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

Alexander Teague, David Fiflis, Zach Wodushek, Alex Babinski, Brian Walczak, DO
University of Wisconsin-Madison

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. Moreover, the impaction technique does not allow for the graft to be removed after implantation. Consequently, this limits the surgeon's ability to fine-tune the graft's placement in the patient to ensure that it aligns with the surrounding tissue.

Hypothesis: Using the screw-in method of inserting osteochondral allografts will avoid direct impaction forces on the articular cartilage to maintain chondrocyte viability. With this approach, the screw can also be removed after insertion, allowing the surgeon to adjust the graft-height to improve cartilage surface congruity despite the challenge of coupled graft rotation and translation in the screw configuration.

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

Methods: A novel OCA transplant system based on a screw-in allograft was constructed. This system was tested against the current surgical procedure in ex-vivo porcine femoral condyles ($n = 5$). Calcein AM & Ethidium Homodimer staining with confocal microscope imaging and CellProfiler analysis was used to quantify chondrocyte viability. Graft placement accuracy was assessed by threading simulated grafts and receiving sites in a Sawbone model and measuring the resulting angular alignment. The graft angular deviation from a target mark on the receiving site was translated to a height offset for evaluation.

Results: The novel screw-in system retained a chondrocyte viability of nearly 89%, which showed a significant improvement ($n = 5$; $p < 0.01$) 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 with an average graft height offset of 0.36 mm ($n = 26$; $p < 0.0001$).

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: The threading approach showcases improvements to current osteochondral allograft transplantation systems which have taken minimal efforts to preserve chondrocyte

viability during graft insertion, and to afford the surgeon post-insertion graft adjustment ability. Considering all these factors, the screw-in approach will likely 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].

All current protocols for osteochondral allograft transplant rely on this impaction for to seat the allograft into the patient[7,8]. 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[9]. Chondrocyte death in response to

impaction has also been shown to be dose dependent where higher impaction forces result in more proliferative cell death[10].

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[11]. 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]. Here, we propose a novel method of graft insertion, that eliminates the need for impaction forces in graft placement. In this strategy, threads are created in the subchondral bone of both the graft and the recipient site, and the graft is then screwed into the recipient site.

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.

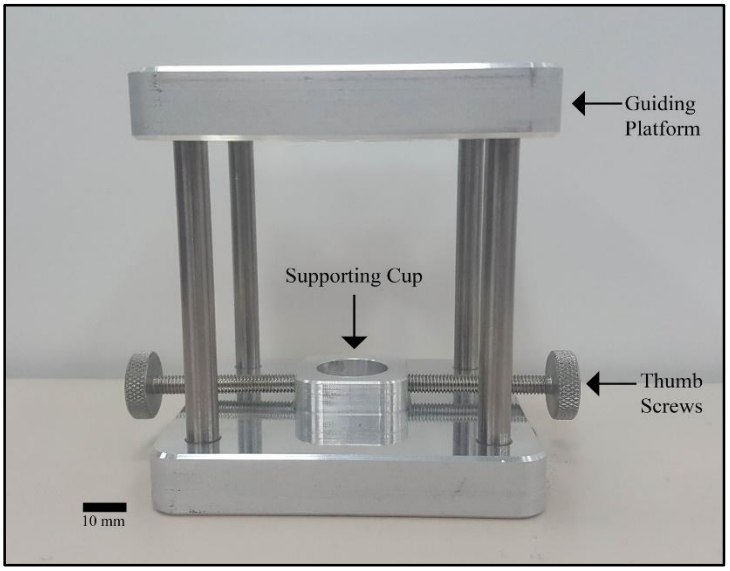

Figure 1. Guiding platform with graft holder cup. This component ensures axial alignment of the threads defined on the graft. The allograft would be inserted cartilage side up into the supporting cup, and the thumb screws would tighten around the allograft.

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, which are fine enough to allow the surface of the graft to always remain within 0.75 mm of the native surface, yet coarse enough to be cut well defined threads in the relatively soft bone. 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.

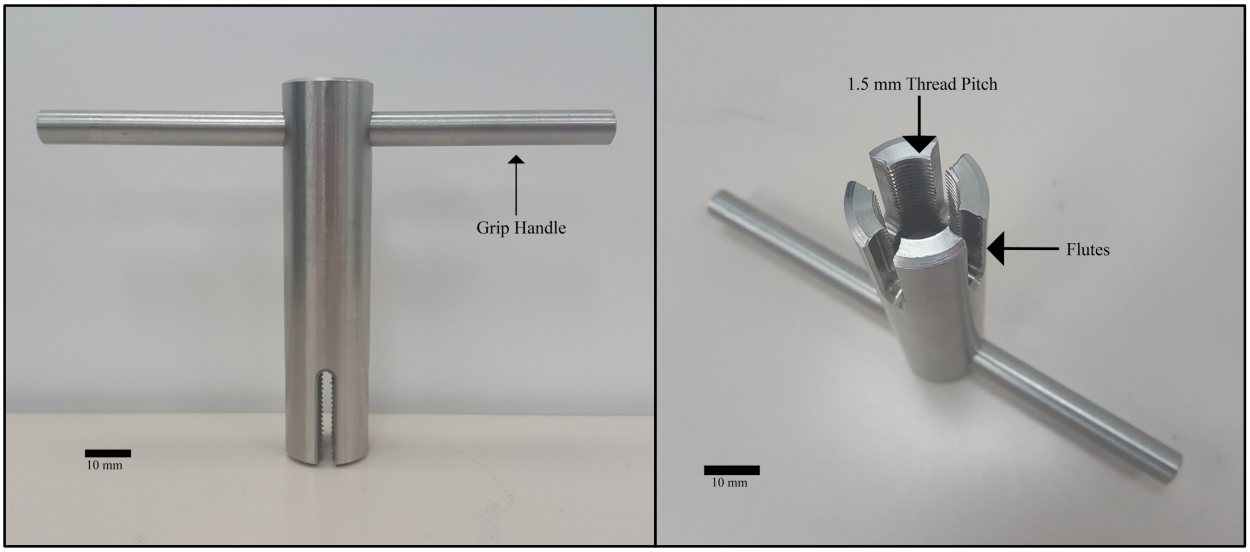

Figure 2. Left: Stainless steel die used to create threads on the external profile of the graft before insertion into the recipient site. The die would be inserted through the guiding platform to maintain

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.

