Ex-Vivo Evaluation of a Novel Approach to Osteochondral Allograft Transplantation

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Background: Osteochondral allograft transplantation is a procedure to repair end stage focal cartilage defects by impacting a cadaveric cartilage and bone graft into the patient. Counterproductively, this impaction is detrimental to chondrocyte viability which is a key determinant of graft integration and long-term clinical success.

Hypothesis: Using a screw-in method of inserting osteochondral allografts and avoiding direct impaction forces on the articular cartilage will lead to increased chondrocyte viability.

Study Design: Controlled *ex-vivo* laboratory study.

Methods: A novel OCA transplant system based on screw-in insertion of the allograft was constructed. This system was tested against current surgical procedures in ex-vivo porcine femoral condyles (n= 5). CaAM & EthHD staining was used to quantify chondrocyte viability, and laser scanning of the articular surface was used to quantify graft placement.

Results: The novel screw-in system retained a chondrocyte viability of nearly 89%, which shows a significant improvement ($p < 0.01$) over traditional surgical methods, which had a mean chondrocyte viability of 55%. The screw in system also does not compromise graft placement, as the height difference between the graft and original articular surface was well below 1 mm.

Conclusion: Through this novel approach to osteochondral allograft transplantation, chondrocyte viability at the time of implantation can be preserved without compromising graft placement accuracy and resulting surface congruence.

Clinical Relevance: Showcases a likely improvement to current osteochondral allograft transplantation systems which have taken minimal efforts to preserve chondrocyte viability during graft insertion as a means of promoting successful graft integration.

Key Terms: Osteochondral transplantation, osteochondral allograft, cartilage repair, chondral defects, bone screw.

Introduction

Osteochondral defects can arise from traumatic injury, or degenerative cartilage diseases like osteoarthritis or osteonecrosis [1–3]. The leading concomitant knee pathology for this defect is a tear in the medial meniscus, which reduces support of the knee and results in greater joint contact forces [4]. Other pathologies leading to osteochondral defects include abnormal bone growth and excessive stress in the knee [5]. OCA transplantation represents an end-stage solution to cartilage repair after other repair techniques (like debridement, microfracture, or autologous chondrocyte implantation) have failed [1]. The rate of OCA transplantations performed is increasing by 5% annually, and is expected to reach 3500 procedures by the year 2020 [6].

The most common surgical approach to implanting an osteochondral allograft is the dowel technique. This procedure begins by preparing the recipient site for the allograft. The focus of this preparation is to create a cylindrical void that is perpendicular to the surrounding cartilage. To ensure perpendicularity, a guide wire is inserted orthogonal to the condyle at the defect site. A cannulated dowel reamer is passed down the guidewire and advanced to a depth of between 7 mm -14 mm, clearing a void 10 mm-25 mm in diameter.

The allograft is created from fresh cadaver tissue, and its geometry is matched to the recipient site on the patient. To harvest the graft, a surgical hole-saw is passed through a guide ring on the articular cartilage creating a cylindrical dowel. Then, measurements of the recipient site depths are used to guide the surgeon as they cut the graft to a complementary length with an oscillating saw. The allograft is then positioned directly above the recipient site, and impacted into the patient with a hammer and impact rod until the graft lies flush with the surrounding cartilage [7].

The impaction force used to press fit osteochondral allografts into place during a transplant procedure induces cell death in the superficial portion of the articular cartilage. The impaction impulse deforms mechanoreceptors in the cell initiating an intracellular signaling cascade ultimately activating executioner caspases, triggering cell apoptosis. It was found that impacted grafts have an average of 47% greater cell death compared to control grafts, particularly on the articular surface. These grafts also showed increased levels of caspase 3 activity, which is a known enzyme involved in programmed cell death [8]. Chondrocyte death in response to impaction has also been shown to be dose dependent where higher impaction forces result in more proliferative cell death [9].

The effects of impaction on chondrocyte viability is an important medical concern for this procedure as chondrocyte viability at the time of impaction is the primary determinant of allograft success. A study was performed in canine models to assess the effects of chondrocyte viability at the time of impaction on allograft success. Subjects received an osteochondral allograft and graft cell viability was assessed at the time of impaction where viability ranged from 23-99%. Six months post-surgery, procedural success was compared to initial chondrocyte viability. The researchers found that no graft with an initial chondrocyte viability below 70%

was successful [6]. While other factors contributed to procedural success, none were as significant as initial chondrocyte viability.

