Osteochondral Transplant Delivery System

Preliminary Report

Biomedical Engineering Design 301
Department of Biomedical Engineering
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Team Members:
Rodrigo Umanzor (Leader)
Nicholas Zacharias (BSAC & BWIG)
Eduardo Enriquez (Communicator)
Bilin Loi (BPAG)

Client: Dr. Brian Walczak, DO
Department of Orthopedics and Rehabilitation
University of Wisconsin Hospital

Advisor: Dr. Krishanu Saha, PhD
Department of Biomedical Engineering
University of Wisconsin Madison

Abstract

Current osteochondral graft surgical procedures repair articular cartilage defects on the femoral condyle using impaction for insertion of the allograft into the recipient site. The forces associated with impaction has a detrimental affect on the chondrocyte viability within the cartilage layer and can cause long-term failure of the allograft. Therefore, we proposed a novel design that utilized threading of the allograft and the recipient site for delivery through rotational means rather than impaction. We found that threading was a viable option and a live/dead assay demonstrated that chondrocyte viability after allograft delivery was comparable to impaction, but the results were rather inconclusive. Further testing is necessary to evaluate the potential for a difference between chondrocyte viability associated with two insertion methods. Confocal microscopy is a precise and accurate option to evaluate the viability of chondrocytes. Imaging with a confocal microscope allows for observations of live/dead cells a various depths within a section of sample tissue. Three bone plugs will be obtained for each time point in a threaded, impacted and controlled condition. The cartilage will be removed from each plug and subjected to a live/dead assay. The cartilage imaged using a confocal microscope to obtain cell counts and later analyzed for cell viability percentages.

Table of Contents

Introduction

Motivation

Articular cartilage defects in the knee are commonly detected in roughly 60% of patients undergoing knee arthroscopies [1]. Disease or blunt trauma to the knee induces a degenerative cascade of articular cartilage that can ultimately result in osteoarthritis (*Figure 1*). Symptoms experienced by individuals with osteoarthritis include varying degrees of pain and loss of anatomical movement or altered function of the diseased bone. Current treatment for osteoarthritis defects involve preventative care and surgical procedures that aim to replace the diseased cartilage, such as osteochondral allografts. However, osteochondral allograft surgeries exhibit a 75-80% success rate as mentioned by the client on account of current methods used to deliver the graft. Current methods have detrimental effects on the long term survival of the cartilage due to the mechanical loads associated with graft delivery [2]. As of now, there are no clinical procedures that are consistent and reliable in regenerating cartilage. Hence, there is a need for a novel procedure that will minimize the magnitude of mechanical loads required for proper delivery of the graft to reduce adverse effects in transplanted cartilage.

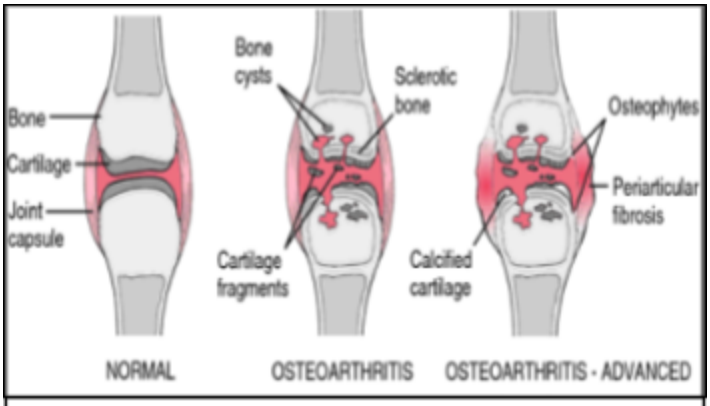

Figure 1: A diagram depicting differences between healthy bone and various cases of osteoarthritis [4]

