United States, transplantation occurs, on average, 24 days after procurement (range 15–43 days).[38,39] This prolonged period of time in storage before transplantation compromises chondrocyte viability and graft quality. Chondrocyte viability is a major determinant of graft performance in-vivo.[40,41] Implantation of viable chondrocytes can assure maintenance of the ECM components, like sulfated glycosaminoglycans (S-GAG) and collagen, as well as biomechanical properties that ensure the integrity of the articular surface after transplantation.[40,41] Multiple studies report that chondrocyte viability falls below the generally acceptable level of 70% after 28 days of storage at 4°C.[40,42,43]

We know that chondrocyte viability gradually decreases with time ex-vivo in storage, however clinical studies evaluating the effects of storage time on patient outcomes after OCA transplantation remain controversial.[44] In a study by Nuelle et al.,[44] patients with grafts transplanted after storage time >28 days had a 2.6 times higher likelihood of unsuccessful outcome (define by persistent postoperative pain >0 and within 2 points of preoperative levels on visual analogue scale (VAS)). Conversely, Schmidt et al.[45] demonstrated no difference in postoperative clinical outcomes in a matched-pair analysis of patients who received early released grafts (mean storage, 6.3 days) versus patients who underwent OCA transplantation with late released grafts (mean storage time, 20.0 days). In another matched-control study, Rauck et al. found no association between OCA storage time and rates of postoperative graft delamination, with an average storage time of 30 days in the delamination group and 31 days in the intact control group. [46]

With the majority of OCAs transplanted within 15-28 days, these studies fail to evaluate the effects of OCA storage time on in-vivo graft performance within the working window of practice here in the United States leading a significant knowledge hiatus behind.[47]

Furthermore, another obstacle regarding OCA storage is that the 14 days window after release is often insufficient for size matching, scheduling surgery, and transporting tissues and therfore results in wasted grafts, long wait lists, and high product prices.[48] Consequently, there is an unmet need to extend the current storage condition and improve the quality of the OCA after storage. Recently, transition from 4˚C storage to 37˚C has been proposed by multiple investigators.[40,42,49,50] Under 37˚C storage improved chondrocyte viability was reported at 28 days, especially at the vulnerable surface zone. [42] However, chondrocyte viability at 37˚C still remained low throughout all zones of the graft at 28 days and therefore its use remained controversial.[40,42,43]

Articular cartilage is normally exposed to a hydrostatic loading environment in vivo and shows abundant intratissue water under weight-bearing conditions.[51,52] During everyday activities, chondrocytes within the joint cartilage experience cyclic hydrostatic pressure (HP) levels of 0.2 - 20 MPa.[53] In previous studies our group and others demonstrated that application of HP in culture medium promotes cell viability and anabolic turnover in chondrocyte constructs *in vitro*.[54-59] [54-57] Therefore, we believe that the 37˚C storage do need the addition of hydrostatic pressure to better maintain the quality of the graft resulting in even superior outcomes compared to the current 4˚C storage condition, which was one of the objectives of my thesis.

Donor recipient sex mismatch

Sex mismatch has often been regarded as a potential contributor toward adverse outcomes in solid organ transplantation outcomes. Indeed, sex mismatch has been negatively associated with lung, heart, pancreas, kidney and liver transplantation,[60-64]

There are numerous potential immunologic mechanisms by which donor-recipient sex mismatch might affect outcomes following transplantation; including minor histocompatibility antigen present on the Y chromosome, increased immunologic response during normal pregnancy in women, and differing hormonal composition between the sexes.[65]

While intact articular cartilage is believed to be an immunoprivileged tissue bone has antigenicity. Previous reports have shown that human leukocyte antigen (HLA) antibodies do in fact develop after OCA transplantation.[32,66,67] However, the potential role of donor-recipient sex mismatching in OCA transplantation has not yet been characterized.

2. Objectives:

The overarching purpose of this thesis is to enhanche the clinical outcomes following OCA transplantation by understanding the graft-specific predictors of OCA failure and by improving the current OCA storage.

Graft-specific predictors

1. Our study examines a narrowed focus on practical working window (storage time 15-27 days) here in the United States; seeking to distinguish differences in graft survival that may exist within this timeframe, and ultimately inform best practices in the context of current regulations here. We hypothesized that OCAs transplanted within 19-24 days would have lower failure rates at 5 years than those transplanted at 25-27 days.
2. To explore the potential impact of donor-recipient sex mismatching in OCA transplantation, this current study aims to evaluate the hypothesis that donor-recipient sex-mismatching in OCA transplantation in fact results in increased rates of OCA failure.