Despite the prevalence of osteochondral allograft transplantation, the failure rate is as high as 15.5% at 5 years and can certainly be improved [1]. Nevertheless, the benefit of this procedure over total knee arthroplasty is the promising possibility of restoring full-range of motion, and maintaining the patient's quality of life [10]. The motivation in this project, therefore, is to improve full-graft integration and long-term integrity by protecting chondrocyte viability—a significant factor in determining procedure success [4].

Materials and methods

Surgical Instruments

A novel OCA transplant system was constructed to create threads on the donor graft and patient receiving site. This system consists of three components: a tap, a die and die base, and a graft screwdriver. The die base, shown in Figure 1, consists of two parallel plates separated by vertical stainless-steel pins. In the bottom plate, a removable supporting cup holds the graft. Two thumb screws tighten down the graft and prevent it from rotating during threading. In the top guiding platform, there is a hole cut through it that matches the size of the die. This hole lies directly over the supporting cup, which ensures axial alignment between the threads and the graft.

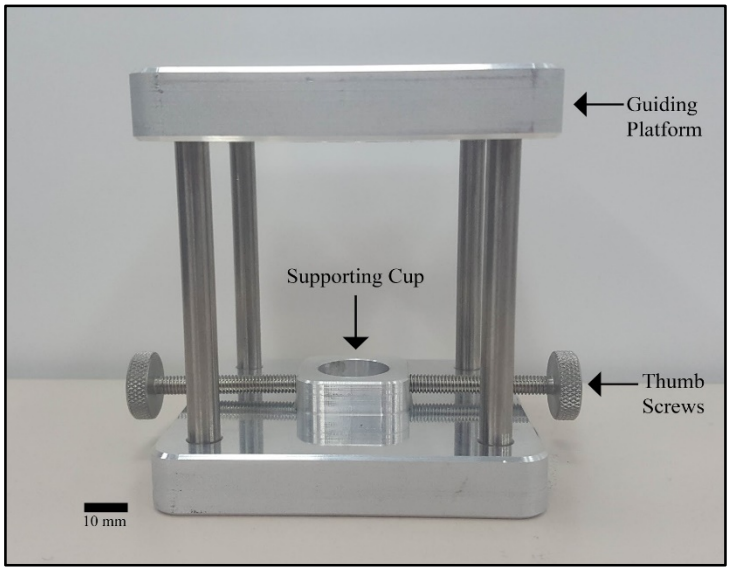

Figure 1. Guiding platform with graft holder cup. This component ensures axial alignment of the threads defined on the graft. The allograft would be inserted cartilage side up into the supporting cup, and the thumb screws would tighten around the allograft.

The die, as depicted in Figure 2, consists of a stainless-steel body and handle. The handle is removable and offers the surgeon a comfortable grip when using the tool. The die body consists of an open-ended cylinder. The open end has 4 flutes built in to allow the bone shavings created during the threading process to escape. The threads have a 1.5 mm pitch, allowing the surface of the graft to always remain 0.75 mm of the native surface. The die threads begin as a taper and lead in to allow more consistency during the threading process while requiring less pressure from the surgeon.