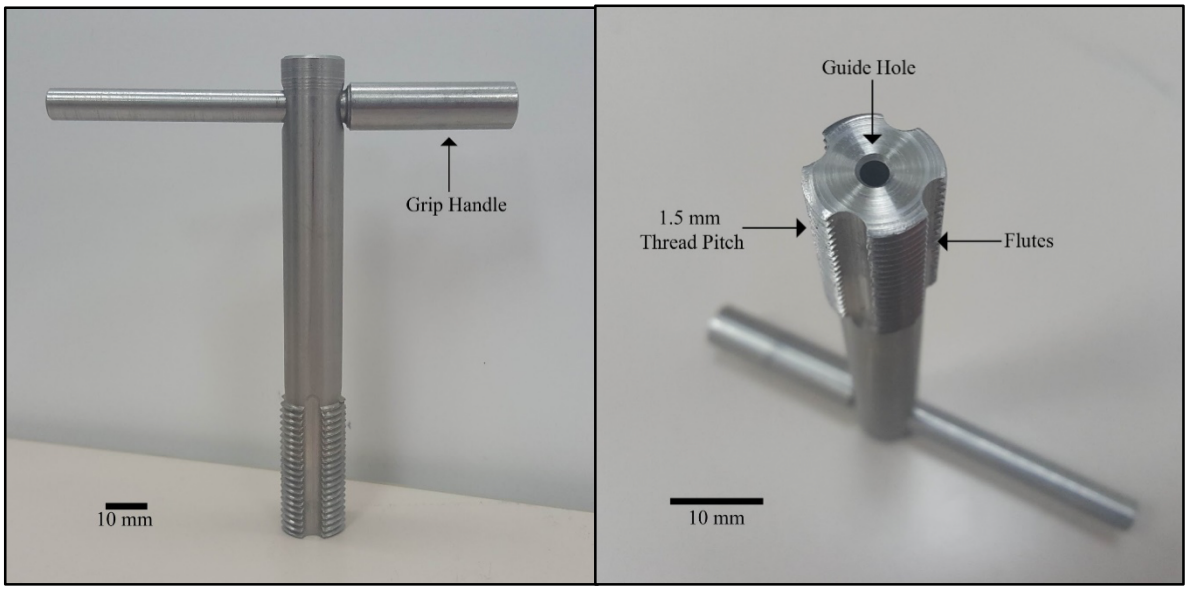

Figure 3. Left: Tap system used to create threads within the recipient site. A guide wire is to be slid through the guide hole and inserted into the center of the recipient site to ensure proper alignment. Right: Closeup of the threads and flutes on the tap as well as the hole along the tap's central 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[12].

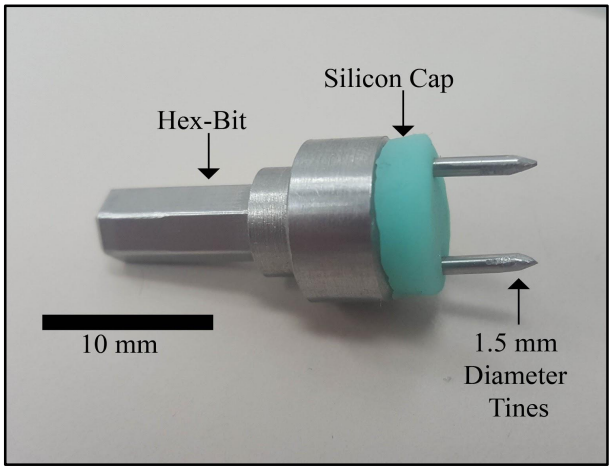

Figure 4. Bident screwdriver tool used to aid in inserting the graft into the recipient site. The bident attaches to a standard screwdriver via the hex-bit extrusion. The silicon cap is a failsafe intended to protect the cartilage from unwarranted impact in the case of accidental over-insertion of the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

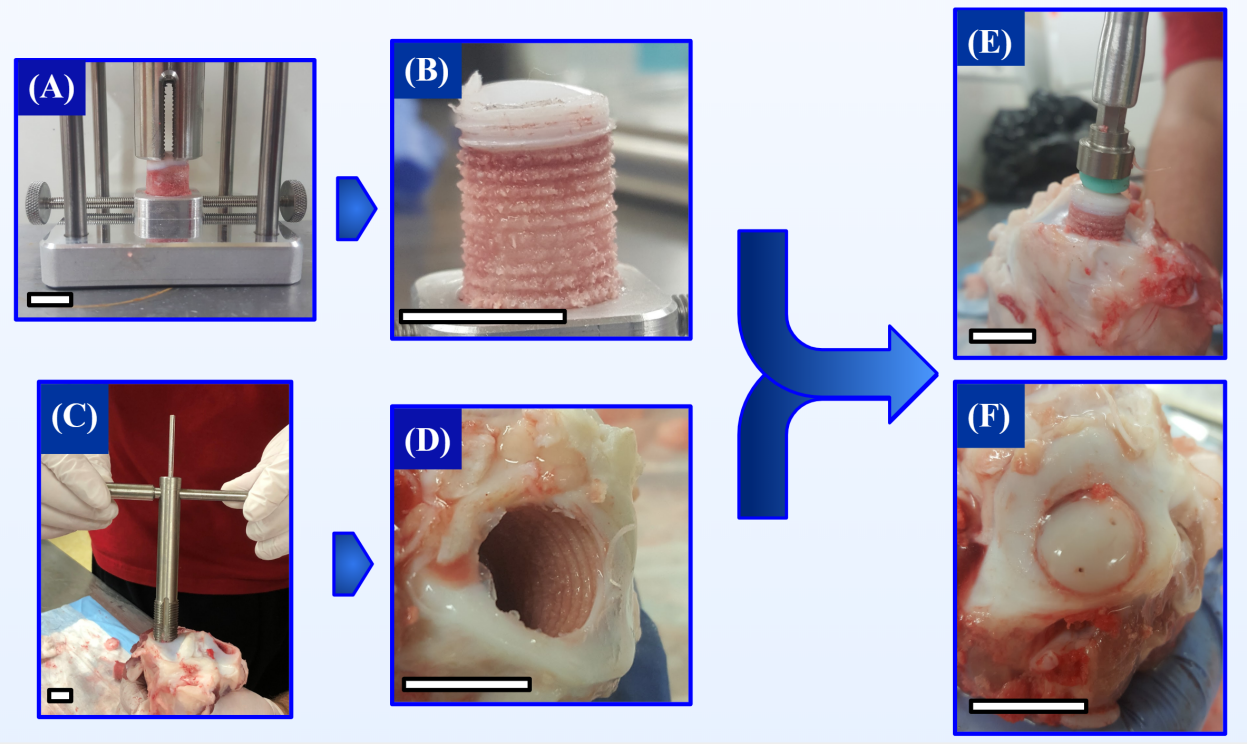

Figure 5. Graft threading procedure workflow. A: Graft in the supporting cup being threaded by the die. B: Threads defined on the graft. C: Tap inserted over the guidewire to define threads on the receiving site. E: Graft screwdriver being used to screw in the graft. F: Fully inserted graft.

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.

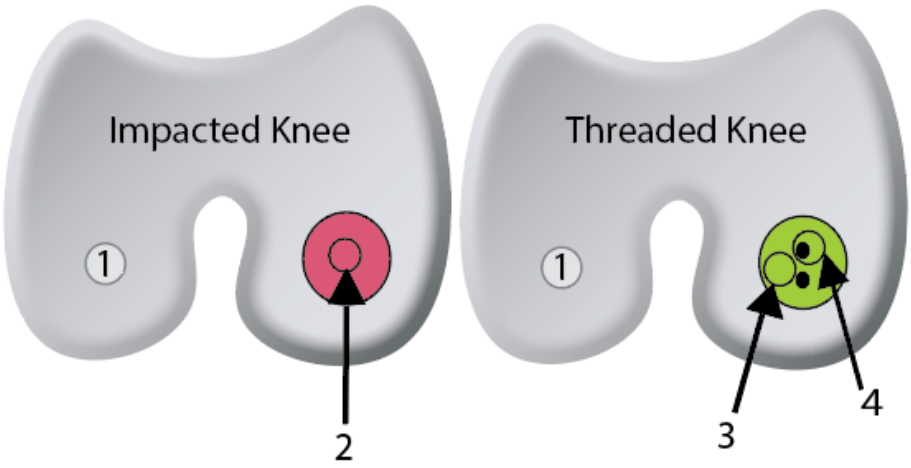

Figure 6. Diagram of biopsy locations in each sample set. A control biopsy (1) can be taken from a non-grafted section of cartilage. One biopsy (2) is taken from the impacted allograft (left), another is taken from the bulk of a threaded graft (3), and the last is take from a location surrounding a tine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

Graft Placement Testing

A Sawbone model was used to evaluate the accuracy of the threading tools as well as evaluate placement of the graft in the receiving site. Biologic bone samples are expensive, have complex geometry, and variable mechanical properties making controlled testing challenging. To better represent the mechanical properties of bone that our devices will be threading we performed testing in Sawbone. Sawbone is a synthetic polyurethane foam bone analog with material properties closely matching those of actual human bone. The density and Young's Modulus of the foam was chosen to mimic cancellous bone. The average density of cancellous bone in human femoral condyles is 0.346 g/cm^3 which was closest to the 0.32 g/cm^3 density foam which was used to conduct this threading testing[13].

