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

PhD thesis

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Abbreviations

ACI: autologous chondrocyte implantation

ACL: Anterior crucial ligament

AMIC: autologous matrix-assisted chondrogenesis

ANOVA: analysis of variance

AP: atmospheric pressure

AUC: area under the curve

BMI: body mass index

bOC: bovine osteochondral

CI: confidence interval

Col-2: collagen type II

DAB: 3,38-diaminobenzidine

DFO: distal femoral osteotomy

DMEM/F12: Dulbecco's modified Eagle's medium/Ham’s F12 nutrient mixture

DPBS: Dulbecco’s phosphate buffered saline

ECM: extracellular matrix

FtF: female-to-female

FtM: female-to-male

HHGS: histological-histochemical grading system

HLA: human leukocyte antigen

HP: hydrostatic pressure

HTO: high tibial osteotomy

JRF: Joint Restoration Foundation

KS: keratan sulfate

MACI: matrix-assisted chondrocyte implantation

MAT: meniscal allograft

MPFL: medial patellofemoral ligament

MRI: magnetic resonance imaging

MST: marrow stimulation techniques

MtF: male-to-female

MtM: male-to-male

N: number

OA: osteoarthritis

OAT: osteochondral autograft transplantation

OATS: Osteochondral Autograft Transplant System

OCA: osteochondral allograft transplantation

OCD: osteochondritis dissecans

PGs: proteoglycans

PJAC: particulated juvenile allograft cartilage

ROC: receiver operating characteristic

SD: standard deviation

S-GAG: sulfated glycosaminoglycans

TTO: tibial tubercle osteotomy

Y: year

4˚C-AP: 4˚C atmospheric pressure

37˚C-AP: 37˚C atmospheric pressure

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37˚C-HP: 37˚C hydrostatic pressure

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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]

Articular cartilage defects are commonly found in healthy subjects by magnetic resonance imaging (MRI) evaluation, or incidentally during arthroscopic meniscectomy or anterior cruciate ligament reconstruction.[3] Although the true incidence of cartilage lesions is unknown, numerous studies report articular defects in 60-66% of knees undergoing arthroscopy.[4-6] Accordingly, cartilage injuries of the knee affect approximately 900,000 Americans annually, resulting in more than 200,000 surgical procedures.[4] Since articular cartilage injury comprises a spectrum of disease entities ranging from single, focal chondral defects to progressive degenerative disease and end-stage OA, it is important to understand that only a small percentage (~5%) of all chondral defects seen on MRI or arthroscopically require cartilage repair. Hence the identification of patients requiring cartilage repair is a significant challenge that requires careful consideration of various different factors.[7]

Articular cartilage has a poor spontaneous healing capacity as a consequence of its relatively hypocellular and avascular structure.[8] Consequently, after a cartilage injury, spontaneous healing is usually not expected.[2] In fact, a regenerative process can only be observed when the subchondral bone is affected, leading to a subsequent release of bone marrow derived mesenchymal cells. However, the formed scar is usually composed of fibrocartilaginous tissue with lower biomechanical properties when compared to the native cartilage tissue.[8,9] Such tissue has far inferior properties, leading to early wear, incomplete subchondral bone coverage, possible pain and inflammation slowly propagating the clinical progress towards OA.

1.1 Etiology and natural history

The nature of chondral lesions is not clearly understood but numerous factors might contribute to its development and progression to OA. Several studies reported that certain

individuals are genetically predisposed for early deterioration and breakdown of the articular cartilage and subsequent OA.[10] Other studies showed that the prevalence and severity of knee cartilage defects increases with increasing age and body mass index. In fact, both are also risk factors for knee cartilage degeneration in healthy individuals.[11,12]

Moreover, lesion-specific characteristics including depth, size, location, chronicity, and associated bony abnormalities have been found to influence the clinical outcome after surgical treatment. Hence, the presence of significant subchondral edema, subchondral cysts or intralesional osteophytes as well as an injured or degenerative osteochondral unit should be appreciated during treatment.[13,14] Furthermore, staging of OA is required as cartilage restoration demonstrates unfavorable clinical outcomes in the setting of advanced degenerative OA.[15,16]

Cartilage injury may be of various etiologies including acute traumatic injuries, early posttraumatic degenerative changes, chronic repetitive microtrauma, developmental defects (osteochondritis dissecans [OCD]), or acquired metabolic factors such as avascular necrosis.[5,6] Patients are approximately evenly split in reporting a traumatic versus an insidious onset of symptoms with cartilage lesions occurring in all three compartments of the knee. Lesions are most commonly found on the weight-bearing femoral condyle (43-58%) with the majority of them located on the medial condyle. While patellar lesions account for 11-36% of all lesions, trochlear lesions are less frequently encountered with only 6-16%.[5,6,17] In 90% of cases, the defect size is reported to be less than 4 cm^2 and athletic activities are frequently associated with the diagnosis of chondral lesions.[5,18] In a retrospective study, Widuchowski et al. analyzed 25,124 knee arthroscopies and reported that 60% of knees contained chondral or osteochondral lesions, of which 68% were focal chondral lesions, 3% were OCD lesions and 29% were osteoarthritic lesions.[19] Interestingly, only 5-11% of patients younger than 40 years are diagnosed with high-grade chondral lesions, while up to 60% of older patients (up to 65 years) are affected. However, cartilage defects caused by traumatic events or developmental causative agents such as OCD are more common in the younger population.[5,6,18,20]

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1.1.1 ***Alignment, meniscal function, ligamentous patholaxity***

Articular cartilage requires a fine and regulated environment to properly maintain its homeostasis and overall health. Any insult that disrupts the intra-articular environment might impair this critical equilibrium, potentially leading to cartilage damage.

Abnormal forces across the knee joint can also lead to cartilage damage and subsequent degeneration. Leg axis malalignment plays a crucial role in articular cartilage deterioration by placing abnormal stresses on the articular surface of the respective tibiofemoral compartment.[21,22] Also, patellar maltracking contributes to an unfavorable force distribution across the patellofemoral compartment, resulting in patellofemoral pain and cartilage damage. This malalignment, eventually, increases the chance of patellar dislocation, which in turn may lead to traumatic cartilage damage.[23] Ligamentous instability may also result in abnormal forces to the knee joint ultimately contributing to cartilage degeneration.[24] Anterior crucial ligament (ACL) rupture is the most common ligament injury in the knee and frequently associated with meniscal tears, causing laxity and instability, leading to pain and varying levels of disability ranging from limited sports participation to difficulties performing activities of daily living.[24,25] ACL deficient and instable knees are at increased risk for future cartilage injury with high potential of progression to posttraumatic OA.[24] Although ACL reconstruction reinstates knee stability, which is necessary to preserve meniscus integrity, individuals that underwent ACL reconstruction are still at increased risk for developing knee OA compared to the general population.[26]

Intact menisci are essential to maintain the physiological force distribution in the knee as its functional properties are shock absorption, joint lubrication, nutrition of the articular cartilage, joint stability and proprioception in the knee joint.[27] This role is commonly impaired following meniscal injury, resulting in increased contact stresses in the tibiofemoral joint, thus leading to further degeneration and progression of OA.[28] Consequently, preservation and restoration of the meniscal function is paramount in order to prevent or delay degenerative changes within the knee joint. Essentially, all mentioned comorbidities (malalignment, ligamentous and meniscus deficiency) are expected to compromise the outcome after any cartilage repair procedure. Therefore, not only addressing the cartilage defect but also these comorbidities is of utmost importance to obtain successful biologic joint reconstruction.[29]

1.2 Clinical presentation

A crucial factor to adequately manage cartilage injuries is the early recognition and identification of the features of the disease.[30] Delayed diagnosis favors the exacerbation of symptoms and progression to more severe cartilage damage.[31] However, symptoms are not always readily detected or reported, and when they are, a wide variety of clinical presentations might occur, making early diagnosis challenging. Because cartilage does not contain nerves, damage does not directly cause pain, and even large chondral defects can be totally painless for a long period of time. In addition, there is likely a significant delay between the original chondral injury and the actual loss of chondral tissue. Therefore, pain related to cartilage damage, if any, is usually associated with insults to the surrounding structures. In this regard, the adjacent soft tissue, the underlying subchondral bone and the synovium may be the source of pain.[23] Besides pain, patients with a damaged cartilage may experience mechanical symptoms, such as catching or locking. Moreover, acute effusion derived from an acute injury (e.g. hemarthrosis) or recurrent effusion may also be present and is widely considered one of the most important signs of clinical progression.[32]

Several key aspects must be assessed in order to get the most accurate clinical picture of the patient, and to be able to subsequently tailor the succeeding treatment to the individual. On the initial evaluation, it is important to determine the patient's history of symptoms that may be related to cartilage injury and the presence of the aforementioned co-morbidities such as alignment, meniscal function, ligamentous patholaxity. Left unaddressed, all restorative efforts will have a suboptimal outcome. Further, patient's age and body mass index (BMI) should be documented. Numerous studies reported that increased age negatively influences clinical outcome in patients undergoing microfracture.[33] However, this may not hold true for all biological techniques, as recent studies using cell transplant techniques showed that the overall benefit after cartilage treatment in patients older than 40 years was not significantly different from the outcomes achieved in younger patients.[34] While a BMI $\leq$ 35 does not seem to affect overall outcomes in patients undergoing cell-based cartilage procedures, comparable BMI seems to adversely affect the results after microfracture treatment.[35] Moreover, overweight is a well-known risk factor for OA, which in turn adversely affects outcomes in

patients undergoing cartilage restoration.[15,16] Family history of OA is often considered a negative predictive factor for cartilage restoration treatment. However, no clear evidence exists to actually link it to clinical outcomes. Social history and smoking in particular should also be explored as this demonstrated to have an adverse effect on many orthopedic conditions such as fracture healing, spinal fusion, wound repair, bone mineral density, lumbar disk disease, and rate of hip fracture knee ligament and articular cartilage surgery. [36] The duration of symptoms is another valuable information as prolonged symptoms have been associated with worse clinical outcome following cartilage repair.[37] Previous surgeries on the knee, especially prior cartilage repair procedures, should also be noted as autologous chondrocyte implantation demonstrated worse clinical outcomes in patients with prior marrow stimulation techniques.[38,39] Lastly, appropriate patient selection with regards to compliance is fundamental for the successful treatment of symptomatic cartilage defects. Hence, determining patient personality and compliance plays an important role in anticipating the expected clinical outcome.

1.3 Importance of the intact subchondral unit

Articular cartilage is a hypocellular and highly specialized tissue, with only 4% of its wet weight consisting of chondrocytes. The main components of articular cartilage are water (65% to 85% of weight) and the extracellular matrix (ECM) composed of type II collagen (15-20% of weight) and proteoglycans (PGs) (3-10% of weight).[40] The ECM of mature articular cartilage production is related directly to the chondrocyte volume or function, and is composed of 3 major types of macromolecules: fibers (collagen and elastin), proteoglycans, and glycoproteins, which are synthesized and maintained by chondrocytes.[41] Together, these components help to retain water within the ECM, which is crucial to maintain its unique mechanical properties. Another important feature of articular cartilage is that despite its uniform macroscopic appearance it is not a uniform tissue, displaying distinct biochemical and morphological differences between the superficial, middle and deep layers. In particular, articular cartilage has a highly organized structure composed of 4 zones: the superficial (tangential) zone, middle (transitional) zone, deep (radial) zone, and calcified zone.[8] (Figure 1). The chondrocyte phenotype, cell shape, and the ECM structure vary among

the different zones.[42] The deep zone is separated from the calcified zone by the tidemark, which is a thin basophilic line that usually can be seen in a slide stained with hematoxylin and eosin. This represents the boundary between the mineralized and unmineralized regions.[41] Underneath the calcified zone the subchondral plate can be appreciated. (Figure 1.) The relationship between cartilage and the underlying subchondral bone has particular importance when assessing joint health and determining treatment strategies. The articular cartilage is anchored to the subchondral bone via an interface of calcified cartilage, which as a whole makes up the "osteochondral unit". Subchondral bone lies under the calcified cartilage, separated by the cement line. The subchondral bone plays a key role in mechanically and metabolically supporting the articular cartilage. The subchondral bone attenuates about 30% of the impact load, whereas cartilage only attenuates 1-3%.[43] Therefore, the physiologic cartilage subchondral bone unit is critical for the transfer of load to allow for normal joint articulation and movement. In contrast to articular cartilage, the subchondral bone is innervated richly by sensory and sympathetic nerve fibers, which modulate bone regeneration, bone remodeling, and articular surface homeostasis and can be involved in pain generation.[44] Furthermore, the subchondral bone contains blood vessels which, penetrate into the calcified cartilage and metabolically support the deep layer through diffusion.[45] These vessels provide at least 50% of nutrient supply of the cartilage including glucose, oxygen and water and can also transfer signaling molecules between the bone, cartilage and surrounding tissues.[46]

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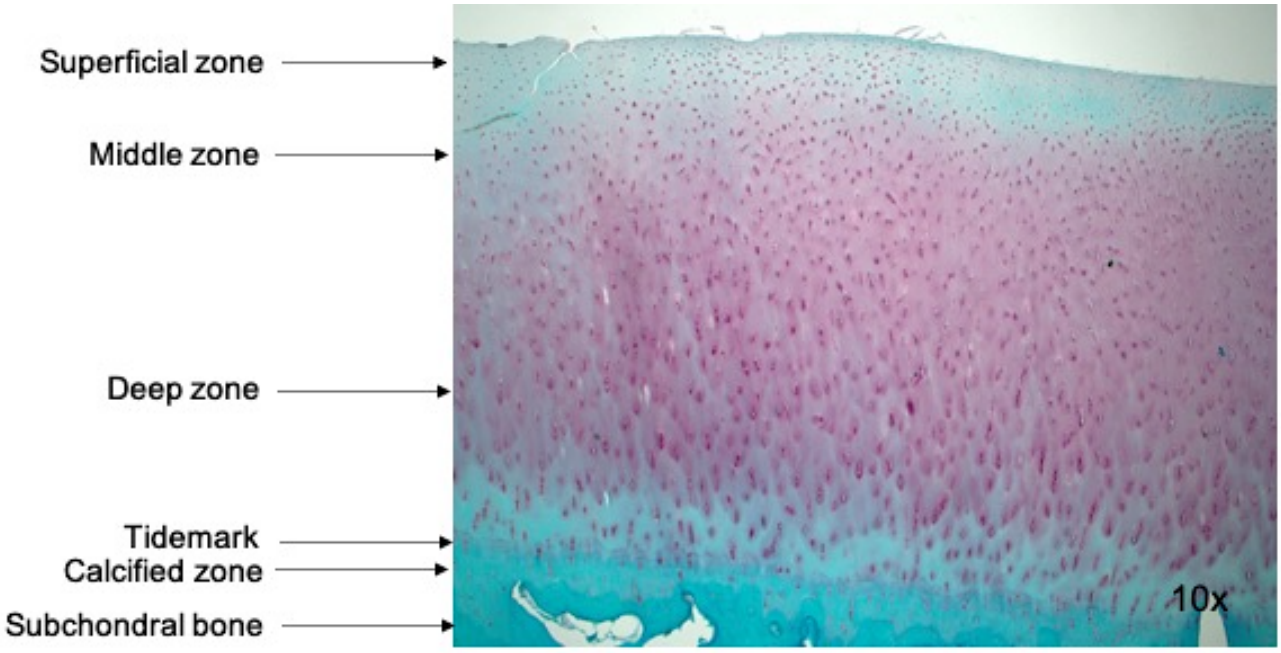

Figure 1. Histology-stained osteochondral allograft with safranin-O and fast green (original magnification, x10). Matrix and chondrocytes are well organized in each zone. The superficial zone has decreased safranin-O staining and less chondrocytes than in fresh healthy human cartilage which is the consequence of the storage. In the middle zone, where safranin-O staining appears, the cells are rounded or ovoid and seem to have random distribution; in contrast, in the deep zone, the cells are arranged in short columns.

Given the aforementioned tissue complexity, treatment of cartilage injury is indeed challenging and must take the integrity of the whole osteochondral unit into account. For instance, surface repairs, such as autologous chondrocyte implantation (ACI), are extremely sensitive to subchondral bone changes as subchondral edema and subchondral sclerosis as a result of previous microfracture has been linked to a significant decline in long-term results.[47] Preoperative MRI is an important tool to evaluate the status of the subchondral bone. The subchondral bone changes include: underlying subchondral cystic change, bone marrow edema (Figure 2, Figure 3 and Figure 4.), intralesional osteophyte and sclerosis. Generally, bone marrow edema is recognized as a non-specific reaction of the bone to trauma, both acute and chronic repetition from overload, and may represent numerous non-specific histological characteristics that can include microtrabecular fracture/fracture healing.[16,48] Most

importantly, subchondral bone and the adjacent calcified articular cartilage can undergo repetitive microinjuries having the potential to initiate a chronic repair mechanism and eventually result in the formation of new bone (subchondral sclerosis). This is a common finding particularly after prior marrow stimulation techniques (MST) (intralesional osteophyte formation, bone marrow edema), which might be the underlying reason behind a recently reported 3 to 8 times higher failure rate and decreased satisfaction rate among patients who underwent MST prior to ACI.[38,39,47,49-52] Consequently, in our practice, in the presence of severe subchondral edema, large subchondral cyst or intralesional osteophyte, surface treatment is not indicated, instead replacement of the entire subchondral unit through osteochondral allograft transplantation is the preferred technique.