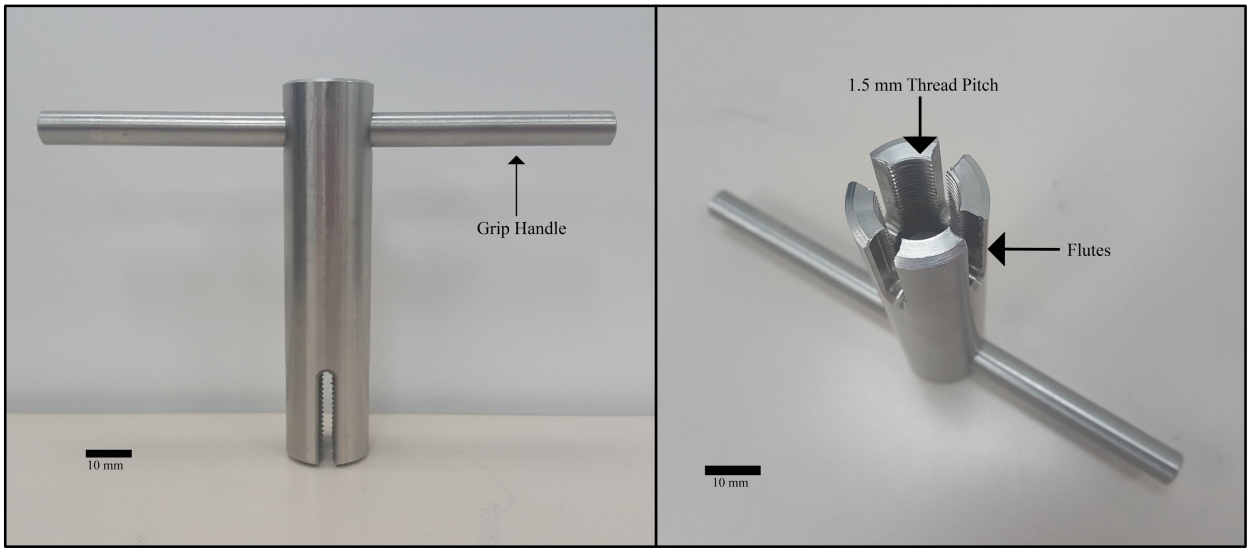

Figure 2. Left: Stainless steel die used to create threads on the external profile of the graft before insertion into the recipient site. The die would be inserted through the guiding platform to maintain axial alignment. Right: Overhead view of the die, showing the internal threads and flutes used to thread the bone.

The tap, as depicted in Figure 3, consists of a stainless-steel body and handle. The die body consists of a cylinder with a hole along the central axis, and threads protruding from working end. The central hole matches the guidewire currently used in surgical systems and sliding the tap along the guidewire ensures the threading axis is perpendicular to the articular surface. The tap has 4 flutes built in to the threads that allow the bone shavings created during the threading process to escape. The threads have a 1.5 mm pitch, matching that of the die. Finally, the tap threads begin as a taper and lead in to allow more consistency during the threading process while requiring less pressure from the surgeon. The handle is removable and has a guide hole to slide over the guide wire.

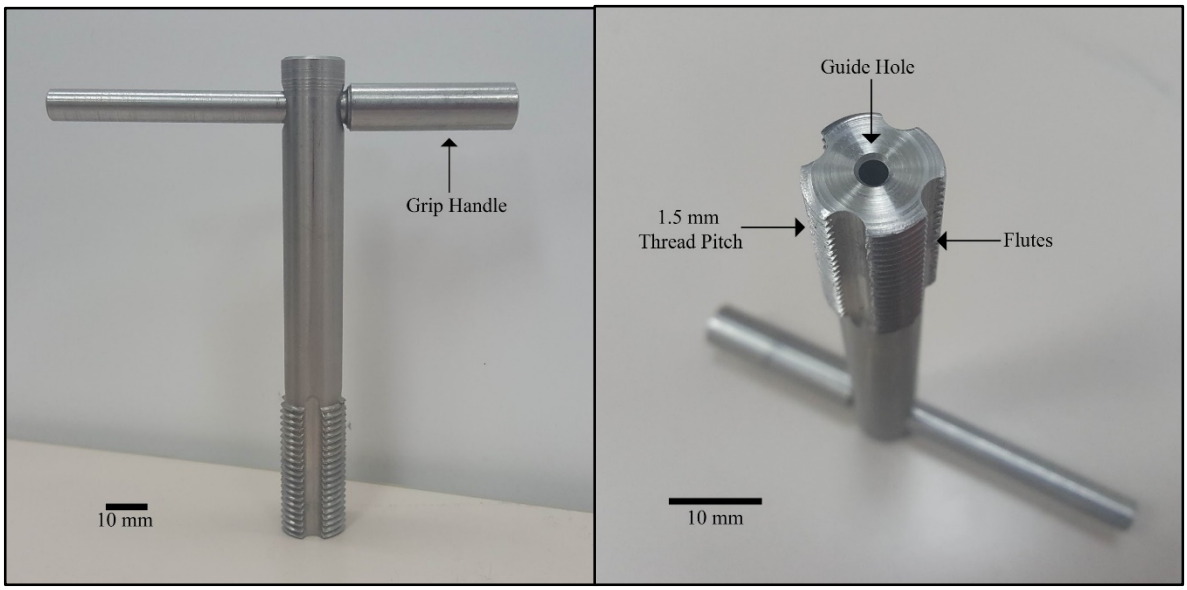

Figure 3. Left: Tap system used to create threads within the recipient site. A guide wire is to be slid through the guide hole and inserted into the center of the recipient site to ensure proper alignment. Right: Closeup of the threads and flutes on the tap as well as the hole along the tap's central axis through which the guidewire will be inserted.