The aim of this testing was to ensure the surface of the graft, when inserted into the receiving site, does not differ in height from the native surface by more than 1 mm. The first step

to evaluate the device is assessing how consistently it can thread the graft and receiving site. Consistent threading is important because if it is unable to consistently define threads where intended, then it will be impossible to develop a reliable procedure that ensures rotational alignment of the graft. Essentially, we need to evaluate if the device can start threading exactly where the threading tools are placed.

To perform the testing, receiving sites were milled to the appropriate 14.5 mm diameter and either 6 mm or 9 mm deep. These depth measures are multiples of the thread pitch (1.5mm/revolution) so if threading was performed as expected, the tap rotation should end where it started. For the receiving site threading, an arbitrary reference mark was made, and it was aligned with a mark on the tap denoting the start of the threads. Light downward pressure was applied to the tap handle as the tap was twisted to initial threading. The tap was threaded into the receiving site until it hit the bottom of the hole at which point the location of the thread starting point on the tap was transferred to the surrounding Sawbone to mark where the tap ended. This mark is known as the tap error and is quantified as the angular deviation from the arbitrary reference mark on the receiving site where tapping started.

A similar procedure was repeated in threading the graft. An arbitrary reference mark was placed on the graft and the starting location of the die threads was aligned with the graft mark before threading. Light downward pressure was applied to thread the graft and threads were cut at least 9 mm down the graft to ensure that there was enough thread to screw into the receiving site. After threading, the graft was cut to a length matching the receiving site depth. The bident was tapped into the graft and the graft was screwed into place. After screwing the graft into place, the graft reference mark was compared to the tap end mark and this angular offset was taken to be the die error. Similarly, the angular offset between the graft reference mark and receiving site mark was measured and is interpreted as the total error corresponding to the accuracy with which the graft can be inserted given errors in the tap and die.

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 7). 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 8a (showing a cross-section of cartilage from an impacted bone graft), and 8b (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 8c). 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.

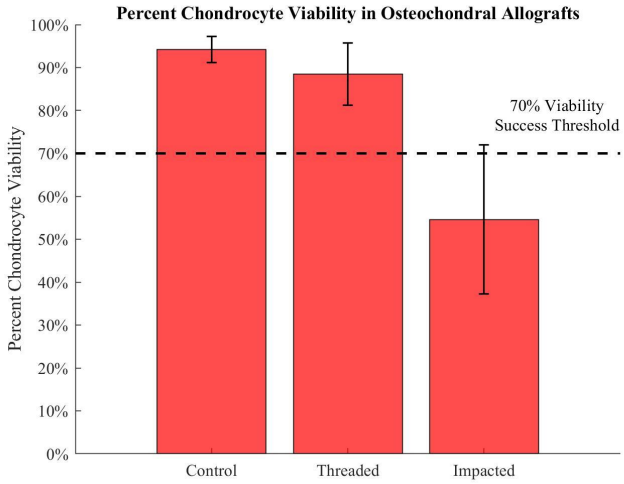

Figure 7: The average percent of living chondrocyte cells is recorded for each treatment group: non-impacted control (94.25% viability) threaded (88.46% viability) and impacted (54.61% viability) allografts (n = 5). A one-tailed t-test compared the viability between the threaded an impacted group to reveal a statistically significant reduction in chondrocyte death with the threading method (p = 0.0098).

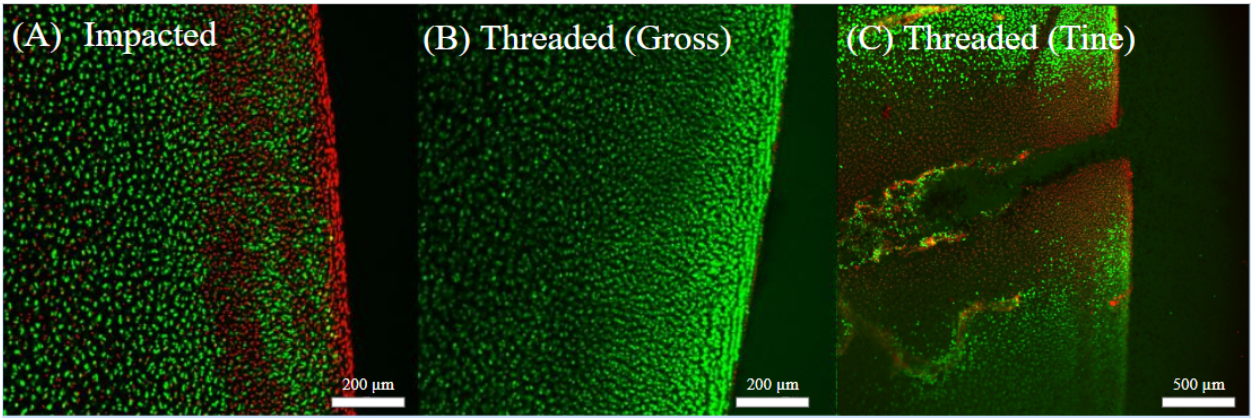

Figure 8: Cross sections of graft cartilage biopsy collected with confocal microscopy (Nikon A1Rs Confocal Microscope; UW Optical Imaging Core) 18 hours after staining. (A) Impacted graft under 10x magnification. (B) Threaded graft under 10x magnification. (C) Threaded graft at

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.

Graft Placement Results

Angle measurements of the tap error, die error, and total error in each mock surgery in Sawbone were quantified using ImageJ (Figure 9, left). The angle offsets were then translated into vertical height offsets by multiplying the offset angle with the thread pitch of 1.5 mm /360°. The average total height offset between the graft and receiving site surfaces on this idealized model was 0.36 mm, which is significantly less than the clinically relevant standard of 1 mm (Figure 9, right).

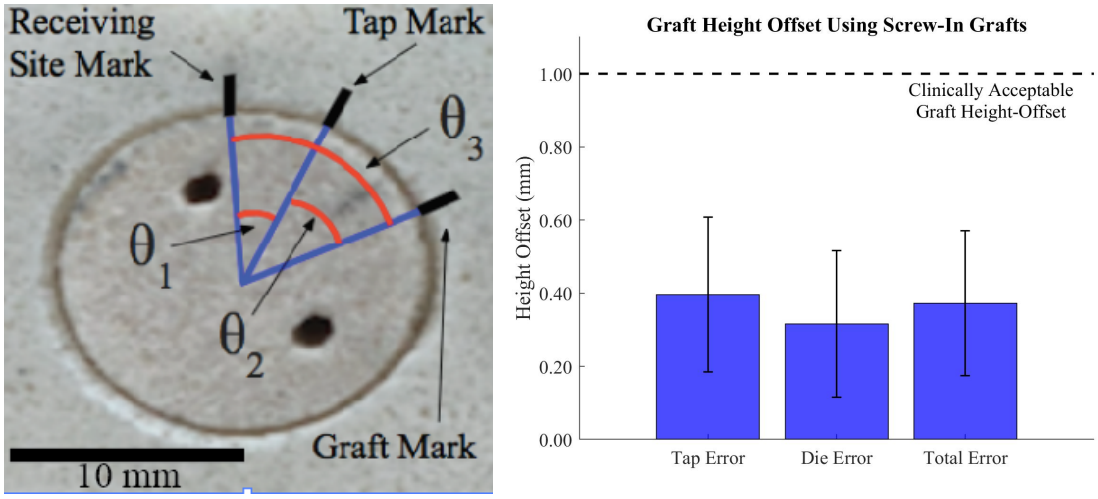

Figure 9: Left: Sawbone graft inserted into Sawbone receiving site. The receiving site mark indicates the intended position for the tap and graft alignment marks. The tap mark indicates the tap position when fully inserted, and the graft mark shows the alignment when the graft is fully inserted. θ_1: tap angle error, θ_2: die angle error. θ_3: total angle error. Right: Mean height offset between the graft and receiving site as a result of measured angle difference (n=26). Error bars indicate one standard deviation. The total height difference was less than the clinically acceptable graft height-offset threshold of 1mm.