Improvement of the current OCA storage

3. In a basic science study Our goal was to understand if early and continuous conditioning of fresh osteochondral allografts using HP may preserve the level of chondrocyte viability in fresh OCAs. We hypothesized that OC explants stored under cyclic HP at 37˚C retain both higher chondrocyte viability and greater amounts of ECM compared to explants stored at 4˚C or 37˚C under atmospheric pressure (AP).

3. Methods

3.1.Graft specific predictors

3.1..1. *Patient Cohort*

With institutional review board approval, informed consent was obtained from all patients at the time of enrollment into the study database. Patients with a minimum two-year follow-up were included.

The indications for treatment of cartilage defects with OCA were one or more full-thickness, chondral or osteochondral defects of the knee with symptoms matching the site of defect location. Diagnosis of chondral or osteochondral defect as well as concomitant pathology, was confirmed via patient history, physical examination, radiographic, and MRI, before OCA transplantation, and the concomitant procedures were also considered during data analysis. Patient data was recorded preoperatively, and patients were followed prospectively for up to five years post-operatively.

Contraindications for OCA transplantation included inflammatory joint disease, unresolved or recent septic arthritis, metabolic or crystalline arthropathies, and established advanced osteoarthritis (Kellgren-Lawrence III-IV). Clinical notes were reviewed to determine patient and lesion specific factors. All grafts were commercially obtained from Joint Restoration Foundation (JRF) Ortho (Centennial, CO), who also directly provided OCA storage time and sex data.

3.1.1.1. OCA storage time

In this review of prospectively collected data we analyzed data from 132 patients who underwent OCA transplantation for symptomatic cartilage defects between February 2014 and December 2016 by Dr Andreas H. Gomoll. Based on our previous experience studying OCA

chondrocyte viability in the laboratory, grafts were stratified into 2 study arms based on their length time in storage before transplantation: an early transplant group (19-24 days storage) and a late transplant group (25-27 days storage). For all grafts in our study population, the shortest time in storage after we acquired the OCA graft was 19 days, while the longest time in storage was 27 days.

3.1.1.2. OCA sex mismatch

In this review of prospectively collected data we analyzed data from 202 patients who underwent OCA transplantation for symptomatic cartilage defects between November 2013 and November 2017 by Dr Andreas H. Gomoll.

3.1.2. Definition of graft failure

Allograft failure was defined as 1) subchondral collapse of the OCA as confirmed on postoperative magnetic resonance imaging or by direct second-look arthroscopy in the setting of persistent symptoms, 2) removal or revision of the primary OCA, or 3) conversion to any form of arthroplasty. All postoperative assessments were performed by the senior author.

3.2. Improvement of the current OCA storage

3.2..1. Preparation of bovine osteochondral (bOC) explants

We purchased bovine forelimb joints of 2- to 3-week-old calves from a local slaughterhouse (USDA certified). We aseptically harvested bOC explants as cylindrical osteochondral columns consisting of articular cartilage and subchondral bone (6 mm in diameter, 8 mm in length) from humeral heads using a set of standard instruments (Osteochondral Autograft Transplant System (OATS), Arthrex, Naples, FL). Ten explants were harvested from each humeral head. We thoroughly rinsed the explants in Dulbecco's phosphate buffered saline

(DPBS, Gibco®, Life Technology, Carlsbad, CA) and removed marrow elements by flushing with DPBS using a syringe attached to a 22-gauge needle. We measured cartilage thickness of each OC explant with a ruler (0.5 mm resolution) and allocated the explants to 1 of 2 groups: 2.5 and 3 mm. Subsequently the same number of bOC explants from each group were allocated into study groups, achieving similar average cartilage thickness in every condition. (Figure 2) Altogether we harvested 60 bOC explants from 6 bovine humeral heads: one fresh (0 days) control and nine OC explants for storage.