The graft screwdriver, as shown in Figure 4, is designed to aid in screwing the graft into the receiving site. It utilizes a hex-bit to attach to a standard screwdriver handle. The working end utilizes two 1 mm diameter tines and a disposable silicone cap to protect the chondrocytes from overhead force when the device is in use. The tines are tapped through the cartilage into the subchondral bone, securing the graft for the surgeon to screw into the receiving site. The tines are comparable in size to wires that are used to secure large osteochondral allografts into the patient.

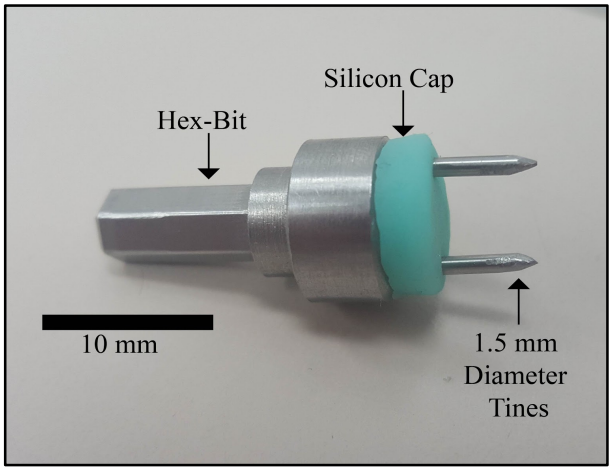

Figure 4. Bident screwdriver tool used to aid in inserting the graft into the recipient site. The bident attaches to a standard screwdriver via the hex-bit extrusion. The silicon cap is a failsafe intended to protect the cartilage from unwarranted impact in the case of accidental over-insertion of the bident into the cartilage.

Graft Threading Protocol

Five-month-old porcine femoral condyle tissue was used for testing. Figure 5 models the threading procedure workflow. Similar to current surgical procedures, a cannulated reamer was inserted over a guidewire and used to drill a hole in the model recipient site. The tap was inserted over the guidewire and used to define threads perpendicular to the articular surface (Figure 5C-D). A hole saw was used to collect a graft from the model donor site. The graft was inserted into the holder cup and the die was used to define threads complementary to the receiving site (Figure 5A-B). The bident screwdriver was inserted into the graft and used to screw the graft into place (Figure 5E-F).