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.

Results obtained from graft placement testing were promising. As a screw-in approach was used, the angular and vertical displacement of the graft in the receiving site were coupled. However, testing in Sawbone indicated the surgical tools were accurate enough to rotationally align the graft to the receiving site such that there was only an average of 0.36 mm height difference, which was well below the design constraint of 1 mm. With this system, however, the surgeon also has the ability to unscrew the graft and trim the graft from the bottom surface which may allow for even better graft placement accuracy.

While impressive results were observed in Sawbone, it is important to emphasize that this was an idealized model, and further complications could arise from testing the device on irregular geometries found in-vivo. In order to translate findings from an idealized model to real bone, a laser scanning method to measure the height offset between the graft surface and the original native articular surface was developed. Future work will include additional surgeries that utilize both cell staining and laser scanning to directly compare the current OATS method to the novel screw-in approach.

The team was fortunate enough to receive feedback from an independent orthopedic surgeon who tested our device, which hold promise for future clinical translation. The surgeon indicated that the system was simple to use and integrated well with the current surgical workflow. The surgeon also noted that the threaded mechanical interface between the graft and the patient would likely be beneficial to prevent premature graft loosening. Moreover, they appreciated how threading would allow the graft to be removed for adjustment beyond the initial insertion.

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.

References

1. Sherman SL, Garrity J, Bauer K, Cook J, Stannard J, Bugbee W. Fresh osteochondral allograft transplantation for the knee: Current concepts. *J Am Acad Orthop Surg*. 2014;22(2):121-133. doi:10.5435/JAAOS-22-02-121

2. Bugbee W. Osteochondral Allograft Transplantation: Clinical Outcome and Return to Sport. *Aspetar Sport Med J*. 2016:246-251. http://www.aspetar.com/journal/upload/PDF/201672616231.pdf. Accessed December 25, 2017.

3. Bugbee WD, Pallante-Kichura AL, Görtz S, Amiel D, Sah R. Osteochondral allograft transplantation in cartilage repair: Graft storage paradigm, translational models, and clinical applications. *J Orthop Res*. 2016;34(1):31-38. doi:10.1002/jor.22998

4. Long WJ, Greene JW, Cushner FD. Early Clinical Outcomes Associated with a Novel Osteochondral Allograft Transplantation System in the Knee. *Adv Orthop Surg*. 2016;2016:1-6. doi:10.1155/2016/1979348

5. Drosos GI, Pozo JL. The causes and mechanisms of meniscal injuries in the sporting and non-sporting environment in an unselected population. *Knee*. 2004;11:143-149. doi:10.1016/S0968-0160Ž03.00105-4

6. Torrie AM, Kesler WW, Elkin J, Gallo RA. Osteochondral allograft. *Curr Rev Musculoskelet Med*. 2015;8(4):413-422. doi:10.1007/s12178-015-9298-3

7. Garrett JC. *Allograft OATS ® Resurfacing Technique for Articular Cartilage Restoration*. Atlanta; 2016.

8. DePuy Synthese Mitek Sports Medicine. *COR ® PRECISION TARGETING SYSTEM Repair of Osteochondral Defects in Canines*. http://synthes.vo.llnwd.net/o16/LLNWMB8/US Mobile/Synthes North America/Product Support Materials/Technique Guides/SUTGCORSystemJ12821A.pdf. Accessed December 22, 2017.

9. Borazjani BH, Chen AC, Bae WC, et al. Effect of Impact on Chondrocyte Viability During Insertion of Human Osteochondral Grafts. *J Bone Jt Surg*. 2006;88(9):1934. doi:10.2106/JBJS.E.00992

10. Kang RW, Friel NA, Williams JM, Cole BJ, Wimmer MA. Effect of Impaction Sequence on Osteochondral Graft Damage: The Role of Repeated and Varying Loads. *Am J Sports Med*. 2010;38(1). doi:10.1177/0363546509349038

11. Pallante AL, Görtz S, Chen AC, et al. Treatment of Articular Cartilage Defects in the Goat with Frozen Versus Fresh Osteochondral Allografts: Effects on Cartilage Stiffness, Zonal Composition, and Structure at Six Months. *J Bone Jt Surg*. 2012;94:1984-1995. doi:10.2106/JBJS.K.00439

12. Gracitelli GC, MMeric G, Pulido PA, McCauley JC, Bugbee WD. Osteochondral Allograft Transplantation for Knee Lesions after Failure of Cartilage Repair Surgery. *Cartilage*. 2015;6(2):98-105. doi:10.1177/1947603514566298

13. Rohlmann A, Zilch H, Bergmann G, Kolbel R. Material properties of femoral cancellous bone in axial loading. *Arch Orthop Trauma Surg*. 1980;97(2):95-102. doi:10.1007/BF00450930

Appendix A: Product Design Specifications

Osteochondral Graft Tapping System

Product Design Specifications

Team: Alex Teague
Alex Babinski
David Fiflis
Zach Wodushek

Function: Osteochondral allografts (OCAs) are used to repair chondral defects in young, active patients. The current procedure involves cutting the graft from cadaveric tissue, then using impaction to drive the graft into a low-clearance receiving hole drilled over the defect. The large impulse associated with graft impaction often leads to decreases in grafted chondrocyte viability, and negatively affects procedure outcomes [1, 2]. To avoid deleterious impaction, we created a screw-in system which taps the patient receiving site and threads the donor graft allowing the graft to be screwed into the patient. Testing revealed that this new system has significantly higher implanted chondrocyte viability when compared to the impaction protocol. A challenge unique to our system, however, is that the one degree-of-freedom (DOF) nature of a screw mechanism limits graft adjustment relative to the traditional two DOF impacted graft. Therefore, the aim of this project is to develop a protocol for threading the graft and receiving site such that desired graft rotation and height can be achieved simultaneously when the graft is fully inserted into the patient.

Client Requirements

1. The protocol must permit a graft height offset from native tissue of no more than ±1.0mm.
2. After graft preparation and insertion, chondrocyte viability must be consistently greater than 70%, which has been shown to be a threshold to successful graft integration [1].
3. The entire system must be sterilized before use in surgery.
4. The threading protocol must be quick and easy to learn so as not to drastically alter the current surgical practice.
5. Damage to the chondral surface must be no greater than what presently occurs during OCA transplantation.