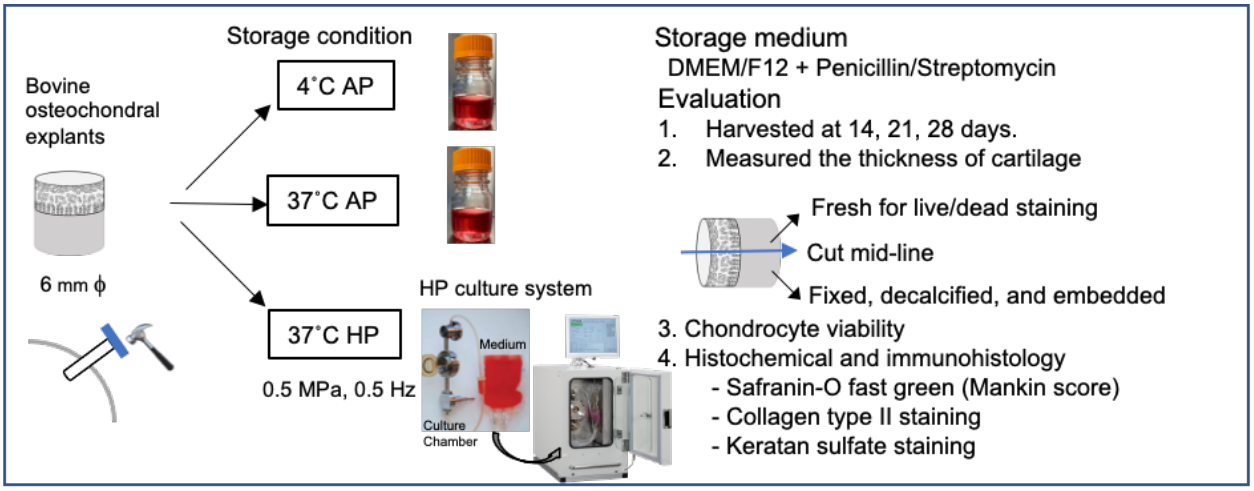

Figure 2. Experimental design. (Based on a manuscript under review.)

3.2..2. Storage conditions of bOC explants

We compared three storage conditions for their effects on chondrocyte viability, longitudinal thickness of cartilage, and histological quality of cartilage ECM (Figure 8.):

1. Atmospheric pressure (AP) at 4˚C (4˚C-AP)
2. AP at 37˚C (37˚C-AP)

3. Cyclic HP 0 – 0.5 MPa, 0.5 Hz with medium replenishment at 0.1 ml/min at 37˚C (37˚C-HP)

From each humeral heads a fresh control was also harvested to account for the possible differences between bovines.

We stored bOC explants in Dulbecco's modified Eagle's medium/Ham's F12 nutrient mixture (DMEM/F12, Gibco®, Life Technology, Carlsbad, CA) with 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco®). For 4˚C-AP storage conditions, explants were stored in 50 ml of the storage medium using two 50-ml conical tubes and kept in a cold room at 4˚C. For 37˚C-AP conditions, explants were stored in 50 ml of the storage medium in a 100-ml glass bottle with a loosely closed cap at 37˚C, 5% CO2 in air. For 37˚C-HP storage conditions, explants were placed in a pressure-proof culture chamber and stored with cyclic HP at 0 – 0.5 MPa, 0.5 Hz and 50 ml of storage medium replenished at 0.1 ml/min at 37˚C, 5% CO2 in air using a HP/perfusion culture system. (Figure 2.) The medium was changed twice a week. [54,56,57]

3.2..3. Evaluation of bOC explants

We harvested bOC explants from each storage condition at 14, 21, and 28 days, cut the cylindrical explant in half longitudinally with a 0.23-mm thick single-edge blade (GEM®, West Hempstead, NY), measured the thickness of the articular cartilage, and subjected the explants to viability assays.

3.2..4. Measurement of cartilage thickness

Thickness of the cartilage was measured using a ruler (0.5 mm resolution) at both peripheries and longitudinal center of the articular cartilage of the hemicylindrical bOC explants. The average thickness of these 3 locations in each explant was used as the thickness of the sample.

3.2..5. Chondrocyte viability

From each explant two 0.5-mm-thick (cartilage with subchondral bone) slices were cut with a blade (GEM®). The slices were incubated in a calcein-AM 5 µg/ml and ethidium-homodimer 10 µg/ml (Live/dead cell viability kits, Life Technology) and fluorescent images of longitudinal explants were acquired at 10x and 20x magnification with filter sets G2A for dead-cell imaging and B2A for live-cell imaging, respectively using an inverted fluorescent microscope (TMD, Nikon, Long Island, NY).