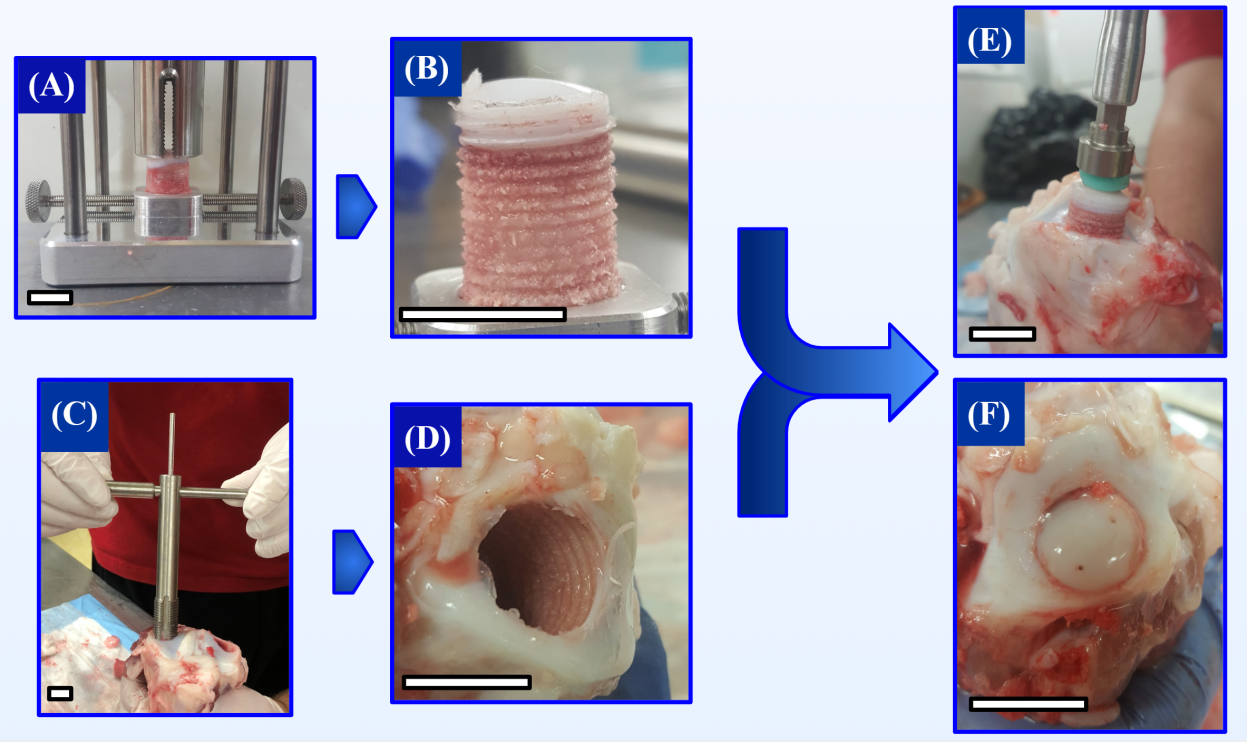

Figure 5. Graft threading procedure workflow. A: Graft in the supporting cup being threaded by the die. B: Threads defined on the graft. C: Tap inserted over the guidewire to define threads on the receiving site. E: Graft screwdriver being used to screw in the graft. F: Fully inserted graft.

Immunochemistry

Immediately following implantation, a set of four 3 mm biopsies of the articular cartilage are collected for each replicate (Figure 6). All samples were washed in Dulbecco's Phosphate Buffered Saline (PBS) and incubated in serum-free Dulbecco's Modified Eagle Medium (DMEM) containing 10 µM Calcein AM (CaAM) and 100 nM Ethidium Homodimer-1 (EthHD) for 24 hours at 37 ℃ and 5% CO_2. All samples were then sectioned with a scalpel blade to expose a cross section of the cartilage from the articular surface to the subchondral bone. Samples were then washed in PBS and subsequently imaged with a Nikon A1RS confocal microscope.

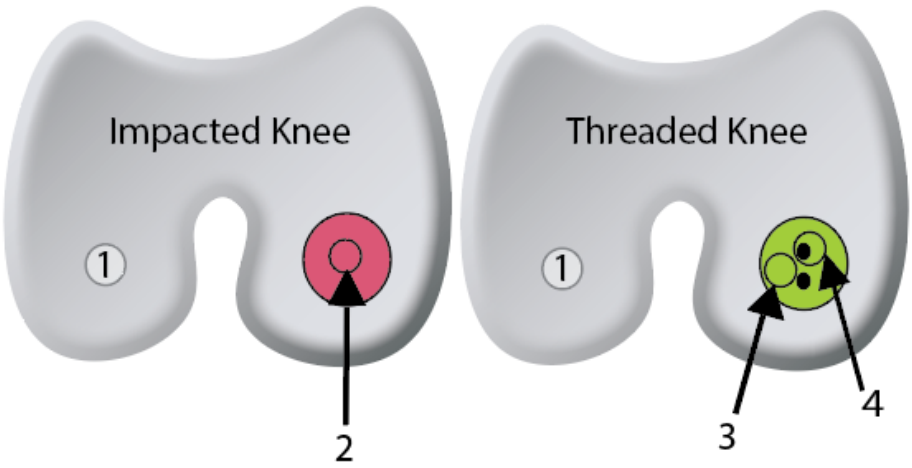

Figure 6. Diagram of biopsy locations in each sample set. A control biopsy (1) can be taken from a non-grafted section of cartilage. One biopsy (2) is taken from the impacted allograft (left), another is taken from the bulk of a threaded graft (3), and the last is take from a location surrounding a tine insertion point (4) of a threaded graft.