Design Requirements

1. **Physical and Operational Characteristics**
    a. **Performance Requirements**

i. Threading the graft and receiving site should not damage the articular cartilage
    1. It should limit gouging, scratching, and other mechanical alterations to the native, or graft cartilage.
    2. It should not result in significant chondrocyte death after use
ii. Insertion of the graft must be easily executed to minimize the risk of tissue damage.
iii. During the procedure, the graft should be easy to insert and remove allowing the surgeon to adjust the graft depth.
iv. The threading protocol must cut threads in the graft and receiving site that result in predictable graft placement.

b. **Safety**
    i. The threading system should not increase the chances of postoperative complications, including (but not limited to) infection, tissue death, or graft dislocation.
    ii. Long term, the threaded graft must not lead to an associated cartilage disorder, significant fissuring or fibrous tissue infiltration, or improper tissue integration.

c. **Accuracy and Reliability**
    i. The threading protocol should allow for successful graft integration into the recipient site. This means that the procedure should maintain at least 70% chondrocyte viability after implantation.
    ii. The measurement protocol should ensure that, after graft insertion, the donor curvature closely matches that of the recipient site within ±1.0 mm of height difference.

d. **Life in Service**
    i. Non-disposable components must be serializable to allow for repeated use
    ii. Life of device materials will vary depending on chosen stainless steel alloy.
    iii. Disposable components should be minimized in the design to prevent excessive recurring costs.

e. **Shelf Life**
    i. Capable of storage at room temperature.
    ii. Must be compliant with hospital regulations of storage.
    iii. Shelf life is not likely to present as a significant design consideration.

f. **Operating Environment**
    i. Protocol must not compromise sterility of the device or surgical field.
    ii. Must function within range of operating room temperatures, in addition to *in vivo* conditions.
    iii. Must be usable in concurrence with all other orthopedic tools and materials.

g. **Ergonomics**
    i. The devices must be designed for comfortable handheld use by the orthopedic surgeon during the procedure.
    ii. To promote easy rotation, the tool must be easy to locate over the central-axis of the graft.
h. **Size**
    i. Tools will be appropriately sized for handheld usage by orthopedic surgeon.
    ii. The device should accommodate bone graft sizes 10 mm - 25 mm in diameter and 7 mm - 14 mm deep.
i. **Weight**
    i. Since the device will be hand-held, its total weight should not be so heavy that it is cumbersome or fatigues the surgeon during use.
j. **Materials**
    i. All materials must pass ISO regulations to corrosion resistance and excessive wear from use [3].
    ii. Tools involved in the procedure must be sterilizable or disposable.
k. **Aesthetics**
    i. Aesthetics will serve as a secondary initiative to the function of the final product.

1. **Production Characteristics**
    a. **Quantity**
        i. One prototype capable of inserting the graft into the patient.
            1. The prototype may have more than one component.
    b. **Components**
        i. The final product must consist of a mechanism for inserting the graft into the recipient hole.
            1. A component must hold the graft in place and align a threading mechanism.
            2. An external threading component must create threads on a harvested graft.
            3. An internal threading component must create threads in the patient receiving site.
            4. A component will function as a screwdriver to screw the graft into the recipient site.
            5. A final component must define the starting threading position on the graft threading component to ultimately allow for predictable graft placement.
2. **Miscellaneous**
    a. **Standards and Specifications**

i. The final product must comply with the FDA standard for manual surgical instruments as stated by CFR 21 - Subchapter H - Medical Devices [3]

b. **Customer**
   i. Orthopedic surgeons implanting an osteochondral allograft

c. **Patient Related Concerns**
   i. Decreasing chondrocytes cell viability leads to diminished graft integrity.
   ii. Unwanted debris and fragments of the graft may be released into the synovial fluid environment and cause other complications.
   iii. A graft with an articular surface homologous to the native tissue is necessary for long term grafting success and patient health.

d. **Current Systems**
   i. Arthrex Osteochondral Allograft Transfer System (OATS). This system is the prototypical system used in osteochondral transplant procedures (and is most similar to the system Dr. Walczak uses). It uses a sizing guide, guide wire, and cannulating reamer to size, locate, and ream the chondral defect. The allograft is prepared using the hole saw which is guided by a manually held ring. The impaction rod forces the graft into the receiving hole [5].
   ii. Zimmer Chondrofix Osteochondral Allograft. This system uses a hollow punch hammered into the bone to guide the drill bit during receiving site preparation. There is no need to prepare an allograft since it comes with a pre-made, decellularized allograft that fits precisely in the hole created by the punch and drill bit. The graft is inserted most of the way using the insertion tool and is pounded in the remainder of the way using an impaction rod [6].
   iii. COR Precision Targeting System. This is the only surgical system that claims to address chondrocyte viability concerns associated with OCA transplantation. The tool encloses the graft during harvesting and insertion to protect it from mishandling. The surgical guide also claims to use “low impaction insertion” but does not describe how impaction forces are minimized relative to traditional tools. Despite the promise with the system, it is not currently in use in human OCA transplantation. [7]
   iv. There are no direct competitors, and of the ones currently in use, all rely on graft impaction.

References

[1] Borazjani BH, Chen AC, Won CB, et al. Effect of impaction on chondrocyte viability during insertion of human osteochondral grafts. J Bone Joint Surg Am 2006;88-A(9):1934-1943.

[2] Sherman SL, Garrity J, Bauer K, Cook J, Stannard J, Bugbee W. Fresh osteochondral allograft transplantation for the knee: Current concepts. *J Am Acad Orthop Surg*. 2014;22(2):121-133. doi:10.5435/JAAOS-22-02-121

[3] "CFR - Code of Federal Regulations Title 21," *accessdata.fda.gov*. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=888. [Accessed: 10-Oct-2017].
[4] W. Bugbee, "OSTEOCHONDRAL ALLOGRAFT TRANSPLANTATION CLINICAL OUTCOME AND RETURN TO SPORT", *Aspetar Sports Medicine Journal*, vol. 10, pp. 246-251, 2016.
[5] Garrett JC. *Allograft OATS ® Resurfacing Technique for Articular Cartilage Restoration*. Atlanta; 2016.
[6] Zimmer, Inc., "Zimmer® Chondrofix® Osteochondral Allograft Surgical Technique", *Zimmer*, 2017. [Online]. Available: http://www.zimmer.com/content/dam/zimmer-web/documents/en-US/pdf/surgical-techniques/biologics/zimmer-chondrofix-osteochondral-allograft-surgical-technique.pdf. [Accessed: 04- Oct- 2017].
[7] DePuy Synthese Mitek Sports Medicine, "COR ® PRECISION TARGETING SYSTEM Repair of Osteochondral Defects in Canines Repair of Osteochondral Defects in Canines Repair of Osteochondral Defects in Canines."

Appendix B: Previous Designs

The design of this prototype has undergone a few iterations but has largely retained the same structure. The threads on the tap and die initially had a 2 mm thread pitch but were reduced to a 1.5 mm pitch in order to increase the stability of the mechanical interface when the graft is inserted. Additionally, the reduction in thread pitch would allow the graft to always be within a single 360-degree rotation of the 1 mm threshold for surface alignment. The guiding platform was initially shorter but did not have the necessary room to easily slide the graft cup through it. The bowl inside the graft cup was shortened to allow for longer graphs to be threaded. Initially we secured the graft in the graft cup with screws extending through the opposite side but switched to thumb screws for simplicity. One major addition to the current design was the addition of the graft screwdriver, which the original design did not have. This was necessary because the original method of screwing was using the surgeon's fingers. This became quite difficult near full insertion of the graft when grip failed. The graft screwdriver is a tool that allows the graft to be inserted flush to the surface of the patient recipient site with minimal damage to the articular cartilage.