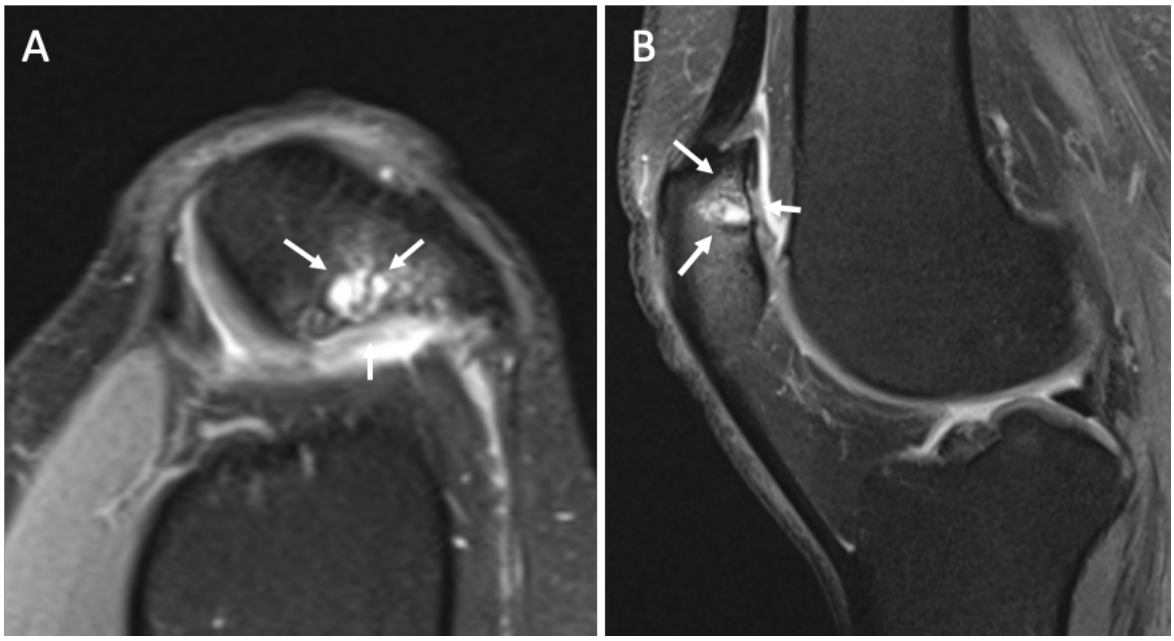

Figure 2. Magnetic resonance imaging (3 Tesla) of a full thickness cartilage defect with a subchondral cyst. Axial (A) and sagittal (B) fat-suppressed intermediate-weighted images demonstrating a full thickness cartilage lesion with an underlying subchondral cyst.[53]

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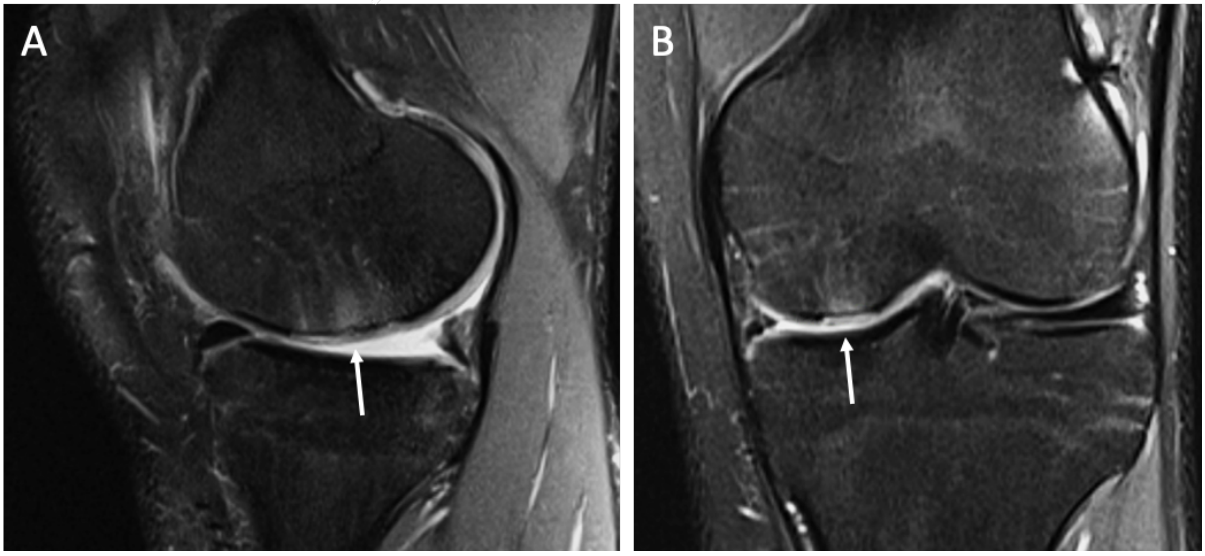

Figure 3. Magnetic resonance imaging (3 Tesla) of a subchondral bone edema and delaminated fibrocartilage following microfracture. MRI also indicates a posterior meniscus root tear. Sagittal (A) and coronal (B) fat-suppressed intermediate-weighted images demonstrating a subchondral bone edema and delaminated fibrocartilage following failed microfracture.[53]

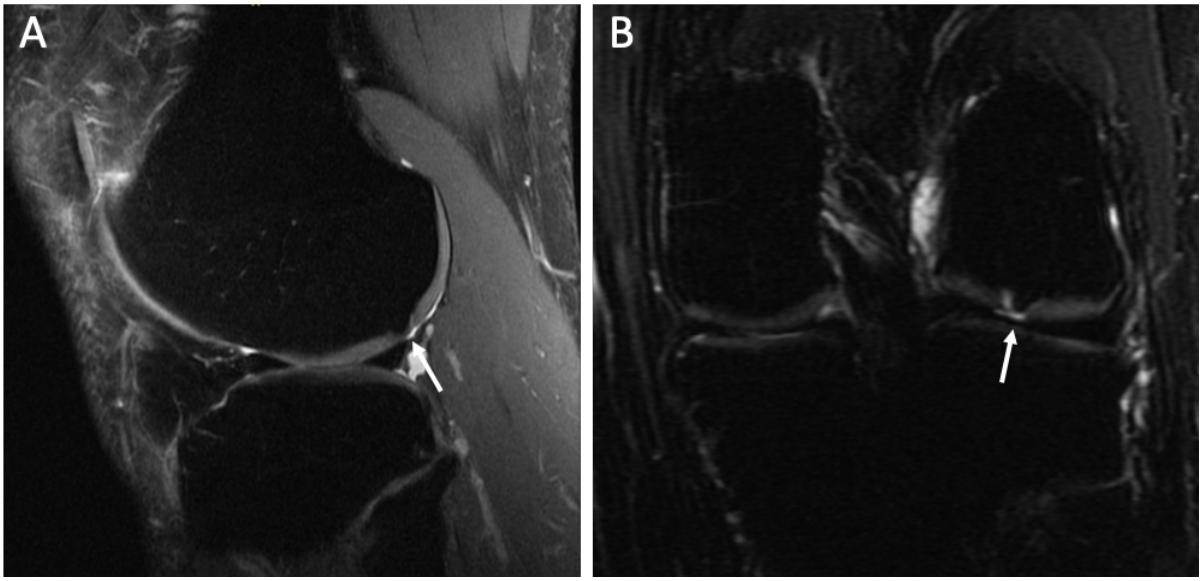

Figure 4. Magnetic resonance imaging (3 Tesla) of an intralesional osteophyte after prior failed microfracture. Sagittal (A) and coronal (B) fat-suppressed intermediate-weighted images demonstrating an intralesional osteophyte after prior failed microfracture.[53]

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1.4 Treatment algorithm

The treatment of cartilage injuries has markedly improved over the years. Despite the historical register of osteochondral allograft transplantation in the beginning of the 1900s, and bone marrow stimulation techniques in the 1950s, the knowledge of the cartilage structure and physiology, and its interaction with the underlying bone has only been explored recently.[54,55]

Recognizing which patient needs surgical cartilage interventions and what procedure would be most suited are still challenging, and the treatment usually is personalized based upon the patients' specific situation. Imperative for success of any cartilage repair procedure is the active participation of the patient, as the rehabilitation requires prolonged periods of time, sometimes in excess of 12 months.[7,53,56] However, as we discussed above the cartilage has a very limited healing potential.[53] 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.[13,41,57] Consequently, the restoration of a symptomatic cartilage lesion is essential to avoid or slow down the OA progression.[7,53,58-60] To delay or avoid a prosthetic arthroplasty, multiple treatment options are available to restore the injured cartilage depending on patient and lesion characteristics including MST, autologous matrix-assisted chondrogenesis (AMIC), osteochondral autograft transplantation (OAT), particulated juvenile allograft cartilage (PJAC), aragonite-based osteochondral scaffold, ACI, and osteochondral allograft transplantation (OCA).[7,53,61,62] (Figure 5.)

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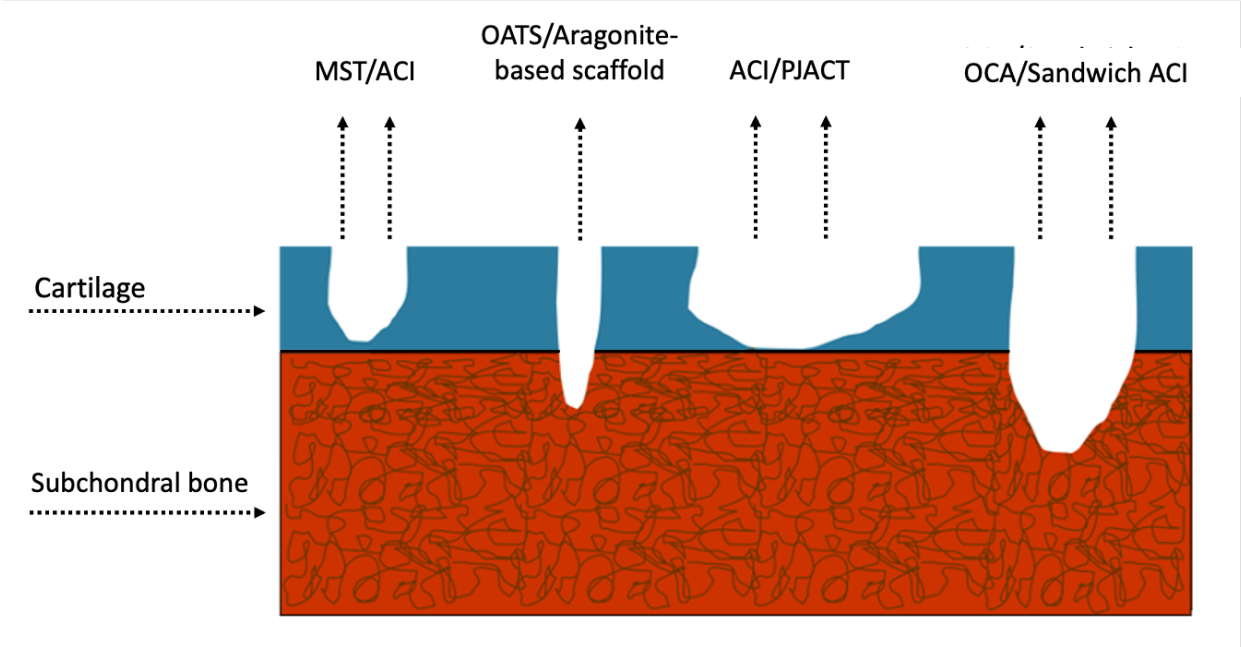

Figure 5. Cartilage repair options for different cartilage defects.[60]

1.4.1 Marrow stimulation techniques (MST)

The aim of MST is to induce the migration of potential repair cells into the chondral/osteochondral lesion. By stimulating bleeding using a sharp material into the vascularized bone marrow, stem cells are allowed to move to the cartilage defect.[2] These cells induce a fibrocartilage tissue formation, which tends to fill the defect. However, this fibrocartilage tissue, mainly formed by collagen type I, has inferior biomechanical properties than the native hyaline cartilage (type II collagen), with reduced stiffness and higher predilection for deterioration over time.[63,64]

Drilling, microfractures and, more recently, nanofractures, which uses smaller diameter and deeper subchondral bone perforations, are described as MSTs. Microfracture is the most frequently performed surgical procedure to treat focal chondral injuries, as it is a relatively easy and accessible technique with low cost. In this method, all unstable pieces of cartilage are removed, creating a well-contained defect ideally surrounded by a stable and perpendicular-edge cartilage. In addition, a complete exposure of the subchondral bone is required. Multiple fracture holes of approximately 3 to 4 mm apart, and 4 mm in depth are

created using a specific device that penetrate the subchondral plates, allowing bone marrow cell migration and clot formation. A systematic review has shown satisfactory outcomes after microfracture in a short-term period follow-up, particularly in young subjects with small defects. However, the positive results tend to be short lived and decline over time.[63] Additionally, bone overgrowth was observed in more than 60% of cases, which may increase the risk of failure.[65]

The nanofracture technique is a new concept based on the same principles of microfractures, but uses thinner devices (awls 1 mm-thick) that produce controlled and deeper perforations of 9 mm-depth,[66] which seems to preserve the trabecular bone.[67] In fact, benefits of the nanofracture over the usual microfracture were demonstrated in an animal model study,[67] but clinical evidence is still lacking.

Marrow stimulation should not be opportunistically performed in all small defects. Indeed, recent guidelines suggest that even smaller lesions should be treated using cell-based therapies rather than microfracture. In our practice, marrow stimulation technique is only indicated in young, athletic patients, with isolated and acute defects less than 2 cm^2 in the femoral condyle.

1.4.2 Autologous matrix-assisted chondrogenesis (AMIC)

The use of biodegradable hydrogels or tri-dimensional scaffolds to improve the clot stability has also been studied. AMIC combines microfracture with the use of a collagen scaffold, aiming to enhance the mechanical stability of the clot along with stimulating the chondrogenic differentiation.[62] A randomized, controlled clinical trial showed superior quality and quantity of the repaired tissue using the AMIC technique in comparison to the microfracture alone; however, no differences in clinical outcomes were observed.[68] To date, there is no sufficient evidence to indicate these techniques over the established procedures.

1.4.3 Osteochondral autograft transplantation (OAT)

OAT is a therapeutic modality best indicated for osteochondral defects that are less than 2 cm^2.[69] OAT consists in harvesting cylindrical osteochondral plugs from a low-weight-

bearing area of the knee - usually the intercondylar notch or the trochlea's periphery - and transfer these plugs to fill a chondral defect. Multiples plugs can be used as a mosaic (i.e. Mosaicplasty) in case of larger defects.[70] The major advantage of OAT is that the lesion is promptly filled by mature, hyaline cartilage using an autologous tissue. In addition, for being an osteochondral plug, the unhealthy underlying subchondral bone can also be addressed. In case of numerous plugs, the gaps between the plugs and the native cartilage are filled by a fibrocartilage tissue after healing. The difficulty in creating a congruent surface between the donor explant and the defect is a limitation of the OAT.[71] Moreover, because of the increased donor site morbidity, the procedure is not recommended to cover large defects.[71]

Generally considered a technically difficult procedure, osteochondral autograft transplantation yields good to excellent results in terms of durability and functional outcomes as long as defects are not too large and only single plug grafts are utilized.[72] Indeed, a systematic review including more than 600 patients who underwent OAT to treat knee osteochondral injuries showed improved clinical outcomes, with an overall rate of implant survivor of 72% in a mean follow-up higher than 10 years.[73] Increased rates of failure were observed in cases of previous surgery, older age (>40 years), women and defects greater than 3 cm^2.[73,74] Randomized clinical studies have shown that, when compared to microfractures, OAT presented better results in young active patients.[74,75]

Failures related to the OAT technique are usually associated with errors in the plug harvesting or implantation. Inadequate restoration of the joint surface congruity leads to biomechanical wear of the implants,[76] leading to worse graft incorporation, bony resorption and cyst formation. In addition, chondrocytes in the plug periphery might not be viable after harvesting, which can impair the lateral integration of the plug.[76] A randomized, controlled clinical trial has reported inferior results of OAT in comparison to ACI in defects over 2cm^2.[77]

1.4.4 Particulated juvenile allograft cartilage (PJAC)

PJAC consists in the implantation of allograft juvenile chondrocytes suspended in their native extracellular matrix. Considering that immature cartilage potentially has increased chondrogenic activity in comparison to the adult cartilage, PJAC fits as a promising

option for cartilage restoration.[78] In fact, when there is no immunological reaction, juvenile chondrocytes have demonstrated a faster growth and 100-fold increase in proteoglycan synthesis when compared to adult chondrocytes.[53,79] This procedure can be indicated to treat focal, contained chondral defects or in combination with OAT or OCA transplantation, aiming to cover the gaps left behind. Frequently, PJAC is indicated for patellar defects after a failed nonoperative treatment, given its ability to fill different lesion shapes.[80] Similarly to the ACI technique, PJAC requires a prior preparation of the lesion, with debridement of any fibrous tissue and stable vertical walls surrounding the defect. [78] Of note, the subchondral bone should be intact.[78]

A positive aspect of PJAC when compared to ACI is that the PJAC is a one-stage procedure. Short-term follow-up studies have shown a significant improvement in the symptomatology of patellar defects treated with PJAC. In addition, better functional outcomes, hyaline-like cartilage formation on histological evaluation and near to normal cartilage repair findings on MRI have been demonstrated.[81,82] However, randomized controlled studies comparing this technique with other cartilage restoration methods are still lacking.