Cell Viability Quantification

To assess cell viability in the stained biopsy samples, images from confocal microscopy were passed through a custom CellProfiler pipeline. Briefly, all images were resolved into red and green images and events were counted in each residual image. Cell viability was characterized as the number of events in the green separated image divided by the total number of events in both images.

3D Laser Scanning

To characterize the height difference between the implanted grafts and the native joint surface, 3D laser scans and analysis of the resulting point clouds were used. A reference scan was first taken of the exposed joint without any modification (i.e. grafting). This scan served as a reference coordinate system for registration, and as a ground-truth for graft-height comparisons (i.e. how far from this native surface does the graft lie after implantation?). The grafting procedure was then performed using the threading technique. After the grafting was complete, the articular surfaces were scanned again to measure any geometry changes.

These scan data were imported to MATLAB and registered to the unaltered joint scan using the ICP algorithm [11]. This will allow for a direct comparison between the reference and grafted scans. The point cloud data collected from the laser scanning were fit with a scattered data interpolant function to quantify the out-of-plane (z-direction) sample heights as a function of in-plane (x-y plane) position. A one-million cell mesh-grid was applied to the interpolant and the values in each cell in the reference scan were subtracted from those of the grafted scan to yield a height difference between the two states. The resulting height difference is attributed to imprecise grafting and allows it to be easily quantified despite the torturous measurement geometry. Representative scans are shown in Figure 7.

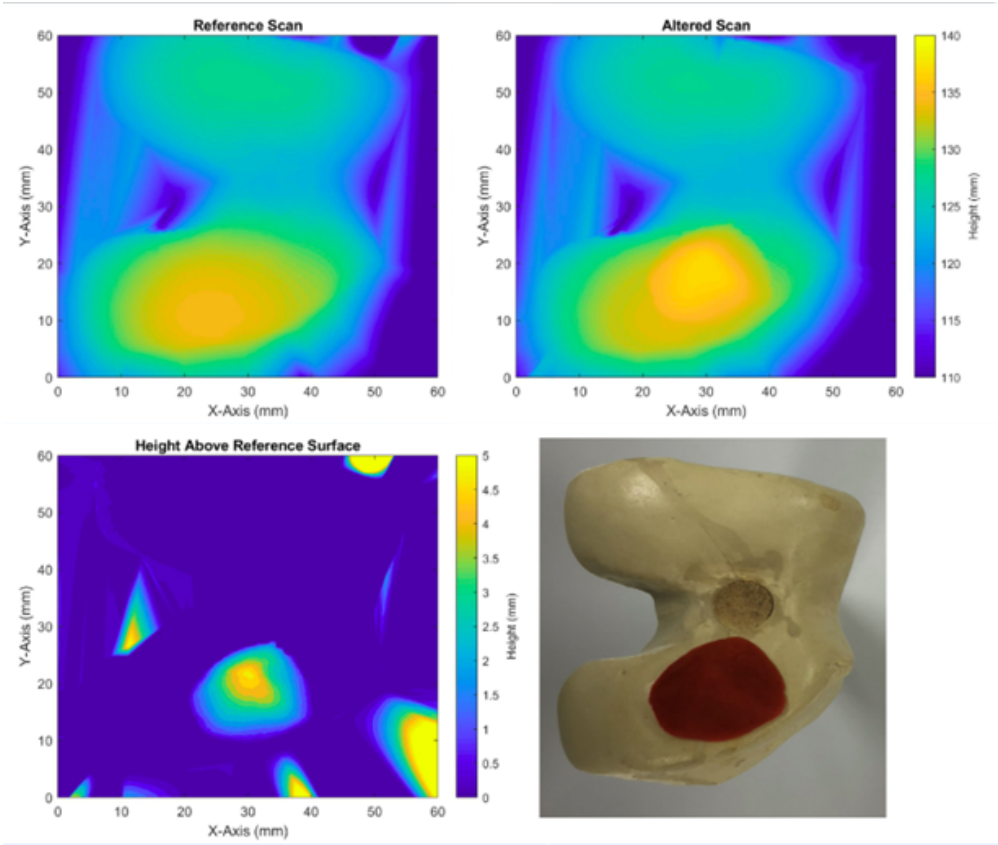

Figure 7: Laser measurements of simulated graft height offset above articular surface in a model femur. The reference scan is the geometry of the unaltered bone. The altered scan shows the bone with the simulated graft and this height offset is quantified by subtracting the reference scan from the altered scan. Image depicts the altered bone model.