Appendix C: Fabrication

<u>Materials</u>

The key material constraints in this prototype were driven by the fact that they must comply with a surgical environment. Traditionally, these include 400-series martensitic stainless steels which offer excellent strength and corrosion resistance. While these alloys are the standard in surgical applications, they tend to be more expensive, harder to machine, and are more challenging to locate in appropriate sizes. Given that the team was simply making a prototype to

demonstrate a proof-of-concept, scrupulous adherence to these standards was not necessary. The team decided to use 300-series stainless steel alloys which are less expensive, easier to machine, and more readily available, but still offer sufficient strength and corrosion resistance. All tap, die, and guidewire components were made from stainless steel to demonstrate functionality when they are not made from traditional high-speed tool steel. The graft holding cup, as well as the die guiding plate and graft cup holding plate were made from 6061-aluminum because it was far less expensive and made manufacturing significantly quicker than using even 300-series stainless steel. The material properties of these components are not critical to proper prototype function, so this was deemed an acceptable substitution.

Methods

The surgical system consists of five primary components: the tap, die, graft holding cup, the alignment platforms, and the graft screwdriver. Each was manufactured separately, while periodically checking for the necessary mutual integration between interfacing components (i.e. matching the threads between the tap and die; ensuring a slip fit between the die body and the guiding hole on the alignment platform, and between the graft holding cup and its supporting plate).

Tap:

The tap was cut to rough length of 100-mm from the raw stock. It was then turned down on a lathe to 16-mm, the major diameter of the M16x1.50 thread to be cut. 30-mm of the rod was left at the 16-mm major diameter, and the remaining 70-mm was turned down to 14.5 mm which is below the minor diameter of the thread—this would allow for proper threading of the tap. An M16x1.50 die was used to cut the external threads along the 30 mm that was left at the 16-mm diameter. Next, a 0.125-inch hole was drilled through the entire length of the die to accommodate the guidewire. Both ends of the hole were countersunk, and both ends of the tap were given a slight chamfer.

At the mill, the tap was placed in a square collet block with the threaded end out. A 0.25-inch ball endmill was used to cut 4 flutes at 90 degrees to one another down the middle of the threaded portion. The tap was flipped in the collet block exposing the unthreaded end into which a 0.25-inch hole was drilled to accommodate the tap handle. Both sides were countersunk, and the tap was complete.

The tap handle was cut to 100-mm in length before being turned down to 0.375-inches in diameter. The diameter was further reduced to 0.25-inches along 70-mm of the handle to allow it to slide into the tap-handle hole. Both ends were chamfered before taking the handle to the mill to drill the 0.125-inch guidewire hole in the middle of the handle.

Die:

The 1-inch die stock was cut to 100-mm in length before turning down to 0.95-inches. A 14-mm tap hole was drilled in the end of the die to allow the M16x1.50 tap to cut internal threads. This hole was tapped and countersunk, before both ends of the die were chamfered. At the mill, the die was placed in a collet block before a 0.25-inch ball endmill was used to flutes on opposite sides. Like with the tap, a 0.375-inch hole was drilled at the opposite end to accommodate the die handle.

Graft Holding Cup:

The graft holding cup was cut from a 2-inch long piece of 1-inch square aluminum stock. This was oriented vertically in the mil vise and CNC was used to mill the external profile. A 0.375-inch hole was drilled all the way through (giving access to push the graft out should it become stuck). Over this hole, a 0.625-inch hole was drilled to a depth of 0.5-inches. The bottom of this hole was flattened, and the diameter enlarged using an endmill. The block was cut to final length, and holes for the bone securing pins were drilled in each of the four sides.

Alignment Platforms:

Both top and bottom support plates were started by squaring the aluminum stock, before using the CNC mill to cut the top half of the external profile, then flipping to mill the other half. A 0.240-inch hole was drilled in the four corners of both plates before they were reamed to 0.249-inches. This would allow for a press fit of the 0.2500-inch stainless-steel support pins. A 0.75-inch hole was drilled in the middle of both plates. This hole was progressively enlarged using the CNC mill until it allowed for a slip fit of the die body. The hole in the bottom plate was cut with a profile matching the graft holding cup and was tuned to allow for a slip fit of the cup. An arbor press was used to press the stainless-steel pins into the bottom plate before the top plate was pressed over the other end of the pins.

Graft Screwdriver

The graft screwdriver consists of three components: the main driver body, the stainless-steel pins, and the protective silicone cap. The stainless-steel pins were purchased pre-fabricated at an appropriate length and diameter (15.875 mm long; 1.5875 mm diameter). The pins are sharpened on a lathe allowing them to pierce the tissue during insertion and minimize local damage.

The protective silicone cap was made using a custom mold. The silicone comes as two liquid parts that solidify after mixing. The liquid silicone was poured into a custom 3D printed mold that exactly matches the final dimensions of the protective cap. After pouring, the silicone and mold was placed in a vacuum chamber to remove residual air pockets from the liquid silicone ensuring a uniform part.

The driver body was fabricated from 303 stainless-steel rod turned to a diameter of 10 mm and a length of 20 mm on the lathe. A 15 mm portion of this 10 mm blank was further turned down to 6.35 mm in diameter, and 15 mm in length to rough out what will become the hex-driver portion of the driver (this allows it to be used in any standard screwdriver).

After the driver profile was roughed out on the lathe, it was placed in a collet block to secure it in the mill. A 3/16-inch ball end-mill was used in the CNC mill to define the standard 1/4-inch hex profile on the back of the driver. A 1.5875 mm drill bit was used to create holes for the sharpened pins to be press-fit into the driver body. The molded silicone was then slid over the sharpened pins, and the graft screwdriver was completed.

Appendix D: Tissue Staining Challenges

Culturing the biopsy samples during cell staining was the challenge with the viability testing. We were limited in the amount of calcian AM & ethidium homodimer staining supplies. Therefore, biopsies were cultured in a smaller volume, 1 mL per biopsy, of DMEM media overnight. However, the DMEM changed colors overnight, indicating acidification of the media. Widespread cell death was observed in all the biopsies, including the control samples, which suggests that 1 mL of media was not enough to maintain cell viability overnight.

Appendix E: CellProfiler Pipeline

Images:

In this phase of the CellProfiler pipeline, 10X images from the confocal microscope were uploaded as RGB TIFF files.

Metadata:

No metadata was extracted from the files.

NamesAndGroups:

A file name was given to the image such that it may be processed by cell profiler.

UnmixColors:

This function separated the two wavelengths of color into individual images. Any pixel containing green of color content 0.5 was transposed into an image in a file named Calcien. Any pixel containing red of color content 0.5 was transposed into an image in a file named Ethd. These were named after the respective fluorochromes that were imaged. The other part of this function was that it creates a grayscale of the two separated images.

ImageMath:

CellProfiler identifies black space as background and white pixels as objects. The UnmixColor function renders output images that will grayscale the images to color objects black and background white. Therefore, the grayscale must be inverted to identify the desired objects. This function inverts the input images grayscale and was applied to the Calcien file. Thus, any pixel that was white becomes black, and vice versa.

ImageMath:

This is the same as the above ImageMath function but for the Ethd image, and the output file was named EthdAfterMath.

Threshold:

In order to separate desired objects from background a threshold was applied. A threshold of 0.5 to 1.0 was applied to the CalcienAfterMathFile, and output as CalcienThreshold.

Threshold:

This is the same as the above Threshold function but for EthdAfterMath, and the output image was named EthdThreshold.

IdentifyPrimaryObjects:

Through an initial run through of identifying primary objects the 10th percentile size of objects in the CalcienThreshold image was 5.1 pixels in diameter and the 90th percentile size of objects in the CalcienThreshold image was 19.4 pixels in diameter. Thus, objects outside of this range were discarded. This function then counted any object within this interval and output the total number of objects counted.