1.4.5 Aragonite-based osteochondral scaffold

Recently, innovative technologies have been developed to improve the chondral or osteochondral regeneration by using materials with interesting biological and mechanical properties. Aragonite-based osteochondral scaffold (Agili-C™, CartiHeal Ltd) is a cell-free, biodegradable and biphasic scaffold that, by stimulating the growth of cartilage and subchondral bone, restores the osteochondral unit to the original structures of the tissue.[83] The mechanism of action for this technique is based on the promotion of bone marrow stem cell adhesion and differentiation, and chondrocyte migration and proliferation from the healthy surrounding native cartilage.[84,85]

CartiHeal is indicated to treat osteochondral defects in both degenerative and non-degenerative joints, including large condylar defects, given that different sizes and shapes of this implant can be designed. Pre-clinical studies have shown that CartiHeal is able to induce

cartilage repair and regeneration, resulting in hyaline cartilage formation and subchondral bone regeneration. These findings were further verified in preliminary clinical trials.[84-86]

1.4.6 Autologous chondrocytes implantation

ACI technique comprises a two-stage procedure, in which an initial arthroscopy is performed to diagnose and evaluate the defect (size and location), and collect a small piece of healthy cartilage for biopsy that will serve as a chondrocyte culture. The biopsy is performed in a low-weight-bearing area of the knee, commonly the intercondylar notch. Next, chondrocytes are isolated and expanded *in vitro*, and stored until their implantation. In the second stage procedure, the lesion's bed is prepared by removing any fibrous tissue, and a contained defect surrounded by vertical edges of normal cartilage is made.[58,87] Prior to 2016 it was necessary to create a sealed chamber using a collagen 1/3 porcine based membrane or periosteum.[87] A chondrocyte suspension was injected underneath the scaffold to deliver the cells into close proximity of the subchondral bone.[87]

Since 2016 a third-generation ACI, the so-called matrix-assisted chondrocyte implantation (MACI) is available. This technique constitutes a substantial improvement of the technique. It consists of a hydrated collagen 1/3 porcine scaffold carrying suspended expanded chondrocytes, that is directly implanted in the defect and secured with fibrin glue. By acting as a cell transporter, MACI allows a more equal distribution of chondrocytes in the defect. Besides that, MACI is an easier and quicker procedure in comparison to the other ACI generations. (Figure 6.) In addition, currently MACI is the only cell-based cartilage therapy approved by the FDA.[71]

A usual indication for ACI is patients with cartilage defects greater than 2 cm^2, when the subchondral plate is intact. Since it is a membrane technique, it can easily be used for defects of different contours and size, and, therefore, can be used for both femoral and patellar lesions. [47,58] In selected cases a unique ACI technique called “sandwich ACI” can be used for large osteochondral chondral defects as well. In this scenario the bone defect is covered with autologous trabecular bone and the ACI/MACI is utilized to restore the cartilage.[58,88]

MACI represents one of the most common cell-based therapies currently employed to treat large full-thickness defects of the femur or patellofemoral compartment. Indeed,

because of the unique anatomy of the patellofemoral joint, MACI appears to be an excellent option considering the difficulty patellofemoral anatomy.[89]

MACI leads to good post-operative outcomes with a survivorship as good as 78% at five years.[90] Regarding clinical outcomes, long-term studies have shown better functional results of ACI/MACI in comparison to OAT. [77,90] When evaluating larger defects, significant functional improvement and satisfactory survival rate of 71% in 10 years follow-up have been reported in lesions sizing 8.4 cm^2 in average.[71] The major disadvantages are that ACI/MACI requires two-stage procedures, long rehabilitation periods and elevated financial costs.[91]

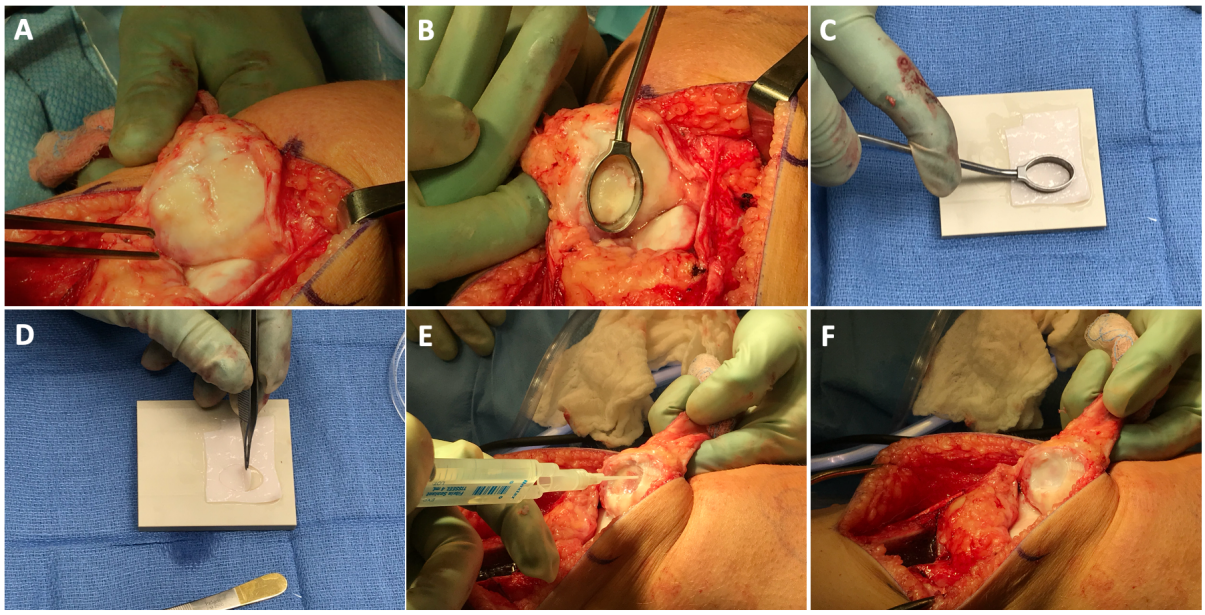

Figure 6. Surgical images of an autologous chondrocyte implantation for a cartilage defect on the patella. (A) Monopolar focal patellar cartilage defect with friable fibrocartilage. (B) The cookie cutter (with defect size marked on the handles) is gently pushed down to the level of the calcified cartilage layer to create a contained defect with stable and vertical edges. (C and D) The cutter is used to cut the appropriate size graft from the MACI sheet. (E) The MACI is secured to the bone with glue and the periphery is sealed. (F) Glue is set and the MACI is stable.[60]

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1.4.7 Osteochondral allograft transplantation (OCA)

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. (Figure 7.) 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.[92-94]

Current indications for OCA transplantation include large focal chondral or osteochondral lesions, failure of previous cartilage repairs, osteochondritis dissecans, osteonecrosis and posttraumatic osteochondral lesions.[59,90,92-96] Even though OCA transplantation is indicated for a challenging population numerous studies have shown good clinical outcomes following OCA transplantation.[59,90,95]

In particular, long-term clinical data following OCA transplantation demonstrates favorable outcomes with success rates reported between 50-89% in 10 years.[93-95,97-100] With such broad reported rates of success however, numerous studies have sought to identify predictors associated with improved graft survival following transplantation. [61,93,101-103] 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.[99,104] 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.[103,105-107]

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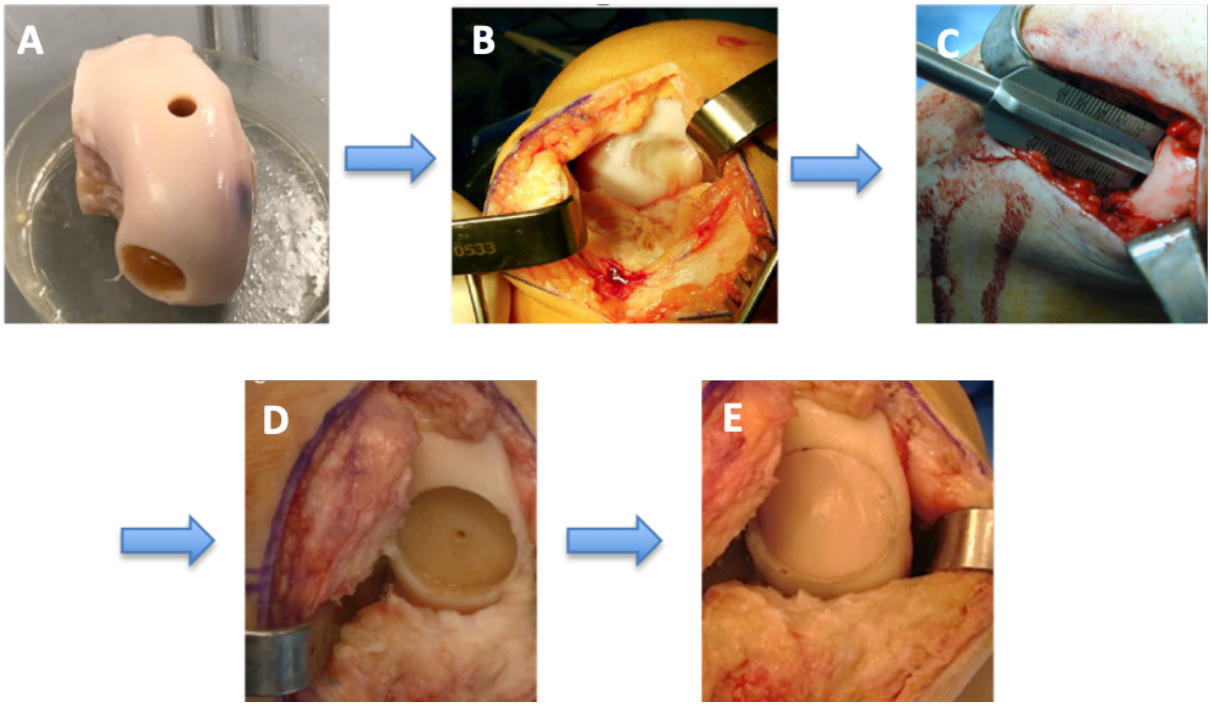

Figure 7. Surgical images of osteochondral allograft transplantation for a cartilage defect on medial femoral condyle. (A) Cadaver condyle. (B) Monopolar focal medial femoral condyle lesion. (C and D) Defect drilling and preparation. (E) osteochondral allograft transplanted to the recipient.

1.4.7.1 The current OCA storage condition

During the 1980s and 90s, OCAs were available only through a limited number of local tissue banks working closely with a few specialized medical centers.[103,105-107] At that time, lack of regulation and an overall low volume allowed an abbreviated procurement and screening process with early transplantation of fresh grafts within 5-7 days of harvest.[29] However, as a wider commercial distribution network came online in the early 2000s, more rigorous safety protocols were implemented by the American Association of Tissue Banks and US Department of Health and Human Services. These requirements ultimately defined a working timeline for allograft transplantation no earlier than 14 days from procurement.[7,108,109] This timeline remains in current practice today.

As we seek to understand the optimal storage conditions for grafts within the constraints of this timeline, extensive investigative efforts have begun to shed light on the

implications of delayed transplantation on chondrocyte viability, graft incorporation, and patient outcomes. 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).[110,111] 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.[112,113] 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.[112,113] Multiple studies report that chondrocyte viability falls below the generally acceptable level of 70% after 28 days of storage at 4°C.[112,114,115]

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.[116] In a study by Nuelle et al.,[116] 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.[117] 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. [118]

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.[119]

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.[120] 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.[112,114,121,122] Under 37˚C storage improved chondrocyte viability was reported at 28 days, especially at the vulnerable surface zone. [114] However, chondrocyte viability at 37˚C still remained low throughout all zones of the graft at 28 days and therefore its use remained controversial.[112,114,115] Articular cartilage is normally exposed to a hydrostatic loading environment in vivo and shows abundant intratissue water under weight-bearing conditions.[123,124] During everyday activities, chondrocytes within the joint cartilage experience cyclic hydrostatic pressure (HP) levels of 0.2 - 20 MPa.[125] 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.*[126-131] [126-129] 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.

1.4.7.2 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,[132-136] Numerous studies reported increased mortality after lung transplantation,[132] lower overall 5 year survival rates in female orthotopic heart transplant recipients,[133] higher risk for graft failure in female pancreas transplant recipients,[134] early graft failure in female renal transplant recipients,[135] and worst outcomes amongst male liver transplant recipients; when donor-recipient sex-mismatching was present.[136]

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.[137]

While intact articular cartilage is believed to be an immunoprivileged tissue bone has antigenicity. Previous reports have shown that human leukocyte antigen (HLA) antibodies

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do in fact develop after OCA transplantation.[102,138,139] However, the potential role of donor-recipient sex mismatching in OCA transplantation has not yet been characterized.

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2 Objectives

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

2.1 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.

2.2 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).

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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. Surgery was indicated in patients who failed a trial of non-operative therapy unless the nature of the defect was such that structural worsening would result from non-operative treatment (such as large irreparable lateral femoral condyle OCD lesion in a young patient). 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). Patients with tibiofemoral malalignment >3º from the neutral mechanical axis of the involved compartment underwent concomitant corrective osteotomy. Similarly, patellofemoral maltracking was addressed with anteromedialization tibial tubercle osteotomy (TTO)[140]; and proximal soft tissue imbalance was addressed via lateral release/lengthening, vastus medialis obliquus advancement, and/or medial patellofemoral ligament (MPFL) reconstruction as indicated. 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.

3.1.1.3 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.

3.1.2 Surgical procedure

OCA transplantation was performed as previously described. [141,142] Concomitant procedures to address mechanical alignment, patellofemoral maltracking, and/or soft tissue imbalance were performed as necessary before proceeding to OCA transplantation. Briefly, a parapatellar arthrotomy was used to expose the cartilage defect on standard cases. Any degenerated cartilage as well as fibrous tissue was outlined. The defect was then sized with

a sizing guide and an identical sized reamer was used to ream to a depth of approximately six to eight millimeters using constant cold irrigation. The donor condyle was prepared using the corresponding sizing guide. After harvesting the allograft dowel, the graft was trimmed using a sagittal saw according to the depth of the recipient site. The allograft was then pulse lavaged to decrease marrow elements at a standardized pressure and length of time. Finally, the graft was placed into the recipient site with manual pressure only, ensuring flush circumferential fit and mechanical stability. Ultimate implantation was standardized and performed by a single surgeon.

3.1.3 Postoperative rehabilitation

Patients were kept touchdown weight-bearing four to six weeks without limitation of range of motion, followed by progression to full weight-bearing as tolerated. During this period, patients were permitted to begin unrestricted range of motion exercises, quadriceps sets, straight-leg raises, and patellar mobilization. A stationary bicycle was permitted at four weeks. Open chain exercises were not permitted in this first phase. Strengthening was added at the six-week point and was increased over the next two to three months. Patients progressed to sport-specific activities by four to six months after surgery for isolated OCA and by eight to twelve months for those undergoing concomitant procedures. [99,143]

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

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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 8) Altogether we harvested 60 bOC explants from 6 bovine humeral heads: one fresh (0 days) control and nine OC explants for storage.