Results

Viability Results

The percentage of viable chondrocytes for the threaded grafts was compared to that of the impacted grafts, and a statistically significant difference between the two groups was observed. The mean chondrocyte viability for the threaded bone grafts was 88.5% with a standard deviation of 8.3%. The mean chondrocyte viability for the impacted samples was 54.6% with a standard deviation of 11.4% (Figure 8). A one-sided paired t-test was performed on the data with a null hypothesis of each treatment having equal effects on chondrocyte viability. A p-value of 0.009 was obtained which is significant at the $\alpha = 0.01$ level; thus, the null-hypothesis was rejected as there is evidence supporting higher chondrocyte viability with graft threading compared to the traditional impaction technique. These results are illustrated in Figures 9a (showing a cross-section of cartilage from an impacted bone graft), and 9b (showing a cross-section of cartilage from a threaded bone graft). The number of dead chondrocytes (red) in the impacted graft is observably higher than in the threaded graft, and there is noticeable localization of cell death closer to the articular surface. Additional analysis examined the distribution of cell death in the areas immediately surrounding the tine insertion points (Figure 9c). The images were progressively cropped to isolate lateral cross-sections of chondrocytes at increasing radial distances from the tine insertion point. Through cell viability testing of each cross-section of these samples, we determined that within an average of 412.5 μm from the tine insertion point the implanted cartilage re-achieves the 70% viability threshold.

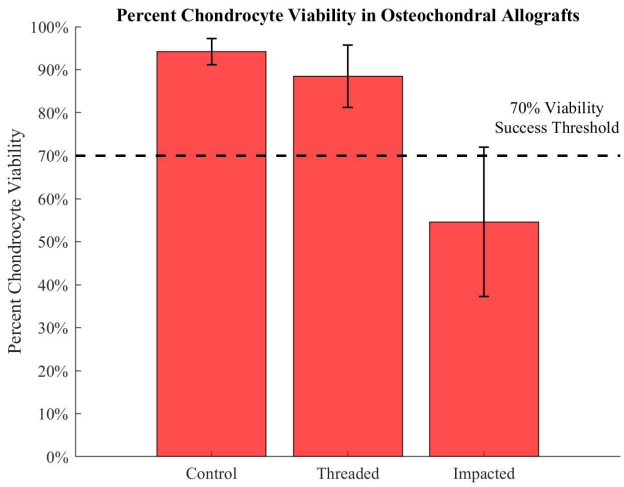

Figure 8: The average percent of living chondrocyte cells is recorded for each treatment group: non-impacted control (94.25% viability) threaded (88.46% viability) and impacted (54.61% viability) allografts (n = 5). A one-tailed t-test compared the viability between the threaded an impacted groups to reveal a statistically significant reduction in chondrocyte death with the threading method (p = 0.0098).