The images were processed to count live and dead cells across the entire tissue slice analyzing the surface, middle, and deep zones of articular cartilage using Image J (version 1.47q, NIH, Bethesda, MD) and the CellProfiler (version 3.1.9, Carpenter lab, Cambridge, MA). Image J was used to analyze four regions of interest (ROI) (500 x 500 pixels area) in each zone, then cells within the ROIs were counted with CellProfiler. [68] We defined the surface, middle, and deep zones longitudinally as 15% from the surface, the next 35%, and the remaining 50% of the cartilage thickness based upon the work of Pallante et al..[42]

3.2..6. Evaluation of the quality of ECM histologically and immunohistologically

Safranin-O/fast green (Fisher Scientific) staining was used to detect the S-GAG matrix, typical in hyaline cartilage, which normally stained in red. Hematoxylin and eosin (Sigma-Aldrich) staining were employed to evaluate the cartilage-bone interface, cell morphology and abnormal calcification. The sections were then evaluated using the histological-histochemical grading system (HHGS) proposed by Mankin et al. [69]

We stained the sections with antibodies against keratan sulfate (KS, clone 5D4, 1:500, MyBioSource, San Diego, CA) and against collagen type II (Col-2, 1:100, MyBioSource) to identify specific ECM components.

3.3.Statistical analysis

3.3.1 OCA storage time

Survival analysis with Kaplan-Meier curves was performed with log-rank analysis to compare both groups (19-24 days storage and 25-27 days storage, based on previous experience studying OCA chondrocyte viability). Multivariable Cox regression analysis was utilized to assess the influence of OCA storage duration on graft survival while adjusting for age and defect size. After evaluating the effect of our study groups on graft survival, we also determined the optimal storage time cut off associated with graft failure by performing receiver operating characteristic (ROC) curve analysis and calculating the area under the curve (AUC) as a descriptor of diagnostic precision. We determined the optimum storage time by identifying the volume cut off value that maximized Youden's J statistic and provided sensitivity, specificity, negative and positive predictive values.

3.3.2 Sex mismatch

Baseline characteristics among the four donor–recipient sex groups (male to male, female to female, male to female, female to male) were compared using 1-way analysis of variance (ANOVA) for continuous variables and the chi-square test for categorical variables. For significant associations, post hoc Bonferroni test were performed.

Cumulative survival was assessed using the Kaplan–Meier method, and results were compared with the log-rank tests. Multivariable Cox regression analysis was utilized to determine the impact of possible confounders on the relationship between donor–recipient sex mismatch and survival. The covariates were: age [35] graft size [37,70] and BMI [71], factors that have been previously associated with clinical outcomes after OCA transplantation.

3.3.2. Improvement of the current OCA storage

One-way ANOVA was used with post-hoc Bonferroni test to determine differences among groups with respect to each outcome measure of continuous data: chondrocyte viability and cartilage thickness.

4. Conclusion

In summary, OCA transplantation is a safe and successful treatment option for large osteochondral defects of the knee with overall excellent rates of graft survival at 5 years. When outcomes were specifically analyzed within the working window for transplantation in the United States, OCAs implanted after 19 to 24 days showed a significantly increased rate of survivorship (93%) when compared to grafts implanted between 25 and 27 days (70.4%). In addition, we found that mismatch between donor and recipient sex has a negative effect on OCA survival following transplantation (2.9 times greater likelihood of failure), particularly in those cases when male donor tissue was transplanted into a female recipient (2.6 times higher odds for failure). These findings stress the importance of prioritizing early transplantation and performing transplantation to the same sex whenever possible, while realizing that there are logistical challenges to doing so, including surgeon, patient and OR availability.

Furthermore, our basic science study demonstrated that OC explants stored with HP at 37°C maintain higher chondrocyte viability and histologically determined cartilage integrity at 28 days compared to the currently used storage technique (4˚C-AP). It seems that under 37°C the application of cyclic HP is essential to utilize the advantages of the maintained metabolic activity of the chondrocytes under this temperature. In the future, HP may offer a significant improvement in culture conditions to increase graft viability for fresh OC grafts. This could extend shelf life and improve clinical outcomes in the long term.

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