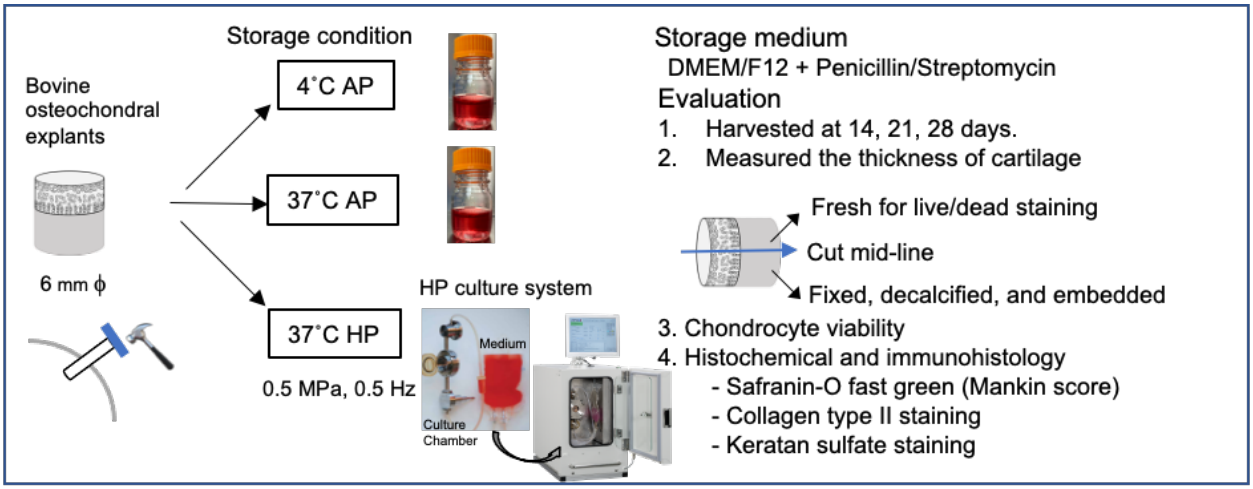

Figure 8. Experimental design. (Based on a manuscript under review.)

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 8.) The medium was changed twice a week. The magnitude of cyclic HP was chosen according to previous work designed to maintain adequate chondrocyte viability. [126,128,129]

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.

3.2.3.1 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. Subsequently, cell viability was assessed, and the remaining samples were fixed for histological and immunohistological evaluation.

3.2.3.2 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) dissolved in DPBS at room temperature for 40 minutes. These concentrations of dyes and incubation

time were chosen to minimize false-positive staining for bOC tissue. After the slices were rinsed in DPBS 3 times in 10 minutes, 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). These images were acquired always at the same shutter speed, 1/8 sec for live cells and 1/2 sec for dead cells. The shutter speeds were optimized prior to experiments and validated each time with dead cells control of bovine articular cartilage slice frozen and thawed twice (data not shown). We took images from the middle of each OC explants. We started at the surface of the cartilage through the middle and the deep zone until the subchondral bone.

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. [144] 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.[114]

3.2.3.3 Evaluation of the quality of ECM histologically and immunohistologically

The remaining half of every bOC graft was fixed in 2% paraformaldehyde/0.1 M cacodylic acid (pH 7.4) for 2 days on a rotary shaker (Fisher Scientific) and decalcified in synthetic decalcified solution (Rapid Decalcifier®, Apex Engineering Products, Aurora, IL) for 1.5 - 2 hours following manufacturer's instructions. Samples were embedded in paraffin and cut into 7-µm thick sections. The sections were deparaffinized in xylene and rehydrated in a series of graded alcohol for staining.

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. [145] Normal

articular cartilage received a score of 0, whereas higher scores indicated deterioration. HHGS is intended for the grading of human osteoarthritic cartilage, however, it has also been used widely and has been shown to be reproducible for animal studies. [112,146]

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. For KS, the sections were incubated with 0.1 unit/ml chondroitinase ABC (Sigma-Aldrich, St. Louis, MO) at 37˚C for 1 h. The sections were rinsed three times with PBS between each incubation. We incubated the sections with KS antibody for 1 h followed by with a biotinylated second antibody (Vectastain™ ABC kit, Vector Laboratory, Burlingame, CA) according to the manufacturer's instructions. For Col-2, the sections were incubated with the antibody followed by with the same second antibody as used for KS. Color was developed with 3,38-diaminobenzidine (DAB kit, Vector Laboratory). Counterstaining was performed with Harris's hematoxylin (Sigma-Aldrich).

3.3 Statistical analysis

3.3.1 Graft specific predictors

SPSS (version 21.0; IBM Corp) was used to perform statistical analyses. Demographic results are reported as mean and standard deviation. Normal distribution of the data was confirmed by utilizing the Shapiro-Wilk test. Descriptive statistics were performed with chi-square or Fisher exact testing for the categorical variables and independent-samples t tests or the Mann-Whitney U test when appropriate for continuous variables. All statistical tests were 2-tailed. A p-value of 0.05 was used to determine statistical significance.

3.3.1.1 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.

3.3.1.2 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 [105] graft size [56,107] and BMI [147], 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. All statistical tests were 2-tailed. A p-value of 0.05 was used to determine statistical significance.

4 Results

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4.1 OCA storage time

4.1.1 Baseline demographics and clinical characteristics

One-hundred and thirty-two patients underwent OCA transplantation between February 2014 and December 2016. Of these, 11 patients (8.3%) were excluded from this study due to incomplete data, and 10 patients (7.6%) were lost during the follow up period. Hence, one-hundred and eleven patients (84.1%) met study inclusion criteria. Of these patients, 56 (50.5%) received OCAs that were stored for 19-24 days, and 55 patients (49.5%) underwent OCA transplantation with grafts that were stored for 25-27 days. The mean age at time of surgery was 36.3 ± 10.2 years (range, 15 – 56 years) with a mean follow-up of 2.8 ± 0.8 years (range, 2 – 5 years). Baseline demographics and clinical characteristics of the study patient population are outlined in Table 1, while graft characteristics for all transplanted OCAs are demonstrated in Table 2.

Table 1. Demographics data of patients who underwent osteochondral allograft transplantation

	Total	**19-24 days storage**	**25-27 days storage**	**(95% CI)**	**P value**
Number of patients (%)	111	56 (50.5)	55 (49.5)		
Age, y, mean SD (range)	36.3±10.2 (15-56)	36.4±10.2 (15-56)	37±10.3 (17-56)	(-4.5 - 3.2)	0.735
BMI (kg/m^2), mean SD (range)	27.6±5.3 (18 - 38.6)	27.6±5.2 (19-38.2)	28.2±4.7 (19-38.6)	(-2.5 - 1.3)	0.547
Female, n (%)	56 (50.5)	27 (48.2)	29 (52.7)		0.634

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Defect size, cm^2, mean SD (range)	2.4±1.2 (2-7.2)	2.5±1.2 (1-6.4)	2.4±1.2 (1-7.2)	(-3.5 - 5.6)	0.642
HTO, n (%)	10 (9)	5 (8.9)	5 (9.1)		1
TTO, n (%)	13 (11.7)	5 (8.9)	8 (14.5)		0.392
DFO, n (%)	1 (0.9)	0	1 (1.8)		0.495
MAT, n (%)	3 (2.7)	2 (3.6)	1 (1.8)		1
ACL, n (%)	5 (4.5)	4 (7.2)	1 (1.8)		0.364
Defect location					
Tibiofemoral, n (%)	73 (65.8)	37 (66.1)	36 (65.5)		0.945
Medial femoral condyle	60 (54.1)	30 (53.6)	30 (54.5)		0.918
Lateral femoral condyle	19 (17.1)	9 (16.1)	10 (18.2)		0.768
Patellofemoral, n (%)	49 (44.1)	24 (42.9)	25 (45.5)		0.783
Patella, n (%)	23 (20.1)	9 (16.1)	14 (25.5)		0.223
Trochlea, n (%)	30 (27)	17 (30.4)	13 (23.6)		0.425
Bipolar defect lesion, n (%)	11 (9.9)	5 (8.9)	6 (10.9)		0.761

Tibio-and patellofemoral, n (%)	6 (5.4)	1 (1.8)	5 (9.1)		0.088
Number of grafts used per patient, n (%)	1.4±0.7 (1-3)	1.4±0.6 (1-3)	1.5±0.7 (1-3)	(-0.4 - 0.1)	0.273
2 grafts to the same location	22 (19.8)	9 (16.1)	13 (23.6)		0.317
Multifocal grafts, n (%)	18 (16.2)	6 (10.7)	12 (21.8)		0.129
Overlapping grafts configuration, n (%)	4 (3.6)	3 (5.4)	1 (1.8)		0.317
Smoker, n (%)	7 (6.4)	5 (8.9)	2 (3.6)		0.438

SD, standard deviation; y, year; n, number; CI, confidence interval; HTO, high tibial osteotomy; (TTO), tibial tubercle osteotomy; DFO, distal femoral osteotomy; MAT, meniscal allograft transplantation; ACL, anterior cruciate ligament reconstruction.

Table 2. Osteochondral allografts' characteristics

	Total	**19-24 days storage**	**25-28 days storage**	**(95% CI)**	**P value**
Donor age, y, mean SD (range)	23.7±6.5 (14-35)	23.2±6.9 (14-35)	24.2±6.1 (14-35)	(-3.4 – 1.5)	0.443
Gender difference (donor-recipient), n (%)	50 (51)	24 (49)	26 (53.1)		0.686
Storage time, days, mean SD (range)	24.2± 2.2 (19 - 27)	22.4±1.6 (19-24)	26±0.8 (25-27)	(-4.1 - 3.1)	< 0.01

SD, standard deviation; y, year; n, number; CI, confidence interval.

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4.1.2 OCA graft failure

Of the 111 patients fulfilling study inclusion criteria, 16 patients' grafts failed. Among these, 4 patients were in the early transplant group (19-24 days) and 12 were in the late transplant group (25-27 days), resulting in failure rates of 7.1% and 21.8%, respectively. (Table 3.)

Table 3. Storage time of each transplanted osteochondral allografts

	Storage day at the time of transplantation								
	Early transplantation (n=56)						Late transplantation (n=55)		
	19 days	20 days	21 days	22 days	23 days	24 days	25 days	26 days	27 days
Intact graft, n (%)	4 (80)	3 (100)	6 (100)	9 (90)	15 (100)	15 (88.2)	14 (82.4)	18 (81.8)	11 (68.8)
Failed graft, n (%)	1 (20)	0	0	1 (10)	0	2 (11.8)	3 (17.6)	4 (18.2)	5 (31.3)
Total, n	5	3	6	10	15	17	17	22	16

n, number.

Patients in the late transplant group had a significantly lower rate of graft survival at 5 years postoperatively (70.4%) compared to patients in the early transplant group (93.1%) ($p = 0.027$) (Figure 9.)

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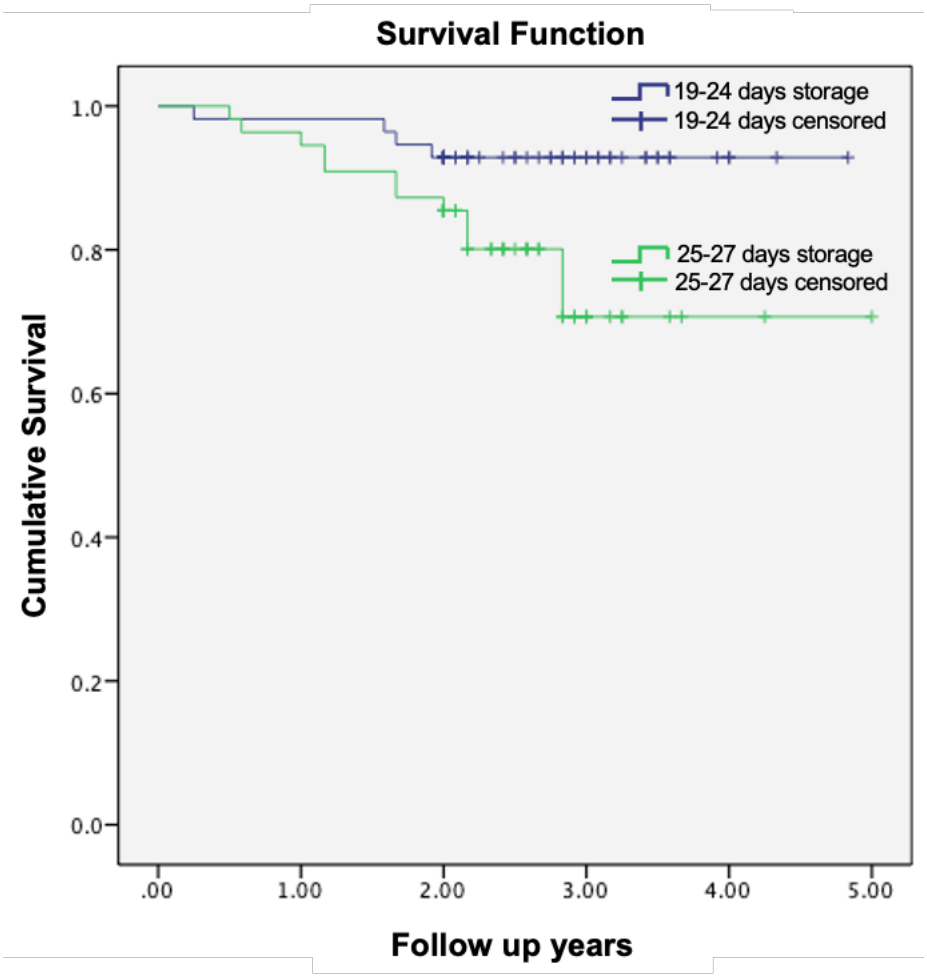

Figure 9. Survival of osteochondral allografts at 5 years for patients who received grafts stored for 19-24 days or 25-27 days.[61]

When correcting for patient age [105] and defect size [107] , both factors that have been associated with clinical outcome after OCA transplantation, multivariable Cox regression revealed that OCAs stored for 25-27 days were 3.39 times more likely to fail compared to grafts that were stored for only 19-24 days (P = 0.035) 5 years after OCA transplantation. (Table 4.)

Table 4. Multivariable Cox regression analysis of the influence of graft 25-27 days storage controlling for age and defect size on the survival of osteochondral allograft transplantation

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	Coefficient	P value	Hazard ratio	(95% CI)
Patient Age	-0.02	0.525	0.99	(0.94 - 1.03)
Defect size	-0.01	0.662	0.99	(0.95 - 1.03)
25-27 days storage	1.22	**0.035**	**3.39**	(1.09 - 10.5)

Subsequently, ROC analysis identified 24.5 days as an optimum cut point of OCA storage time associated with graft failure at 5 years with 75% sensitivity, 54.3% specificity and 0.68 AUC. (Figure 10.)

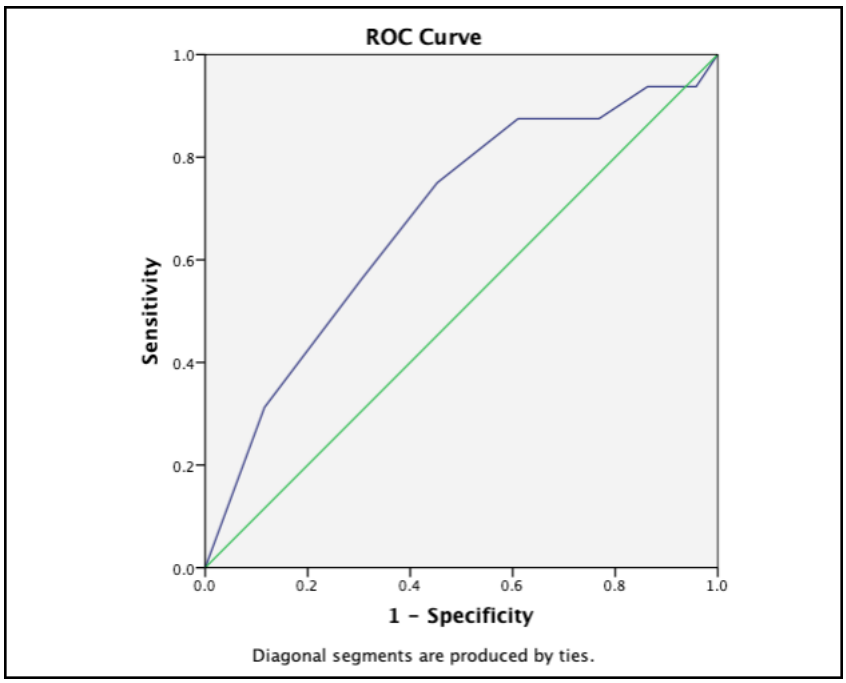

Figure 10. ROC analysis to define the days of storage cut point which was associated with osteochondral allograft failure. ROC, receiver operating characteristic.[61]

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4.2 OCA sex mismatch

4.2.1 Baseline demographics and clinical characteristics

Two-hundred and two patients underwent OCA transplantation between November 2013 and November 2017. Of these, 27 patients (13.4%) were excluded from this study due to incomplete data, and 21 patients (10.3%) were lost during the follow up period. Hence, 154 patients (76.2%) met study inclusion criteria. Of these patients, 102 (66.2%) received OCAs from a same sex donor, while 52 patients (33.8%) underwent OCA transplantation from a donor of different sex. The mean age at time of surgery was 34.7 ± 10.7 years (range, 15 – 56 years) with a mean follow-up of 3.3 ± 1.2 years (range, 2 – 7 years). Baseline demographics and clinical characteristics of the study patient population are outlined in Table 5.