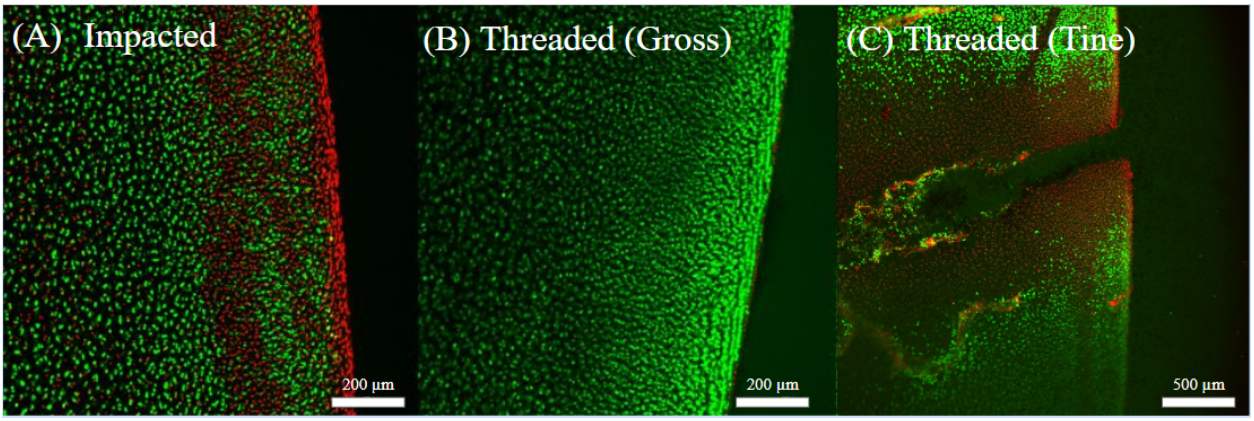

Figure 9: Cross sections of graft cartilage biopsy collected with confocal microscopy (Nikon A1Rs Confocal Microscope; UW Optical Imaging Core) 18 hours after staining. (A) Impacted graft under 10x magnification. (B) Threaded graft under 10x magnification. (C) Threaded graft at the insertion point of the graft screwdriver under 4x magnification. Articular surfaces of each biopsy are on the right side of each image. Red: Dead Cells; Green: Live Cells.

Discussion

The data collected indicate that threading the donor grafts retains greater than 40% more viable chondrocytes than the impaction method. The threading method also retains nearly 20% more viable chondrocytes than the 70% threshold that has been previously linked to successful outcomes in the literature. Additional damage to the chondrocytes due to the tines was found to be minimal. There was localized death around the tine insertion sites, but the viability returned to above the 70% threshold within 400 microns. Moreover, a similar graft treatment is already in clinical practice when two large osteochondral allografts must be overlapped and must be secured with metal pins drilled through the articular surface. The tines used in the graft screw driver are a comparable size and thus do not significantly deviate from current clinical practice. There appears to be minimal effects on the outcome of the procedure, further justifying their use in this device.

The simulated impaction method does not strictly comply with the surgical procedure. In the impaction trials, the grafts faced more receiving site bone interference than is likely typical of the procedure, and this could have altered the forces that the grafts were subjected to. Due to the strict product control by these orthopedic companies, it is quite difficult to obtain accurate measurements of the graft-receiving hole diameter fit tolerances to help better replicate the impaction protocol. Moreover, we did not have a way to quantify the forces associated with the actual impaction process, so it is hard to rigorously justify their clinical relevance. Nevertheless, after observing an OCA transplantation, we are reasonably confident that our impaction forces fell within a clinically appropriate range

These viability results may be affected by multiple factors. One is that the sample size is relatively small, which may produce a less representative data set than a larger sample set could. Due to the young age of the animals, the hardness of this material was significantly lower than expected. Therefore, in order to better simulate the properties of adult human bone, older pigs (or simply harder tissue) should be acquired for testing. Additionally, with such promising results, the possibility of obtaining human cadaver knees to assess the device with may be worth considering.

The novel graft threading device has some advantages over current surgical system. The first advantage is that the graft can be adjusted once it has been inserted by using the graft screwdriver. For example, if the graft is inserted too far, the surgeon can simply back the graft out. However, this may affect the surface geometry congruency between the graft and native surfaces if they were improperly matched. Additionally, the design is simple and intuitive to use, as described by a UW Health orthopedic surgeon who used it for the first time. This surgeon also complimented the mechanical integration of the graft by using the threads to keep it in place with a decreased risk of graft loosening.

Conclusion

OCA transplantation corrects osteochondral defects through the implantation of a donor graft. The existing surgical system involving direct graft impaction is detrimental to chondrocyte viability which can lead to poor surgical outcomes. This novel approach to OCA transplantation addresses this core dilemma by tapping the recipient site, threading the donor graft, and screwing the graft into the receiving site to avoid any impaction force. Following assessment of differences in chondrocyte viability, comparison of impaction to a screw-in graft revealed a significant reduction in cell death. Furthermore, the chondrocyte viability in the screw-in graft remained above the 70% threshold while the impacted graft did not.

There were also concerns about graft placement accuracy given that its rotational and vertical alignment are fully coupled in this screw system and thus may be challenging to minimize surface incongruities. After testing in a Sawbone model, the graft could be matched to the surrounding surface to with 99% confidence that the error would be less than 0.5 mm which is far below the clinically acceptable error of 1 mm. Future work will entail extending these studies to variable porcine tissue models to simultaneously assess chondrocyte viability and graft placement accuracy (using the 3D laser scanning protocol) to evaluate the efficacy of this novel approach to osteochondral allograft transplantation.

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