IdentifyPrimaryObjects:

Through an initial run through of identifying primary objects the 10th percentile size of objects in the EthdThreshold image was 3.1 pixels in diameter and the 90th percentile size of objects in the EthdThreshold image was 12.4 pixels in diameter. Thus, objects outside of this range were discarded. This function then counted any object within this interval and output the total number of objects counted.

Appendix F: High Density Polyethylene Testing

High Density Polyethylene (HDPE) was initially employed for our threading accuracy testing. However, due to the elastic properties of this material, it proved to be an unsuitable model for characterizing threading efficiency of our device. The threading tools did not cut the plastic as expected, but rather formed the material to make the threads. The forming action was not suitable because it altered the effective dimensions of the receiving site and graft making them impossible

to thread together. To actually quantify threading accuracy, a more representative bone model was needed, which led us to using Sawbone.

Appendix G: Testing Tissue Mechanical Properties

The tissue that was available for testing this semester did not accurately represent the mechanical properties of human bone in which this threading system would be used. The tissue was obtained from research pigs at the Wisconsin Institute for Medical Research (WIMR) and all of these animals were juveniles at approximately 3 months old. As such, they were far from skeletal maturity, and had incredibly soft bone that made threading nearly impossible. At 3 months, pig bone has a Young's Modulus of 480 to 700 MPa, compared to 750 to 900 MPa at 7 months [1]. The weak material properties of these young pigs explained why the subchondral bone was soft enough to be crumbled by hand in some cases. When compared to that of a typical human patient undergoing this procedure of over 18 years of age, who has a mean cortical bone Young's Modulus of 20.7 GPa [2], there is a significant difference in mechanical properties. This discrepancy led to poorly defined threading and an inadequate interface between the graft and the recipient site. Moreover, there was a weak interface between the cartilage and subchondral bone which caused the cartilage to often shear off the subchondral bone when cutting the graft. Poor bone threading, and graft cartilage shearing from the bone prevented any effective threading testing or viability evaluation.

[1] T. D. Crenshaw, E. R. Peo, A. J. Lewis, B. D. Moser, and D. Olson, "Influence of Age, Sex and Calcium and Phosphorus Levels on the Mechanical Properties of Various Bones in Swine," J. Anim. Sci., vol. 52, no. 6, pp. 1319–1329, Jun. 1981.

[2] J. Y. Rho, R. B. Ashman, and C. H. Turner, "Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements.," J. Biomech., vol. 26, no. 2, pp. 111–9, Feb. 1993.

Appendix H: 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 MeshLab and registered to the unaltered joint scan using the ICP algorithm [1]. This will allow for a direct comparison between the reference and grafted scans. The point cloud data were then imported into MATLAB and were fit with a scattered data interpolation 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 showing an accurately placed graft, and an inaccurately placed graft are shown in Figure 1.

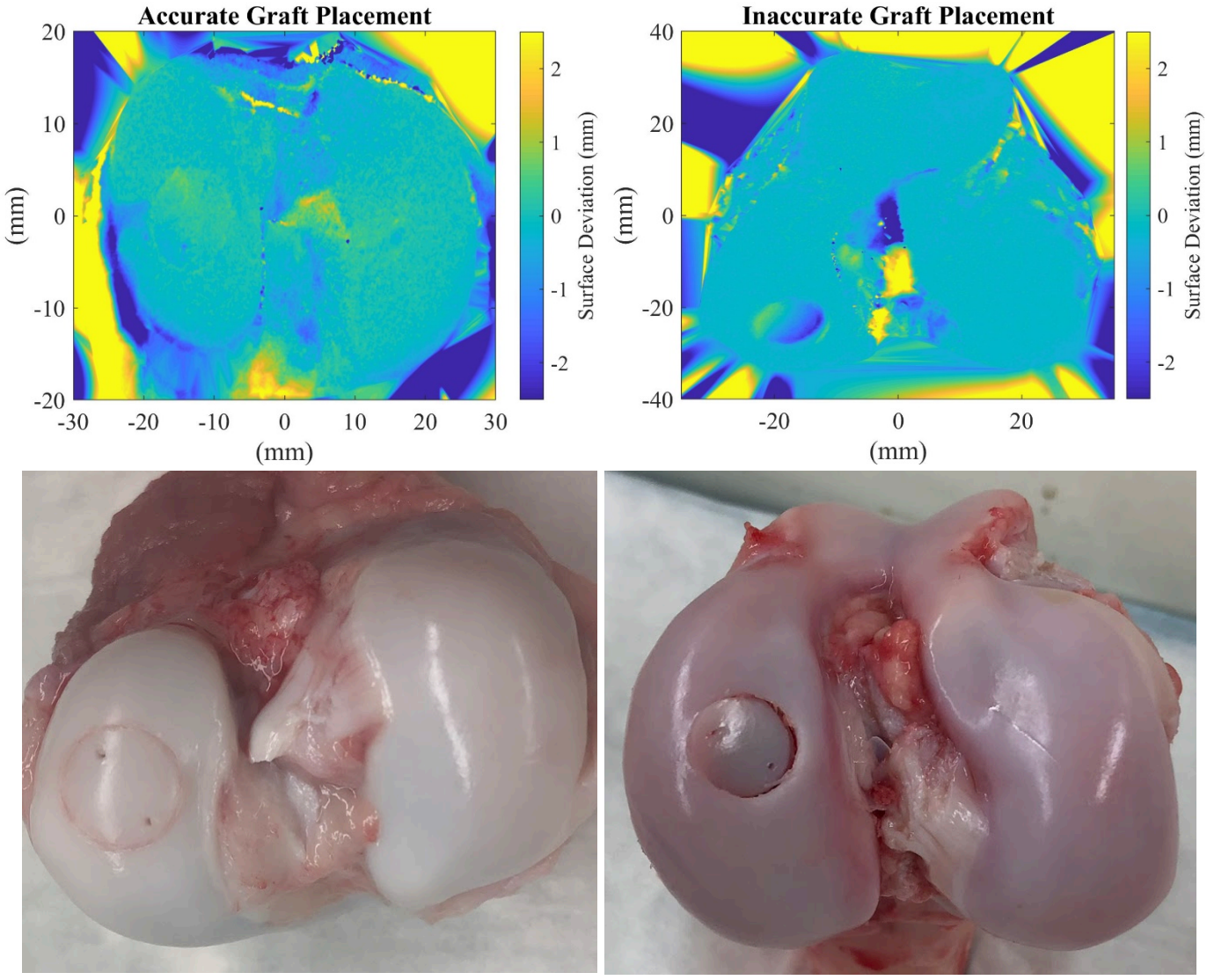

Figure 1: Laser measurements showing surface deviation from native cartilage of an accurately placed graft, and an inaccurately placed graft. Large deviation (height difference) magnitudes

indicate poor graft placement accuracy. Images of the scanned femur are included below the surface deviation plots to show grafting accuracy in the tissue.

[1] P. J. Besl and N. D. McKay, “A Method for Registration of 3-D Shapes,” IEEE Trans. Pattern Anal. Mach. Intell., vol. 14, no. 2, pp. 239–256, 1992.