Table 5. Demographics data of patients who underwent osteochondral allograft transplantation with a graft from a similar sex or different sex donor

	Total	Similar donor-recipient sex	Donor-recipient sex mismatch	(95% CI)	P value
Number of patients (%)	154	102 (66.2)	52 (33.8)		
Age, y, mean SD (range)	34.7±10.7 (15-56)	35±10.7 (15-56)	34.1±10.9 (13-56)	(-2.6 - 4.6)	0.593
BMI (kg/m^2), mean SD (range)	27.4±5.2 (17 - 39.5)	27.4±4.6 (18-39)	27.4±6.2 (17-39.5)	(-1.8 - 1.7)	0.948
Defect location					
Medial tibiofemoral compartment, n (%)	80 (51.9)	52 (51)	28 (53.8)		0.736

Lateral tibiofemoral compartment, n (%)	34 (22.1)	24 (23.5)	10 (19.2)		0.543
Patellofemoral compartment, n (%)	65 (42.2)	42 (41.2)	23 (44.2)		0.717
OCA graft size cm	2.4±1.1 (1-7.2)	2.4±0.9 (1-6.1)	2.4±1.4 (1-7.2)	(-3.5 - 5.6)	0.988
HTO, n (%)	13 (8.4)	11 (10.8)	2 (3.8)		0.143
TTO, n (%)	19 (12.3)	11 (10.8)	8 (15.4)		0.412
DFO, n (%)	1 (0.6)	1 (1)	0		
MAT, n (%)	6 (3.9)	3 (2.9)	3 (5.8)		0.391
ACL, n (%)	5 (3.2)	2 (2)	3 (5.8)		0.207
Smoker, n (%)	7 (4.5)	3 (2.9)	4 (7.7)		0.181

SD, standard deviation; y, year; n, number; CI, confidence interval; OCA, osteochondral allograft; HTO, high tibial osteotomy; TTO, tibial tubercle osteotomy; DFO, distal femoral osteotomy; MAT, meniscal allograft transplantation; ACL, anterior cruciate ligament reconstruction.

4.2.2 OCA graft failure

Of the 154 patients fulfilling study inclusion criteria, 18 patients' grafts failed (11.7%). Among these, seven patients were in the similar donor-recipient sex group and eleven were in the donor-recipient mismatch group, resulting in failure rates of 6.9% and 21.2%, respectively. (Table 1.) Patients who received an OCA graft from a different sex donor had a significantly lower rate of graft survival at five years postoperatively (63%) compared to patients who underwent OCA graft transplantation from a similar sex donor (92%) ($p = 0.01$). (Figure 11.) While the gender of the recipient alone was not associated with lower survival rate at 5 years ($p=0.179$)

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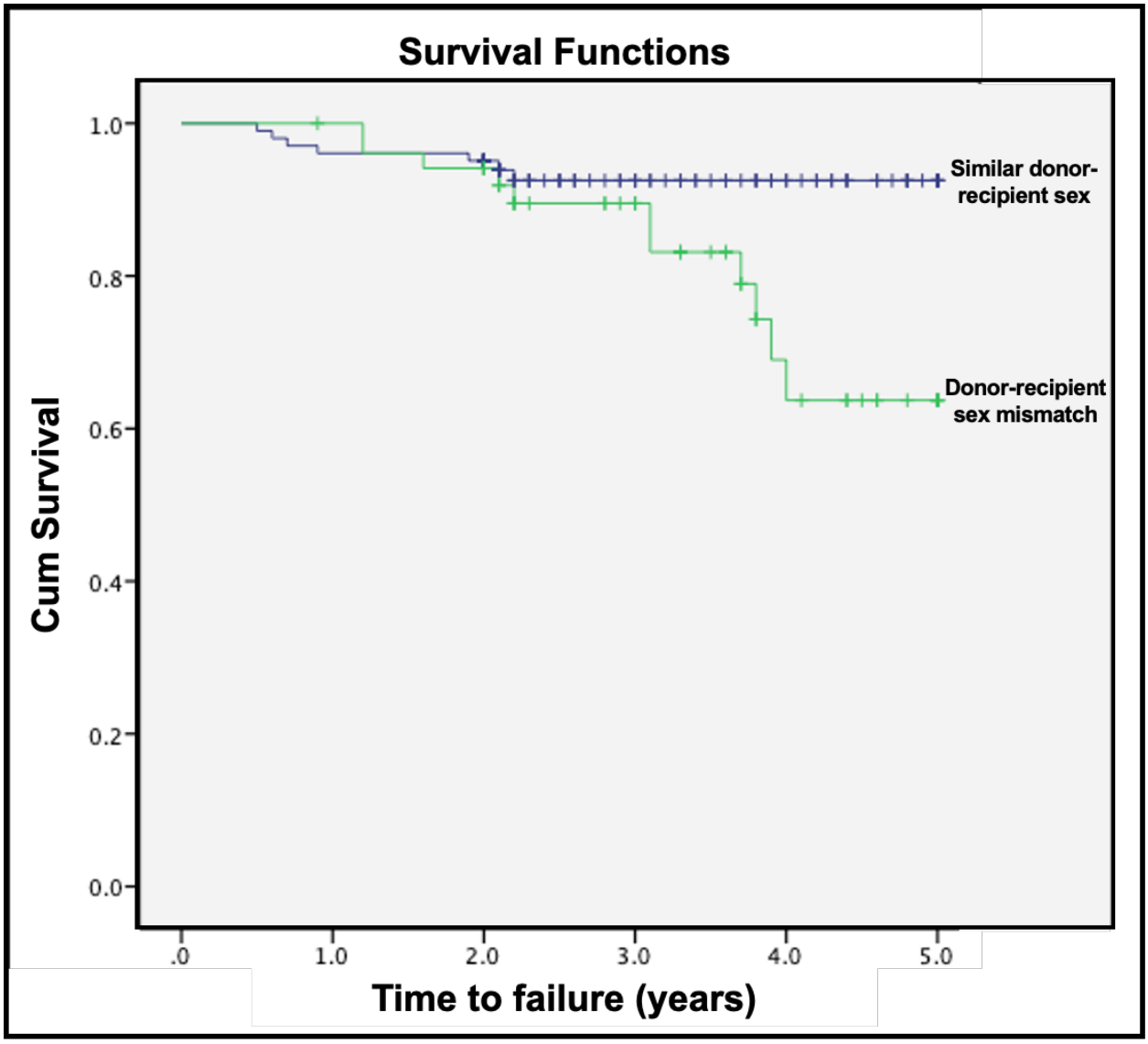

Figure 11. Kaplan–Meier curve of five-year survival by donor–recipient sex. Lower cumulative five-year survival was found in patients who received an OCA graft from a different sex donor compared to patients who had OCA graft from a similar sex donor (p = 0.02).[148]

When correcting for patient age, [105] graft size, [56,107] and BMI, [147] multivariable Cox regression revealed that sex mismatch OCA transplantation is 2.9 times more likely to fail at five years compared to patients who received OCA grafts from a similar sex donor (p = 0.03). (Table 6.)

Table 6. Multivariable Cox regression analysis of the influence of donor and recipient sex mismatch controlling for BMI, osteochondral allograft graft size and age on the survival of osteochondral allograft transplantation

	Coefficient	P value	Hazard ratio	(95% CI)
BMI	0.05	0.26	1.05	(0.96 - 1.15)
OCA size	-0.01	0.78	1.00	(0.96 - 1.03)
Age	-0.02	0.36	0.98	(0.93 - 1.02)
Sex mismatch	1.06	**0.03**	**2.87**	(1.11 - 7.44)

OCA, osteochondral allograft; CI, confidence interval.

Subsequently, patients were further divided into 4 subgroups: male graft transplanted to a male recipient (male-to-male, MtM); female graft transplanted to a female recipient (female-to-female, FtF); male graft transplanted to a female recipient (male-to-female, MtF); and female graft transplanted to a male recipient (female-to-male, FtM). (Table 7.)

Table 7. Demographics data of patients who underwent osteochondral allograft transplantation with a graft from a similar sex or different sex donor further divided into four groups based on donor and recipient gender.

	Male donor, male recipient	Female donor, female recipient	Male donor, female recipient	Female donor, male recipient	(95% CI)	P value

Number of patients (%)	71	31	46	6		
Age, y, mean SD (range)	36±.10.6 (15-56)	32.9±10.7 (16-49)	33.4±10.6 (13-52)	34.7±10.7 (13-56)	(-2.6 - 4.6)	0.593
BMI (kg/m^2), mean SD (range)	28±4.7 (18-39)	25±9.7 (19-34)	26.6±6 (17-39.5)	27.4±5 (17-39.5)	(-1.8 - 1.7)	0.948
Defect location						
Medial tibiofemoral compartment, n (%)	37 (52.1)	15 (48.4)	26 (56.5)	2 (33.3)		0.711
Lateral tibiofemoral compartment, n (%)	16 (22.5)	8 (25.8)	9 (19.6)	1 (16.7)		0.912
Patellofemoral compartment, n (%)	31 (43.7)	11 (35.5)	19 (41.3)	4 (66.7)		0.547
OCA graft size	2.5±1 (1-6.1)	2.2±0.9 (1-4.5)	2.5±1.4 (1-7.2)	2.5±1.1 (1-7.2)	(-3.5 -5.6)	0.988
HTO, n (%)	**11 (15.5)**	**0**	**1 (2.2)**	**1 (16.7)**		**0.016**
TTO, n (%)	8 (11.3)	3 (9.7)	8 (17.4)	0		0.53
DFO, n (%)	1 (1.4)	0	0	0		
MAT, n (%)	2 (2.8)	1 (3.2)	3 (6.5)	0		0.718
ACL, n (%)	1 (1.4)	1 (3.2)	3 (6.5)	0		
Smoker, n (%)	3 (4.2)	0	3 (6.5)	1 (16.7)		

SD, standard deviation; y, year; n, number; CI, confidence interval; OCA, osteochondral allograft; HTO, high tibial osteotomy; TTO, tibial tubercle osteotomy; DFO, distal femoral osteotomy; MAT, meniscal allograft transplantation; ACL, anterior cruciate ligament

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reconstruction. Bold values denote statistical significance at the $p < 0.05$ level; $^{*}p < 0.05$ vs female to female.

There was no significant difference in survival between male-to-male and female-to-female groups; and between female-to-female and female-to-male groups (female-to-female 91%; female-to-male 84%). However, a significantly higher cumulative five-year survival was found in the male-to-male group (94%, $p = 0.04$) and lower cumulative five-year survival was found in the male-to-female group (64%, $p = 0.04$). (Figure 12.)

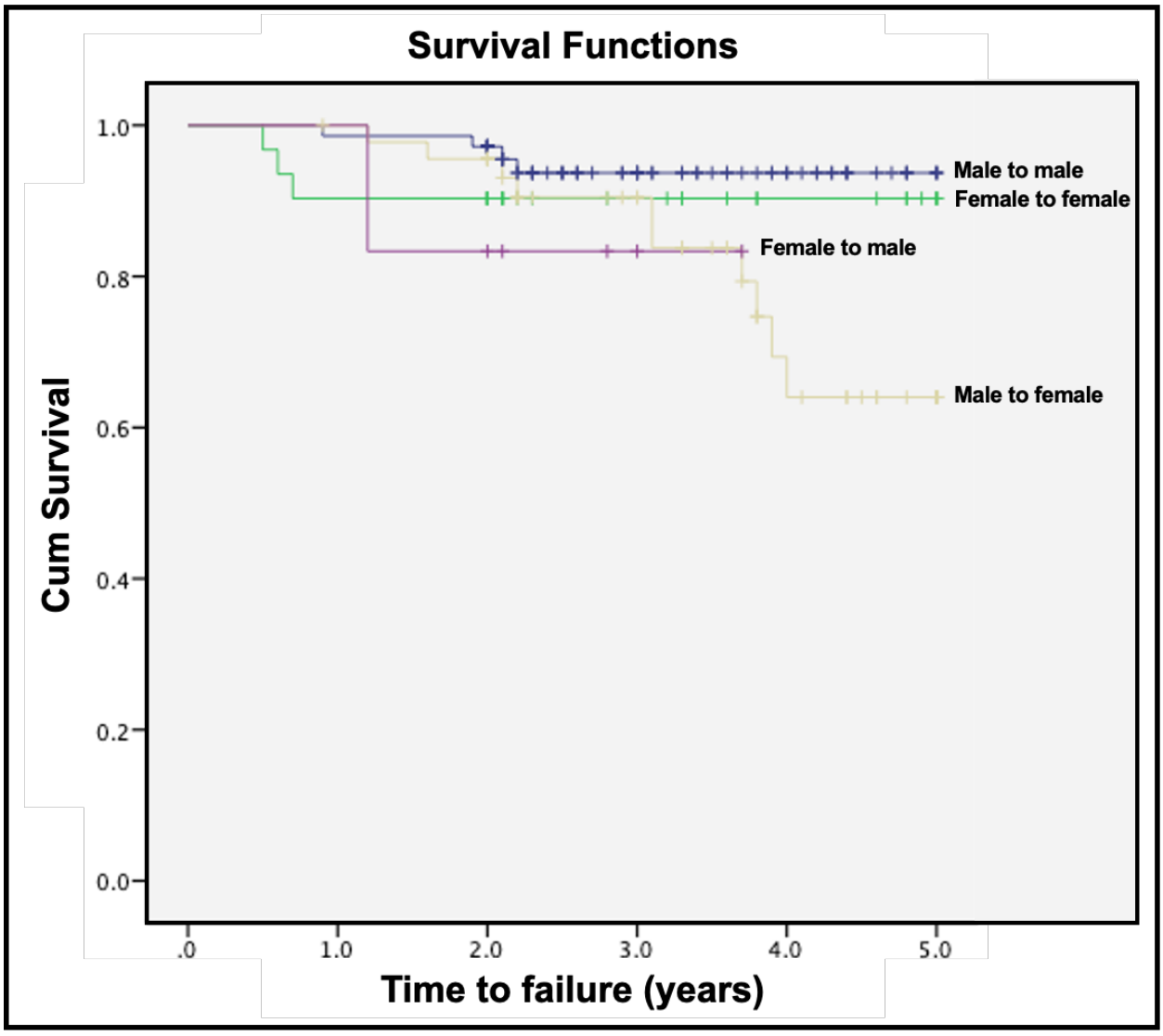

Figure 12. Kaplan–Meier curve of five-year survival by donor–recipient sex. There was no significant difference in survival between male to male, female to female and female to male

groups. However, lower cumulative five-year survival was found in the male to female group. ($p = 0.02$).[148]

Multivariable COX regression indicated that in those cases when a male graft was transplanted to a female recipient, the OCA graft had a 2.6 times higher chance to fail compared to other groups ($p = 0.04$) when correcting for age [105], graft size [56,107] and BMI [147]. Conversely, male graft to male donor showed a strong tendency toward decreased likelihood of OCA graft failure with a 0.33 hazard ratio, however this value did not reach a statistically significant threshold. OCA graft survival was similar between the other groups. (Table 8.)

Table 8. Multivariable Cox regression analysis of the influence of donor and recipient sex mismatch controlling for BMI, osteochondral allograft graft size and age on the survival of osteochondral allograft transplantation

	P value	Hazard ratio	(95% CI)
Male donor, male recipient	0.052	0.33	(0.11 - 1.01)
Female donor, female recipient	0.84	0.88	(0.25 - 3.1)
Male donor, female recipient	**0.04**	**2.63**	**(1.03 - 6.69)**
Female donor, male recipient	0.55	1.904	(0.24 - 15.35)

Bold values denote statistical significance at the $p < 0.05$ level

4.3 Improvement of the current OCA storage

4.3.1 Full-thickness cell viability of the bOC explants after storage

Chondrocyte viability in full-thickness cartilage at 4˚C-AP was significantly lower at 21 days (81.9 ± 5.5%, $p < 0.05$) and at 28 days (71.6 ± 6.5%, $p < 0.05$) than the fresh explant control (92.9 ± 3.7%). (Figure 13 and 14.) Also, the viability at 37˚C-AP was significantly lower at 21 days (78.0 ± 3.9%, $p < 0.05$) and at 28 days (65.8 ± 6.1%, $p <$

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0.05) than the fresh control. However, chondrocyte viability at 37˚C-HP was maintained at 21 days (88.3 ± 5.2%) and at 28 days (88.0 ± 4.2%, p = 0.827) compared to the fresh control. To compare among storage conditions, chondrocyte viability at 37˚C-HP was significantly higher than 37˚C-AP at 21 days ($p < 0.05$) and 28 days ($p < 0.05$), as well as higher than 4˚C-AP at 28 days ($p < 0.05$).

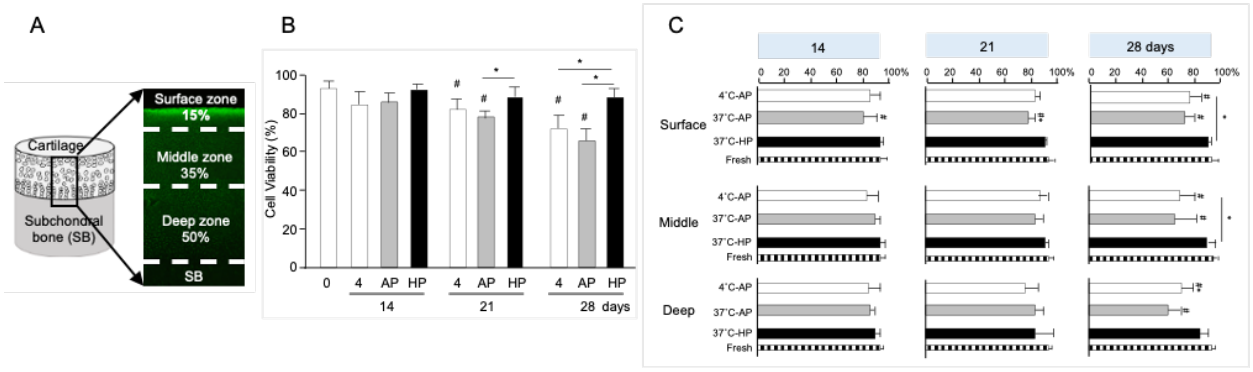

Figure 13. Full thickness and zonal (surface, middle, deep) chondrocyte viability in different storage conditions. A) Surface, middle and deep cartilage zones demonstrated on an osteochondral explant stained with calcein (green demonstrate live cells) B) Full thickness chondrocyte viability in different storage conditions. C) Zonal chondrocyte viability in different storage conditions. * Indicates significant difference compared to 37˚C-HP group ($p < 0.05$), # indicates significant difference compared to a fresh explant ($p < 0.05$). Error bar represents standard deviation. (Based on a manuscript under review.)

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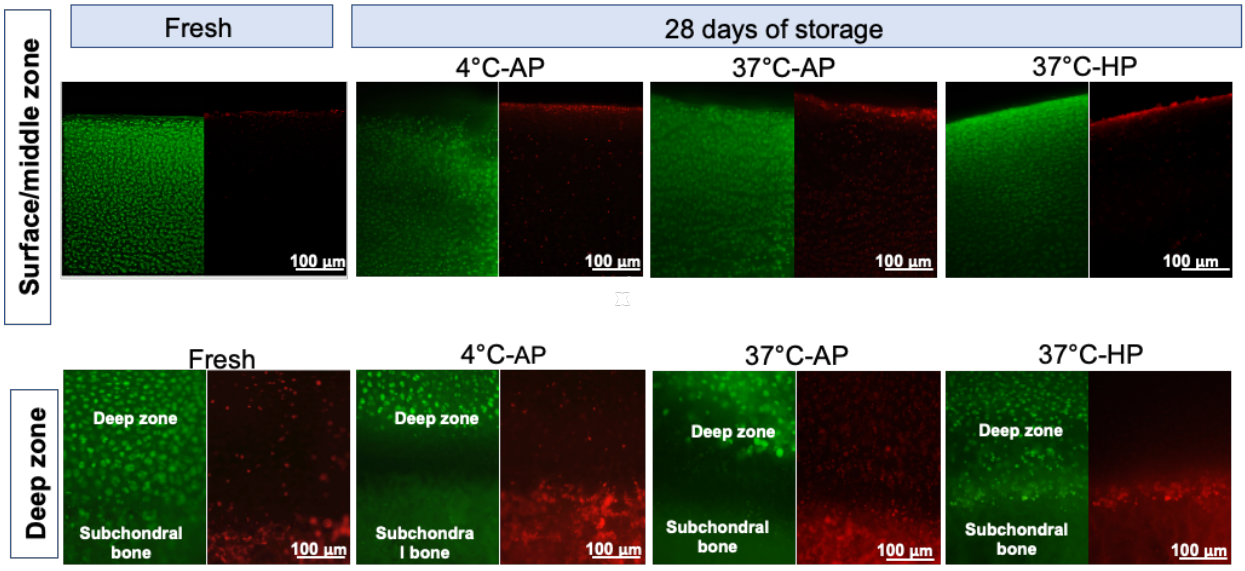

Figure 14. Live and dead chondrocytes in surface/middle and deep zone articular cartilage in defined storage conditions. Green cells indicate the living chondrocytes while red cells indicate the dead chondrocytes. (Based on a manuscript under review.)

4.3.2 Zone-specific cell viability of the bOC explants after storage

In the surface zone, chondrocyte viability at 4˚C-AP was significantly decreased at 28 days (76.3 ± 8.9%, $p < 0.05$) compared to fresh explant control (93.2 ± 5.0%). (Figure 13.) Chondrocyte viability significantly decreased at 37˚C-AP at 14 days (80.9 ± 10.7%, $p < 0.05$), 21 days (78.2 ± 5.5%, $p < 0.01$) and 28 days (72.4 ± 7.3%, $p < 0.05$) compared to fresh control. However, chondrocyte viability at 37˚C-HP was maintained by 28 days (90.4 ± 2%, $p = 0.999$) and did not differ significantly from fresh control. In addition, viability at 37˚C-HP at 21 days (90.8 ± 1.6%) was significantly higher than at 37˚C-AP at both 21 days and 28 days ($p < 0.05$).

In the middle zone, chondrocyte viability at 4˚C-AP at 28 days (68.5 ± 12.3%, $p < 0.05$) was significantly lower than fresh control (94 ± 3.7%). (Figure 13.) Chondrocyte viability after 37˚C-AP storage was also significantly inferior at 28 days (65.4 ± 16.6%, $p < 0.05$) compared to fresh control. However, when stored at 37˚C-HP cell viability was maintained and did not differ significantly from fresh control at 28 days (89.7 ± 6%,

p=0.998). In addition, viability at 37˚C-HP was significantly higher than at 4˚C-AP and 37˚C-AP, respectively at 28 days ($p < 0.05$).

In the deep zone, chondrocyte viability was significantly lower than fresh explant control (91.5 ± 2.9%) at 4˚C-AP at 28 days (70.2 ± 9.3%, $p < 0.01$) and at 37˚C-AP at 28 days (59.7 ± 9.5%, $p < 0.05$). (Figure 13.) However, viability at 37˚C-HP at 28 days (84.1 ± 6.9%, $p = 0.889$) remained similar to that of the fresh control at 28 days. In addition, chondrocyte viability at 37˚C-HP was significantly higher than at 37˚C-AP at 28 days ($p < 0.05$).

At the interface between the deep zone and subchondral bone, only dead cells were seen in all OC explants stored at 37˚C-AP at 28 days. (Figure 14.) We refer to this as the "dead chondrocyte area." This was the case only in the middle of the explant, while in the periphery chondrocytes were living and the dead chondrocyte area was missing. (Figure 15) Under 37˚C-HP and 4˚C-AP the dead chondrocyte area was smaller, with fewer dead cells, and it was present in only 50% of the explants compared to 100% stored at 37˚C-AP.

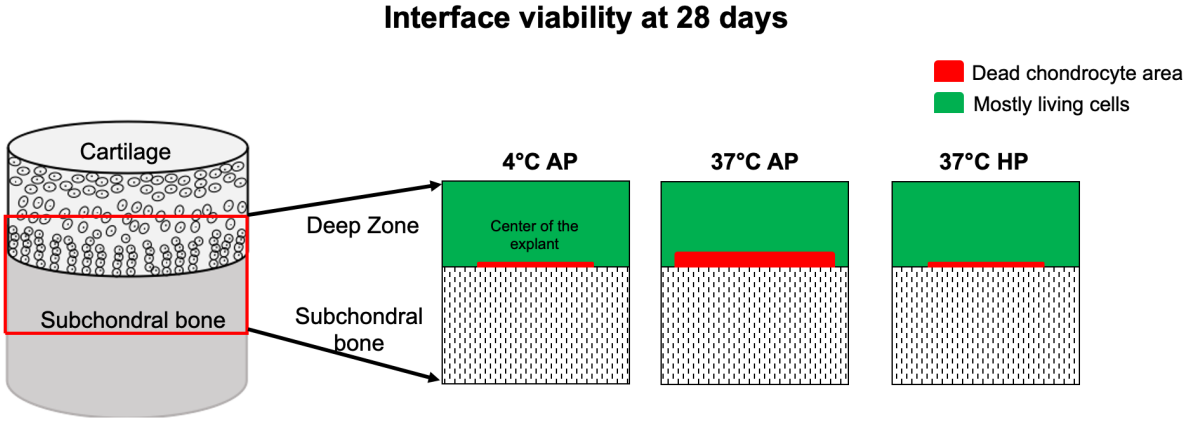

Figure 15. Interface chondrocyte viability at 28 days (dead chondrocyte area) in different groups at 28 days. At the interface between deep zone and subchondral bone, no live cells were observed with only dead cells in all OC explants at 37˚C-AP at 28 days. These findings were only present in the center of deep zone, while in the periphery chondrocytes were living and the dead chondrocyte area was missing. Under 37˚C-HP and 4˚C AP the "dead chondrocyte area" was smaller with less dead cells, and it was present in 50% of the grafts compared to 100% at the 37°C-AP. (Based on a manuscript under review.)

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4.3.3 Cartilage thickness of the bOC explants after storage

The thickness of freshly harvested cartilage was uniform between the groups. Allocation of the bOC explants into the study groups resulted in an average thickness of 2.8 ± 0.4 mm. (Figure 16.) Cartilage thickness was reduced with time when stored at 4˚C-AP and consequently was significantly thinner at 21 days (2.4 ± 0.5 mm, $p < 0.05$) and 28 days (1.7 ± 0.8 mm, $p < 0.05$) compared to freshly harvested bOC explants or 37˚C-HP group (3.0 ± 0.6 mm) at 28 days ($p < 0.05$). On the other hand, cartilage thickness of bOC explants stored at 37˚C-AP was significantly thicker at 21 (4.5 ± 0.6 mm) and 28 days (4.7 ± 0.3 mm) compared to the fresh control, respectively ($p < 0.05$). bOC explants stored at 37˚C-HP maintained their cartilage thickness by 28 days, similar to that of fresh control.

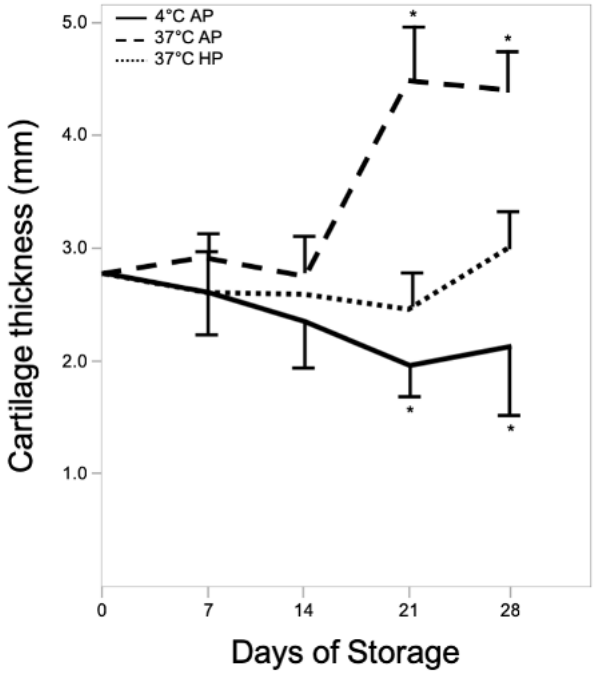

Figure 16. Cartilage thickness in different storage conditions. Cartilage thickness was maintained in the 37°C HP group at 28 days, however, it was significantly thinner at 4°C and thicker at 37°C AP groups at 21 and 28 days compared to freshly harvested control. (*P < 0.05). Error bar represents standard deviation. (Based on a manuscript under review.)

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4.3.4 Histology of bOC explants after storage

We evaluated whether different storage conditions had an effect on histopathology using the HHGS scoring system (Table 9).

Table 9. Histological-histochemical grading system (HHGS) scored in bOC explant. Only those categories of the system are demonstrated which were identified in the samples. Numbers indicate the number of grafts with the morphology at a certain storage condition (n = 6). (Based on a manuscript under review.)

		Fresh (0 days)	14 days			21 days			28 days		
			4°C-AP	37°C-AP	37°C-HP	4°C-AP	37°C-AP	37°C-HP	4°C-AP	37°C-AP	37°C-HP
	Score										
Structure											
Normal	0	6	6	5	6	5	2	4	4	1	5
Surface irregularities	1	0	0	1	0	1	4	2	2	5	1
Cells											
Normal	0	6	4	2	5	0	0	4	0	1	2
Diffuse hypercellularity	1	0	2	0	1	3	1	1	4	1	1
Cloning	2	0	0	2	0	1	1	1	0	1	0
Hypocellularity	3	0	0	2	0	2	4	0	2	3	3
Safranin O staining											
Normal	0	6	1	1	2	0	0	0	0	0	0
Slight reduction	1	0	5	5	3	3	4	4	2	1	1
Moderate reduction	2	0	0	0	1	3	2	2	3	3	4
Severe reduction	3	0	0	0	0	0	0	0	1	2	1
HGSS mean ± SD		0	1.2 ± 0.8	2.7 ± 1.8	1.0 ± 0.9	3.5 ± 1.6	5.0 ± 1.8	2.2 ± 1.2	3.8 ± 1.7	5.0 ± 1.8	3.8 ± 1.9

After 14 days of storage, bOC explants stored at 4˚C-AP exhibited slightly less safranin-O with diffuse hypercellularity (HHGS score 1.2 ± 0.8). bOC explants stored at 37˚C-AP received a score of 2.7 ± 1.8 because of slightly reduced safranin-O staining and hypocellularity with clusters of cells. In contrast, only one explant stored at 37˚C-HP displayed diffuse hypercellularity, accompanied by a slight reduction in safranin-O staining, resulting in a lower score: 1.0 ± 0.9. (Figure 17.)

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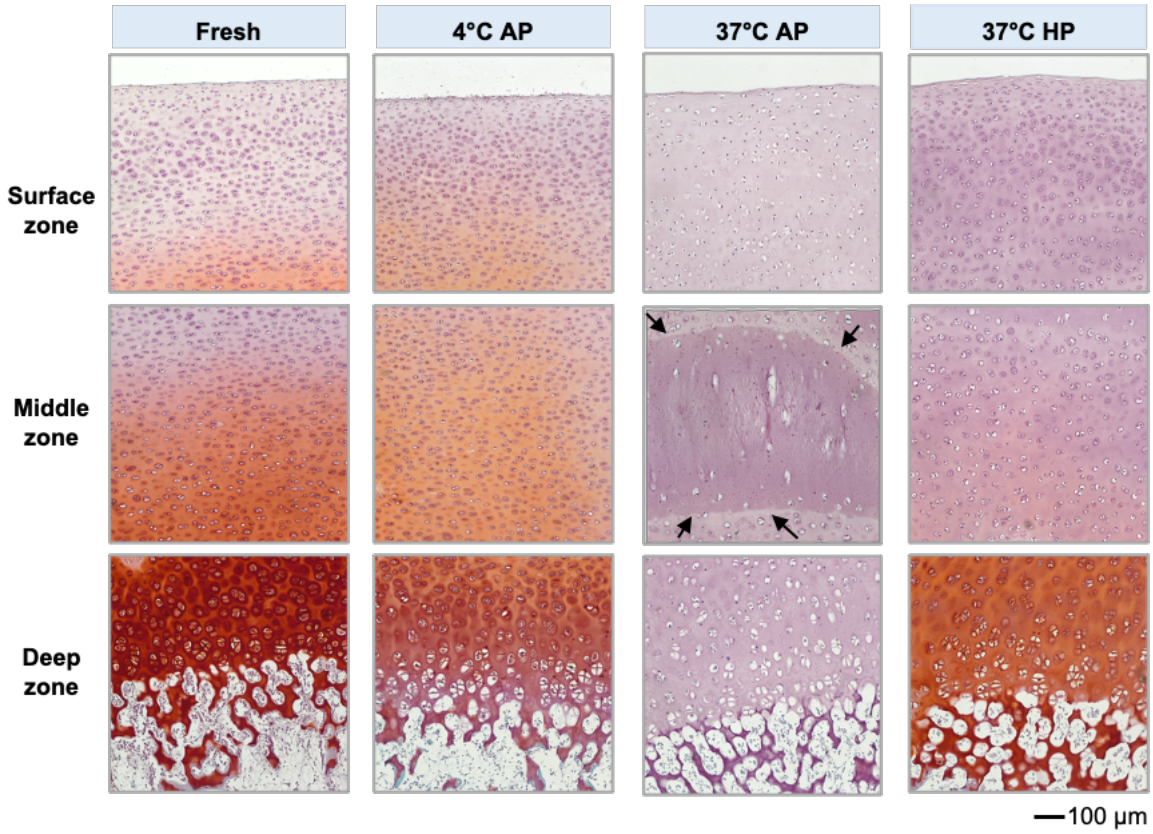

Figure 17. Safranin-o fast green staining of the superficial, middle and deep zone in the different groups at 28 days. Arrows indicate lightly stained cartilaginous matrix between the surface and middle zone in 37°C-AP group. (Based on a manuscript under review.)