Appendix I: Materials and Expenditures

Fall 2017

Use	Product	Part Number	Supplier	Link	Quantity	Unit Price	Total Price
Threaded Tap	Rod,SS,303, 3/4 In Dia x 1 Ft L	2EWZ5	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-2EWZ5?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EWZ4_AS01?$smthumb$	1	$9.80	$9.80
Tap/Die Handle	Rod Stock,SS,1 ft. L,3/8 in. dia.	48KU26	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-Stock-48KU26?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EYA2_AS01?$smthumb$	1	$4.05	$4.05

Die Tube	Rod,SS,304, 1 In Dia x 1 Ft L	2EXG5	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-2EXG5?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EWZ4_AS01?$smthumb$	1	$17.15	$17.15
Die Support Platforms	Aluminum Flat Stock, 6061 Alloy, 0.500" Thick, 12" L X 3" W, Corrosion Resistant	2EZJ3	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Aluminum-Flat-Stock-2EZJ3	1	$9.75	$9.75
Die Platform Support Rods	1/4X2-1/2 416 SS DOWEL PINS	88231915	MSC	https://www.mscdirect.com/product/details/88231915	5	$2.63	$13.14
Graft Cup	Aluminum Square Stock, 6061 Alloy, 1.000" Thick, 12" L X 1" W, Corrosion Resistant	2EZV9	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Aluminum-Square-Stock-2EZV9	1	$6.75	$6.75
Guide Wire	Rod Stock,SS,1 ft. L,1/8 in. dia.	48KU23	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-Stock-	2	$1.12	$2.24

				48KU23?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EYA2_AS01?$smthumb$			
Recipient Site Guide Tube	1 Ft Welded for Plumbing, HVAC And Automotive Stainless-Steel Tubing, 1/2" Outside Dia., 0.440" Inside Dia	48KV02	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-1-Ft-Welded-For-Plumbing-48KV02	1	$6.90	$6.90
Recipient Site Drill Bit	Brad Point Drill Bit,HSS,7/16" x 5-1/2"	19TH11	Grainger	https://www.grainger.com/product/EAZYPOWER-Brad-Point-Drill-Bit-19TH11?breadcrumbCatId=5071&functionCode=P2IDP2PCP	1	$7.60	$7.60
Graft Plug Cutter	Tennon Plug Drill Bit,Steel,5/8"x5-1/2"	29EH39	Grainger	https://www.grainger.com/product/FISCH-Tennon-Plug-Drill-Bit-29EH39?breadcrumbCatId=5071&functionCode=P2IDP2PCP	1	$45.86	$45.86
Testing Tissue	Bovine	n/a	Conscious	https://conscio	6	$7.21	$43.27

	Knuckles		Carnivore	us-carnivore.com/			
Viable Tissue	Bovine Shank, donated by owners	n/a	Johnson's Sausage	http://www.johnsonssausage.com/	4		$0
Tap and tap handle	Standard machining, 14 mm	n/a	Student Shop		1		$0
Die and die handle	Standard machining, 14 mm	n/a	Student Shop		1		$0
Drill bit	m14 14 mm standard drill bit	n/a	Student Shop		1		$0
Drill	Standard drill	n/a	Alex's lab		1		$0
Hammer	Standard claw hammer	n/a	Alex's lab		1		$0
Bone Saw	Standard bone saw	n/a	Grace's Lab		1		$0
DMEM Cell Media	Maintains tissue viability	n/a	BME lab				$0
PBS	Used as biologically neutral tissue wash	n/a	BME lab				$0
Calcein AM/EthD-1	Live/Dead fluorescent stain	n/a	Dr. Walczak				$0
Hoechst Stain	Nuclei fluorescent stain	n/a	Grace's Lab				$0
4% Agarose Gel	Solid gel used for stabilization of samples on	n/a	Grace's Lab				$0

	fluorescent microscope slides						
Fluorescent Microscope	Used to observe cell counts after staining	n/a	BME lab		1		$0
						Material total	166.51
						S+H	$26.28
						Tax	$8.89
						Total:	$201.68

Spring 2018

Use	Product	Part Number	Supplier	Link	Quantity	Unit Price	Total Price
Die Platform Support Rods	1/4" Pin Diam, 3" Pin Length, Grade 416, Precision Dowel Pin	88231923	MSC Industrial Direct Co., Inc.	https://www.mscdirect.com/product/details/88231923	5	$ 2.86	**$ 14.30**
Threaded Tap	Rod,SS,303,3/4 In Dia x 1 Ft L	2EWZ5	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-2EWZ5?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.c	1	$ 9.80	**$ 9.80**

				om/rp/s/is/image/Grainger/2EWZ4_AS01?$smthumb$			
Tap/Die Handle	Rod Stock,SS, 1 ft. L,3/8 in. dia.	48KU26	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-Stock-48KU26?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EYA2_AS01?$smthumb$	1	$ 4.05	**$ 4.05**
Die Tube	Rod,SS,304,1 In Dia x 1 Ft L	2EXG5	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Rod-2EXG5?breadcrumbCatId=17071&s_pp=false&picUrl=//static.grainger.com/rp/s/is/image/Grainger/2EWZ4_AS01?$smthumb$	1	$ 17.15	**$ 17.15**
Die Base	3/4" {T} x 3-1/2" {W} x 18" {L} 6061-	N/A	Speedy Metals	https://www.speedymetals.com/pc-2307-8351-	1	$ 24.45	**$ 24.45**

	T6511 Aluminum Flat, Extruded			34-x-3-12-6061-t6511-aluminum-extruded.aspx			
Trident pins	Unhardened Ground Stainless Steel Dowel Pin, Passivated Finish, 5/8" L, 0.0311 to 0.0313" Pin Dia.	5EDX8	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Unhardened-Ground-Stainless-5EDX8	5	$ 0.74	**$ 3.70**
Pins	Unhardened Ground Stainless Steel Dowel Pin, Passivated Finish, 5/8" L, 0.0623 to 0.0625" Pin Dia.	5EDZ1	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Unhardened-Ground-Stainless-5EDZ1	5	$ 0.73	**$ 3.65**
Graft Secure tool	Thumb Screw, Knurled, 10-32 x 1 1/2 L	5RA86	Grainger	https://www.grainger.com/product/GRAINGER-APPROVED-Thumb-Screw-5RA86	2	$ 5.60	**$ 11.20**
						Material Total:	**$ 88.30**
						Tax:	**$ 6.88**

						S & H	**$ 39.35**
						Total Expense:	**$ 134.53**

Fall 2018/Spring 2019

Use	Product	Part Number	Supplier	Link	Quantity	Unit Price	Total Price
Mock graft for geometric fitting in plastic	Rod Stock, HDPE, ⅝ in., 48 in.	22JL48	Grainger	https://www.grainger.com/product/POLYMERSHAPES-Rod-Stock-22JL48?searchBar=true&searchQuery=22JL48	1	$9.40	$9.40
Mock receiving site for geometric fitting in plastic	Sheet Stock, 12” LX 12” W X 1.000” Thick, 176 Max. Temp. (F), Off-White	1ZAH3	Grainger	https://www.grainger.com/product/POLYMERSHAPES-Sheet-Stock-1ZAH3?searchBar=true&searchQuery=1ZAH3	1	$22.15	$22.15

Mimic of cancellous bone for geometric fitting in physiologically relevant model	Sawbone, 20 pcf Block	1522-03	Sawbone	https://www.sawbones.com/catalogsearch/result/?q=+1522-03	2	$17.75	$35.50
Drill bit for creating recipient sites	Flat-Blade Drill Bit for Wood	2894A65	McMaster-Carr	https://www.mcmaster.com/catalog/125/2524	1	$3.44	$3.44
Testing models for training surgeons on the device	Cow knuckles	N/A	Conscious Carnivore	N/A	5.12 lbs.	$4.99/lb.	$25.54
						Material Total:	$96.03
						Tax:	$3.89
						S+H:	$28.59
						Total:	$128.51
						Project Total:	464.72