After 21 days of storage, moderately reduced safranin-O staining three out of six explants (3/6) was seen at 4˚C-AP with either hypercellularity alone or hypocellularity and cloning (3.5 ± 1.6). At 37°C-AP the majority of the bOC explants displayed only slight reduction in safranin-O staining (4/6 bOC), however, because of surface irregularities, cloning, and hypocellularity, the score was 5 ± 1.8. Moreover, in two explants at 37°C-AP, lightly stained cartilage matrix between the surface and middle zone was seen. (Figure 17.) At 37˚C-HP, 4 out of 6 samples showed a slight reduction in safranin O-staining and 4 out of 6 samples had normal cells, resulting in a lower score compared to other conditions (2.2 ± 1.2). However, in one sample lightly stained cartilaginous matrix was also observed between the surface and middle zone.

After 28 days the histological scores increased in each storage condition. At 4˚C-AP, more diffuse hypercellularity (4/6 bOC), hypocellularity (2/6 bOC) and moderately reduced safranin-O staining (moderate in 3/6 bOC samples and severe in 2/6 bOC) was observed (3.8 ± 1.7). (Figure 17.) At 37˚C-AP, surface fibrillation and hypocellularity was seen together with marked reduction of safranin-O staining (slight reduction in 1/6 bOC, moderate reduction in 3/6 bOC, and severe reduction in 2/6 bOC) for a total score of 5.0 ± 1.8. In addition, lightly stained with safranin-O between the surface and middle zone was seen in 5 bOC explants. At 37˚C-HP, the safranin-O staining was also moderately decreased (slight reduction in 1/6 and moderate reduction in 4/6, severe reduction in 1/6 bOC) compared to fresh explants, with hypocellularity and lightly stained with safranin-O between the surface and middle zone in 3/6 bOC, which resulted in a score of 3.8 ± 1.9.

4.3.5 Immunohistology of bOC explants after storage

KS staining decreased over time in every group. In general changes in KS staining were overall similar to changes in safranin-O staining. However, the large decrease in KS staining was found in the surface zone at 4˚C-AP.

Col-2 staining was similar in the surface zone at 28 days in every group. (Figure 18.) However, Col-2 staining decreased in the middle and deep zone at 4˚C-AP and in the deep zone at 37˚C-AP at 28 days. With the application of HP Col-2 staining was maintained in these cartilage regions.

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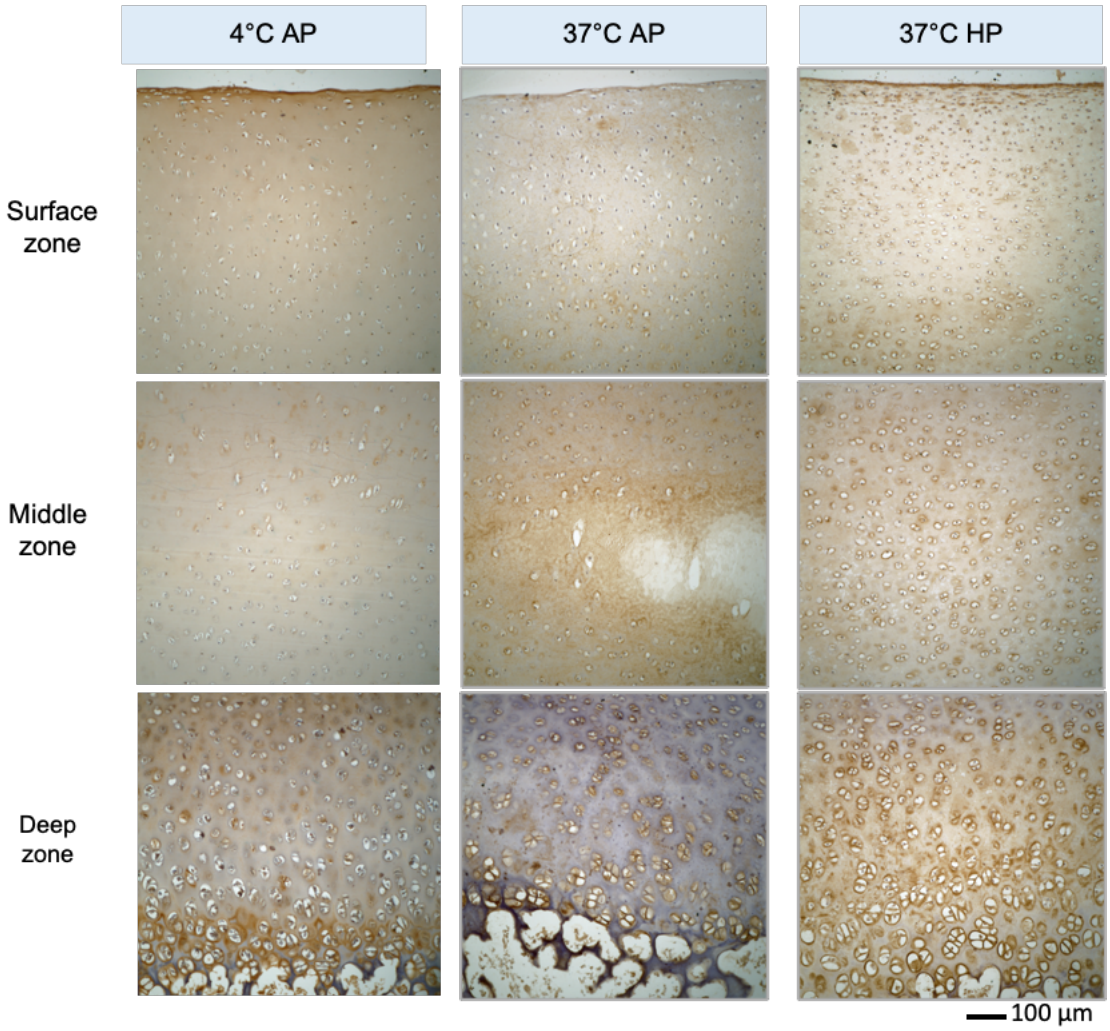

Figure 18. Collagen 2 staining of the superficial, middle and deep zone in different groups at 28 days. (Based on a manuscript under review.)

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5 Discussion

There are three main findings of my thesis:

1. Patients who underwent late OCA transplantation (25-27 days graft storage) had significantly lower graft survivorship at 5 years compared to patients who received early OCA transplantation (19-24 days graft storage) (70.4% vs. 93.1%, respectively). These rates of graft survival and their inverse correlation with graft storage time are in accordance with the findings of previous pre-clinical and clinical studies.[23,26, 28-32] Taken together with the findings of numerous other investigations, our study adds strong evidence to support the successful survivorship of OCAs in the treatment of osteochondral defects of the knee. The improved survivorship of early transplantation grafts at >90% is evident at the 2-year post-operative time point and persists out to 5 years, whereas the survivorship of late transplantation grafts continues to decline. (Figure 9.) Furthermore, storage time and in-situ OCA survival exhibit a strong association at 5 years, with graft storage time beyond 25 days resulting in a 3.4 times greater likelihood of failure (Hazard ratio 3.39, 95% CI 1.09-10.5, p=0.035), even after adjusting for two factors (age[105] and defect size[107]) that have been implicated in worse clinical outcomes after OCA transplantation. (Table 4.)

2. Mismatch between donor and recipient sex results in a significantly lower rate of OCA survival following transplantation (63% vs. 92%, respectively) at five years. (Figure 11.) In addition, donor-recipient sex-mismatch and in-situ OCA survival exhibit a strong negative association at five years, with sex-mismatch associated with a 2.9 times greater likelihood of failure, even after adjusting for age [105], graft size [56,107] and BMI [147] each of which have been implicated in worse clinical outcomes after OCA transplantation. (Table 6.) Sex-mismatch demonstrated the largest effect in those cases when grafts from a male donor were transplanted to a female recipient. In this group the cumulative 5-year survival was markedly decreased with a 2.6 times higher chance of OCA failure. (Table 8.)

3. The application of HP at 37˚C maintained higher viability of chondrocytes within full-thickness articular cartilage explants over storage conditions at 4˚C-AP and 37˚C-AP. (Figure 13 and 14.) This difference was observed in every longitudinal depth zone under HP compared to other storage conditions. Cartilage thickness changes were also observed under 37˚C-AP and 4˚C-AP, with a significant increase and decrease in cartilage thickness at 28 days compared to freshly harvested bOC explants control. (Figure 16.) Storage with HP for 28 days, allowed for articular cartilage thickness to be similar to the fresh explant control. In addition, a smaller reduction in S-GAG stained with safranin-O was observed at 37˚C-HP and at 37˚C-AP compared to 4˚C-AP by 28 days. Morphologically, the bOC explants showed similar cartilage appearance at 37˚C-HP and 4˚C-AP compared to fresh control, while surface fibrillation and structural irregularities in the explants appeared at the 37˚C-AP. (Figure 17.) Consequently, we believe that the addition of HP to 37˚C provides further benefits which results in high chondrocyte viability and cartilage integrity after OC storage.

5.1 OCA storage time

Data recently published by Schmidt et al. and Rauck et al. refute the correlation of increased OCA storage time with clinical outcomes and graft delamination, respectively.[117,118] Schmidt et al performed a matched pair cohort study of 75 patients who received early release OCAs (mean storage 6.3 days) between the years of 1997 and 2002; matched 1:1 by age, diagnosis, and graft size, with 75 patients who received grafts between 2002 and 2008 with a mean storage time of 20 days after regulatory requirements had changed. Evaluating the primary outcomes of knee pain, patient satisfaction, function, graft failures, and reoperations; they concluded that there was no difference in postoperative pain and function noted between the groups.[117] The fact that these matched cohorts underwent noncontemporary interventions represents the greatest limitation of this study. Though controlling for a number of patient factors; there are a variety of confounding variables that may have compromised the results. One of the most notable confounders of the study by Schmidt et al is the shift in graft supply and storage conditions before and after October 2002.

Allografts in the early release group prior to October 2002 were obtained from the University of California, San Diego tissue banks and were stored in Ringer's lactate solution from time of procurement until time of transplantation. Contrastingly, allografts in the late release group after October 2002 were obtained from the Joint Restoration Foundation and stored in their proprietary nutritive culture media after initial processing, and for up to 28 days prior to transplantation. Chondrocyte viability is known to benefit from storage in nutritive media compared to Ringer's lactate solution.[149,150] It is also possible that noncontemporary surgeon and technical factors may have affected outcomes in this study; specifically, surgeon experience and technical learning curve over time may have allowed for improved outcomes in the late release group. The authors of this study acknowledge these confounders, and also make mention of the greater number of shell grafts implanted in the early release group (through a larger surgical approach) compared to dowel grafts (through smaller surgical exposure) in the late release group.[117]

All OCAs in our study were obtained from the same vendor (JRF Ortho); and procured, processed, and stored under identical conditions. Furthermore, all OCAs were transplanted contemporaneously, by the same surgeon, at the same center, and using the same surgical technique.

Rauck et al. examined matched cohorts of patients with and without evidence of OCA delamination; matched based on recipient age, sex, BMI, and chondral defect location.[118] Their match cohort size was established based on a power calculation needed to detect a difference in storage time of 4 days or more between groups (power of 0.80 and significant level of alpha =0.05). Their results showed no association between OCA storage time and graft delamination.[118] However, the average storage time was 30 days in the delamination group and 31 days in the control group; a statistically insignificant difference with p =0.78.[118] The authors did not publish OCA storage time ranges (maximums, minimums) for their cohorts. With the majority of OCAs in the United States transplanted within 15-28 days (average 24 days), a distinguishable difference in average OCA storage time as powered in this study falls on the late extent, and largely outside of the average practice in the United States. [7,108,109,119] Therefore, the objective of the present study was to specifically focus on differences in OCA survival within this timeframe. Given the constraints of current allograft regulations here in the United States, we also sought to inform best practices going forward

by identifying the timepoint for OCA transplantation that maintains an optimal rate of graft survivorship at 5 years. ROC analyses in our dataset calculate a threshold of 24.5 days (with 75% sensitivity, 54.3 % specificity, and 0.68 area under the curve) --- suggesting that OCA transplantation here in the United States should ideally occur before 25 days of graft storage time. (Figure 10.)

It is intriguing to consider whether there are ways to further improve the OCA survivorship and clinical outcomes. One way to enhance the in vivo performance of the graft may be afforded by transplantation at time points earlier than 14 days. Given our knowledge that OCA chondrocyte viability shares an inverse correlation with graft storage time;[112,114,115,151] it is plausible to extrapolate improved graft survivorship and outcomes with earlier "fresh" OCA transplantation.[152] However, this technique would require regulatory changes to allow expedited screening. Alternatively, other venues of prolonging chondrocyte survival by changing and improving the current storage technique to extend the window past 28 days safely are being pursued by multiple authors and were amongst the objectives of this thesis. [112,114,121,122]

5.2 Sex mismatch

Articular cartilage has long been regarded as an immunologically privileged tissue believed to provoke little or no rejection after transplantation,[153] even though both cellular[154] and acellular[155] components of cartilage have each been shown to evoke an immune reaction[138,154,156,157] The dogma that articular cartilage is an immunologically inert tissue stems from a belief that the avascular, intact chondral matrix functions as a physiologic barrier inhibiting egress of cartilage antigens[139] and ingress of immune surveillance cells[138,155,158], thus protecting chondrocytes within.[102,153,154,159] Conversely, allograft bone is known to be antigenic. [138,155,160,161] As such, the subchondral osseous component, rather than the superficial chondral portion, of osteochondral allograft plugs is thought to be responsible for eliciting host sensitization and immune reaction.[133,138,153,162,163] The immunologic load of OCA grafts has been directly associated with 1) the burden/size of osseous transplant tissue, 2) the use of fresh, as opposed to frozen graft tissue, and 3) the resultant systemic serologic response observed after implantation.[153,164,165]

A number of studies have sought to understand the clinical consequences of the host immune reaction to OCAs, as well as the role such a response may play in potential OCA rejection or graft failure. Stevenson et. al. studied the role of histocompatibility leukocyte antigen-matching on systemic immune reaction (measured by serum titers) as well as chondrocyte viability (evaluated histologically) in a canine OCA model; concluding that antigen-mismatched OCAs developed a significantly larger immune response with worse chondrocyte viability/histologic scores than antigen-matched grafts.[139] Sirlin et. al. also showed that human recipients of shell OCAs may develop serum anti-HLA class I antibodies after transplantation.[138] Furthermore, antibody-positive patients also demonstrated signs of increased inflammation and less complete graft incorporation (as evaluated by MRI) when compared to patients who remained antibody-negative after transplantation. Interestingly, despite these findings, the development of anti-HLA antibodies after transplantation did not correlate with increased rates of frank clinical graft failure in this study.[138] Two retrieval studies of human failed fresh OCAs did not appreciate significant immune mediated reactions nor evidence of frank rejection when grafts were examined histologically.[166,167] More recently, Hunt et. al evaluated the development of anti-HLA antibodies and post-operative outcomes in 84 patients receiving fresh, nonmatched OCAs.[102] They concluded that Knee Society function scores were equivalent between antibody-positive and antibody-negative groups. However, they appreciated a non-significant trend in graft survival at a mean follow-up of 52.7 months (range, 24 – 165 months), with antibody-positive patients demonstrating a lower graft survival rate of 64%, compared to a higher rate of graft survival of 79% in antibody-negative patients (P=0.152).[102]

In contrast to our early understanding of the interplay between graft and host factors relevant for successful OCA transplantation, the pathways of host immune response and graft rejection have been well-characterized in the fields of transplantation. As the nuances of donor-recipient factors and immune-modulating therapies have been investigated over the decades, recent work has identified the effect of donor-recipient sex-mismatching in solid organ and hematopoietic cell transplantation.[14,133,137,168-172] Specifically, the male-specific minor histocompatibility antigen H-Y has been implicated in the development of more robust immune responses including increased rates of graft-versus-host disease as well as acute graft rejection following transplantation of male tissues into female recipients.[168,170,173,174] These

associations have been observed even when tissues are otherwise matched at major histocompatibility loci.[170] Minor histocompatibility H-Y antigens encoded on the Y-chromosome present distinct regions compared to the homologous H-X proteins on the X-chromosome that may present high immunogenicity.[171] For instance, kidney transplantation from male donors to female recipients has been associated with an increased rate of rejection due to the recipient lymphocyte alloimmune response against the graft H-Y antigens.[168] In contrast, naive donor lymphocytes carried during hematopoietic cell transplantation may target minor histocompatibility antigens, including H-Y antigens, that generates a combined humoral and cellular alloimmune response, triggering graft-versus-host disease.[172]

To explore the potential impact of donor-recipient sex-mismatching in OCA transplantation the current study evaluated whether sex-mismatching is associated with increased rates of OCA failure at five years. The principal findings of this study demonstrate that sex-mismatching results in significantly lower rates of OCA survival at five years when compared to sex-matched groups. (Figure 11.) Furthermore, sex-mismatching is independently associated with a 2.9 times greater likelihood of failure at five years, even after adjusting for recipient age [105], graft size [56,107] and BMI [147] – each representing potential recipient-specific confounders implicated in worse clinical outcomes after OCA transplantation. (Table 6.) Analyzing the four subcategory combinations of donor-recipient sex pairings, male-to-female grafts demonstrated a significantly lower and worst cumulative 5-year survival rate of 64% (p=0.04), while male-to-male grafts demonstrated a significantly higher and best cumulative five-year survival rate of 94% (p=0.04). (Figure 12.) When adjusting for recipient age, graft size, and BMI; multivariable COX regression identified MtF sex-mismatching to be an independent risk factor for OCA failure, with a 2.6 times greater likelihood of OCA failure in this group, while there was a strong trend toward decreased likelihood of OCA failure in the MtM group. (Table 8.) Recent work by Frank et. al. revealed that though male and female patients had similar patient-reported outcome scores, survival rates, and reoperation rates at 5 years after OCA; female patients <40yr old tended to fail significantly earlier than male patients <40yr old (p=0.039) while male patients >40yr old tended to fail significantly earlier than female patients >40yr old (p=0.046). Overall, however, the authors of this study concluded that recipient sex is not a prognostic factor for success or failure of OCA.[56] The data presented in our study is in contrast with the current

literature, however our study was specifically designed to evaluate the donor-recipient sex mismatch in OCA transplantation that may explain the discrepancy.

The nature of OCA failure is multifactorial. Furthermore, the clinical consequence of immunologic interactions between donor and recipient tissues in OCA transplantation are also likely multifactorial beyond potential sex-specific characteristics alone. Though the present study demonstrates a clear association between sex mismatching and OCA failure; the precise pathways of graft failure and to the extent of which immunologic pathways (e.g. the male-specific minor histocompatibility antigen H-Y) contribute to OCA failure are beyond the scope of the present study.

The effects of sex-mismatching in OCA transplantation are not necessarily immuno-modulated; i.e. differences in graft anatomy/radius of curvature, antigenic burden, tissue density/architecture, or histologic composition properties between mismatched sexes might also account for some degree of the failure outcomes observed in this study. It is conceivable that the biomechanical differences of male and female tissues, for example the biomechanical mismatch of a larger radius of curvature with ticker chondral surfaces in the case of a male OCA transplanted to a female recipient knee, could be a contributing factor to the poorer outcomes observed in the FtM subgroup - separate from potential immunologic differences.

5.3 Improvement in OCA storage

5.3.1 ***Chondrocyte viability in full-thickness cartilage***

Chondrocyte viability in OCA is a major determinant of *in vivo* graft performance following its transplantation.[112,113 150 122] The required cell viability for clinical use is generally accepted to be above 70%.[61,112,114,115, 8] Recently, several investigators reported that OCA stored in serum-free culture medium at 4˚C maintained chondrocyte viability at near-normal (90 - 100%) levels for 14 days after harvest;[149] however, viability decreased significantly to a range of 15% to 70% by 28 days at 4˚C.[112,114,115, 8] To improve viability, other storage conditions *e.g.,* storage at 37˚C were examined, though their effects on OCAs remain unclear because of controversial results.[112,114,121,122]

Mickevicius et al. and Harb and colleagues demonstrated higher chondrocyte viability in goat OC explants stored at 4˚C compared to 37˚C after 14 and 56 days of storage.[112,122] On the other hand, Garrity et al. reported that chondrocyte viability was maintained in human OCAs through 56 days of storage at 37˚C, similarly to the level of fresh explants, while 4˚C did not maintain viability even at 28 days of storage.[121] In our study, the viability of chondrocytes stored at 4˚C-AP and at 37˚C-AP significantly decreased with time compared to fresh bOC explants. Only the application of cyclic HP at 37˚C elicited markedly higher chondrocyte viability (88%) by 28 days considered within the acceptable clinical range. (Figure 13.) Our results therefore indicated that the application of cyclic HP is favorable on chondrocyte viability during storage. Since the cyclic HP is capable to promote mass transfer, the infiltration of solutes into and from dense avascular cartilage *in vitro*, we believe that chondrocytes particularly in the distance from the periphery of the cartilage maintain viability.

Zonal chondrocyte viability of the cartilage

Chondrocyte viability was also compared across the entire articular column from surface zone to deep zone. The surface zone is in contact with synovial fluid and is responsible for most of the tensile properties of cartilage, which enable it to resist the shear, tensile, and compressive stresses generated by weight bearing and joint loading.[8] Hence, loss of surface zone chondrocytes reduces mechanical resistance and begins the degenerative processes within the cartilage.[175,176] High cell viability and retention of ECM structure in surface zone are essential to support the integrity of OCA. Interestingly, at 37˚C-AP and at 4˚C-AP, a significant decline in chondrocyte viability in surface zone occurred at 28 days when compared to fresh explant controls. Only the addition of HP maintained chondrocyte viability in this surface zone through 28 days. (Figure 13.) Therefore, the application of HP seems to be advantageous in the surface zone during OC storage.

We also found a significant higher chondrocyte viability in the middle and deep zone with the utilization of HP. (Figure 13.) In addition, besides the differences in chondrocyte viability among the storage conditions, the area between deep zone and subchondral bone defined as calcified area exhibited markedly lower cell density and higher number of dead cells after 21 days of storage in each storage condition. (Figure 15.) We believe that viability in this calcified area is critical for improvement of post-operative outcomes, because one

complication following OCA transplantation is the delamination of the cartilage from the bone.[118] Loss of living chondrocytes in this area likely increases the risk of graft delamination. It is definitely a novel finding of our study that have not yet been demonstrated in the literature. The reason behind we observed such dead area and others studies did not might be the methodological difference between our study and others.[112,114,121,122] We evaluated cell viability in longitudinally sliced bOC explants composed with both cartilage and bone while previously the cartilage was removed from the subchondral bone before live/dead staining. We believe that the removal of the cartilage posed a risk of underestimating the viability in the interface between deep zone and subchondral bone. The dead area was seen in the longitudinal center of the bOC explants, while the periphery of the explant was well maintained with living chondrocytes. The dead chondrocyte area at 37˚C-HP was present in only 50% of the explants, the dead area was markedly smaller, and contained fewer dead cells compared to explants at 37˚C-AP where the dead area was seen in every explant (100%). (Figure 15.) One possible explanation for this result is that under AP the infiltration of nutrients to the center of the deep zone as well as the removal of waste products from the explant is limited. However, cyclic HP has a capability to improve solute exchange.[177,178] At 4˚C-AP, 50% of the OC explants had similar size of dead area to that found in HP. (Figure 15.) Even though this storage at 4˚C was also under AP, the chondrocytes have decreased metabolic activity under 4˚C, which requires less nutrition and waste product exchange, which explains why fewer explants had a dead chondrocyte area in the deepest part of the deep layer compared to 37˚C-AP.[179] Our finding may point towards a potential danger of culture conditions at 37˚C-AP as opposed to culture conditions at 4˚C-AP or under HP conditions at 37˚.

5.3.2 Cartilage thickness

Cartilage matrix retains fluid to withstand weight-bearing and joint loading; therefore, changes in hydration and cartilage thickness are related to the mechanical properties of the tissue. In our study, compared to fresh OC explants, cartilage thickness had decreased at the 4˚C-AP by 28 days. (Figure 16.) Under storage at 4˚C, the various catabolic enzymes in the ECM are inactive, and chondrocytes' metabolic activity is lower than storage

at 37˚C.[179] Thus, it is possible that as a response to these changes, the cartilage became dehydrated, leading to thinning of the tissue.[8]

On the other hand, at 37˚C-AP we observed a significant increase in articular cartilage thickness and disrupted ECM fibers in the middle zone which may be related to catabolic activity and possibly swelling due to increased tissue hydration during storage.[179] In contrast, we observed maintained cartilage thickness overtime at 37˚C-HP. We speculate that HP reduced catabolic activity, *e.g.,* matrix metalloproteinase-13, allowing it to maintain adequate hydration in the articular cartilage.[126,129]

5.3.3 Cellular and matrix histology of cartilage

In our study, at 37˚C-HP and 4˚C-AP storage, articular cartilage morphology was similar to fresh control by the end of storage, while surface fibrillation and structural irregularities appeared at 37˚C-AP. (Figure 17.) Such observation regarding the surface irregularities under 37˚C storage is concordant with the literature.[112] To quantify the histological changes during storage, we also evaluated the explants' cartilage using the HHGS score. (Table 9.)[180] We found that the cartilaginous ECM stained with safranin-O faded with time in each storage condition by 28 days. Particularly, 37˚C-AP storage had the most grafts with markedly decreased S-GAG, while 4˚C-AP had the most grafts with a slight reduction. It can be explained by the differences in metabolic activity of the chondrocytes stored between 4˚C and 37˚C conditions.

Lightly stained cartilaginous ECM was often seen between the surface and middle zones at 37˚C-AP and 37˚C-HP. (Figure 17.) This area is where parallel aligned collagen fibers of the surface zone become obliquely organized.[8] The lightly stained cartilaginous ECM was observed 5 of 6 bOC explants at the 37˚C-AP and 3 of 6 explants at the 37˚C-HP by 28 days. Interestingly, in these bOC explants, diffuse hypocellularity was also observed. (Figure 17.) We believe that it is a consequence of increased separation in the surface and middle zones in this lightly stained cartilaginous ECM. However, we are aware that bovine cartilage tends to be more susceptible to long term storage than human cartilage, probably due to the different proportion of ECM components, absence of tidemark, different cell

density and stiffness of these two tissue. Consequently, future studies with human tissue are necessary to further evaluate the effects of HP on human OCA storage.

In summary, storage under 37˚C with the application of cyclic HP outperformed both 37˚C-AP and 4˚C-AP. We acknowledge that 4˚C-AP storage is a straightforward storage condition with a low risk of infection. However, the metabolic activity of the chondrocytes under 4˚C is decreasing, and consequently, the deterioration of the tissue is inevitable. 37˚C storage is more challenging however; it provides a similar environment that the OC structure used to in vivo. Based on our study it seems that the addition of cyclic HP to 37˚C is essential and assists an in vitro culture to resemble more to the in vivo condition.

5.4 Limitations

5.4.1 OCA storage time

We acknowledge that there are limitations to the present study. The patients were not directly case matched for age, gender, BMI, lesion location, lesion size, or concomitant procedures. However, there were no statistically significant differences among these factors between the early and late transplant cohorts of this study.

5.4.2 Sex mismatch

One major limitation is the small size of the FtM subgroup (6 patients., 4% of total study population). Male OCAs are more readily available from tissue banks than female donor tissue. This fact is apparent in the current study given the larger MtM and MtF subgroups. The relatively infrequent FtM condition limits the strength of our analysis compared with the conversely mismatched MtF group in this study. Future work to elucidate the precise immunologic pathways, sex-specific characteristics, and additional donor-specific risk factors consequential to possible graft rejection will certainly prove valuable toward optimizing the clinical success of OCA transplantation. Other immunologic determinants; including race, blood type, or post-partum status, might also represent additional variables for near term focus. With a new appreciation for the impact of sex-mismatching in OCA

transplantation, efforts to avoid donor-recipient sex-mismatching, when possible, will enhance our current standard of care.

5.4.3 Improvement in OCA storage

One of our major limitations is that we used bOC explants taken from humeral heads of calves (2 -3 weeks old). However, the goal of this study was to understand if HP may preserve the level of chondrocyte viability in fresh OCAs. As there is no prior data on the application of HP on OC explants, we chose a convenient and cost-effective source for OCA storage studies. Future studies with mature OC explants should be conducted because immature bovine chondrocytes have higher baseline metabolic activity, affecting our results. In addition, fluid flow replenishment was only used during HP and not in the other groups, which might interact with the pure effect of HP. Finally, in this study, we did not use serum contained medium because we focused on the capability of cyclic HP to minimize the reduction of cell viability. In future studies, serum contained medium, and cyclic HP algorithm should be examined to extend storage time.

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6 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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7 Összefoglalás

Az ízületi porc gyógyulási képessége limitált. Ennek következtében, ha egyszer egy porc defektus kialakul, a hyalin porc visszaépülése nem lehetséges, ezzel rontva az ízület funkcióját. Krónikus gyulladásban tartja az adott ízületet és gyakran vezet ízületi artrózishoz. Következésképpen a porcdefektus korai felismerése és helyreállítása elengedhetetlen a későbbi protézis beültetés elkerülése érdekében.

Oszteokondrális allograft transzplantáció (OCA) során allograft porc és csont kerül beültetésre a recipiensbe a porcdefektus korrekciójának érdekében. A beültetés követő 10 éves túlélési ráta 50-89%, ami igen szélesnek mondható. Következésképpen kutatók és klinikusok olyan faktorokat keresnek, amik befolyásolhatják, illetve javíthatják ezt a rátát, növelve a graft túlélést.

A kutatómunkám átfogó célja az OCA beültetését követő graft túlélés javítása. Ennek érdekében allograft specifikus faktorok hatását vizsgáltuk a beültetést követő graft túlélésre, valamint célunk volt a jelenlegi tárolási technika fejlesztése. Allograft specifikus faktorként vizsgáltuk a tárolási idő, illetve a donor és recipiens nemének hatását a graft túlélésre két retrospektív klinikai kutatásban. A tárolási technika fejlesztésére pulzatilis hidrosztatikus nyomást fejtettünk ki a graftokra 37˚C-on, ami a járás közbeni nyomás viszonyokhoz hasonló.

A jelenlegi kutatásainkkal demonstráltuk, hogy a graftok tárolási idejét figyelembe kell venni transzplantációt megelőzően, hiszen a 19–24 napig tárolt graftok túlélési ideje szignifikánsan hosszabbnak bizonyult a 25–27 napig tárolt graftokéhoz képest. Szintén bemutattuk, hogy a donor és recipiens neme fontos faktor az OCA beültetésének sikerességében, hiszen az ellenkező nembe való beültetés csökkent túléléssel járt, legfőképpen a férfiból származó graft női recipiensbe való beültetéskor. Végezetül bebizonyítottuk, hogy a pulzatilis hidrosztatikus nyomás használata oszteokondrális graftok tárolása közben szignifikánsan javítja a porcsejtek túlélését és jobban megtartja a graft szövettani tulajdonságait a jelenleg használt tárolási eljáráshoz képest.

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8 Summary

Articular cartilage has limited spontaneous healing capacity after injury; therefore, once articular cartilage is damaged, full recovery of its structure, function, and biomechanical properties is unlikely and is usually a step toward OA. OCA transplantation is a single-step cartilage repair procedure that uses allograft tissue permitting the treatment of large osteochondral defects with success rates between 50-89% in 10 years. However, with such broad reported rates of success, numerous studies have sought to identify predictors associated with improved graft survival following transplantation.

The overarching purpose of this thesis is to enhance the clinical outcomes following OCA transplantation by understanding the graft-specific predictors of OCA failure and by improving the current OCA storage. In particular, in two retrospective clinical studies, we investigated the effect of OCA storage time, and sex mismatch between the donor and recipient on OCA graft survival. In addition, in a basic science study, we aimed to improve the current OCA storage by applying hydrostatic pressure under 37˚C storage.

We found that OCAs implanted after 19 to 24 days showed a significantly increased rate of survivorship when compared to grafts implanted between 25 and 27 days. In addition, we demonstrated that mismatch between donor and recipient sex has a negative effect on OCA survival following transplantation, mainly in those cases when male donor tissue was transplanted into a female recipient. 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.

These findings stress prioritizing early transplantation and performing transplantation to the same sex whenever possible. Finally, our results underline enhancing the current OCA storage condition by applying HP to the graft during storage.

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