Existing Devices/ Current Methods

The current procedure involves placing an appropriately sized Lesion Gauge up next to the defect, and driving a guide pin through the gauge into the bone. The Lesion Reamer is used along the guide pin to drill down to a section of good bone to create the recipient site. Measurements are taken of this site, and are used for fabrication of the allograft. The lesion gauge is placed into the GraftStation™ to find an appropriate surface on the donor tissue, so that the best fit is obtained in all planes of the articular surface, and the 12 O'Clock position is marked. The GraftMaker™, which is a trephining tool is used after locking it into the GraftStation™ and cylindrical graft is cut. This locking prevents horizontal movement of the GraftMaker™ as movement is inhibited in the x and y plane. This cylindrical portion is removed

by cutting at the lower aspect perpendicular to the cylindrical cut. The cylindrical graft is measured and appropriate modifications are made to match the recipient site. Finally, the graft is inserted by gentle impaction using the tamp tool [3]. A calibrated dilator [4] or bevel of the bone in the recipient site [5] can be utilized as well to obtain a press fit by hand, but often impaction is required to obtain a tight fit and appropriate seating into the recipient site [4]. The cartilage surface of the graft is impacted until it is flush with the articular surface surrounding the recipient site. *Figure 2* provides a schematic for this procedure.

The impaction required to insert the graft frequently causes a decrease in the viability of the chondrocytes, which are the cells in cartilaginous tissue. This can ultimately cause associated cartilage disorders due to the lack of viable tissue. For example, fibrocartilage can form during implantation. Fibrocartilage is a scar tissue that forms due to the limited regenerative capabilities of cartilage. It also has a higher coefficient of friction, which can lead to early degeneration of the tissue [6]. In order to limit these effects, the implanted tissue should contain greater than 70% chondrocyte viability postoperatively [7]. However, this is an estimated value, and an acceptable procedure should allow for the long term success of the graft by reducing the mechanical stimuli necessary for graft insertion to limit cell death that could induce associated complications such as fibrocartilage. If after transplantation the graft is not inserted so that it is flush with the articular surface the surgeon must drill into the plug, remove it from the recipient site and make sizing adjustments of the recipient site or graft as necessary. This causes further damage to the cartilage layer as well and decreases cell viability, and is an inherent risk associated with this procedure.

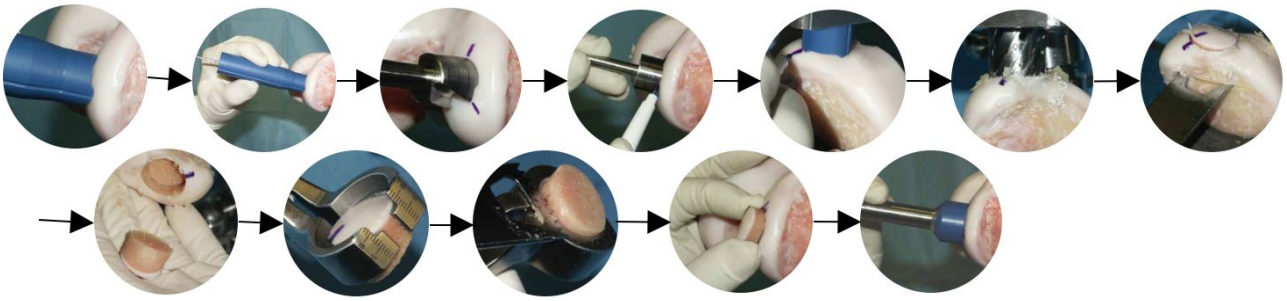

Figure 2: Current clinical procedure used for an osteochondral allograft (ACT Allograft method).

Problem Statement

Osteochondral allografting is a common procedure performed on patients that have a defect in cartilage and bone tissue. Current methods of implantation require the application of mechanical forces that have a detrimental effect on the live chondrocytes present on the graft tissue. Maximizing the amount of viable tissue during and after the surgery is a crucial factor for the success of the procedure. Hence, the client requests a delivery system that will reduce the amount of mechanical forces required to securely place the implant into the recipient site.

Background

Client Information

Dr. Brian Walczak, an orthopedic surgeon, was seeking a novel delivery system for osteochondral allografts that would reduce the compressive loads and in theory increase success rates of the surgical procedure.

Relevant Biology & Physiology

Unlike the majority of other connective tissue found in the human body, articular cartilage is composed of a single cell type, the chondrocyte [8]. Although they are the only cell types found in cartilage, they are present at relatively low densities. Chondrocytes are responsible for producing the collagenous extracellular matrix that makes up the avascular and aneural network of cartilage. The lack of blood supply in combination with the natural sparse presence of chondrocytes contribute to the poor regenerative properties of articular cartilage. Consequently, any significant damage done to the chondral surface of a knee will experience little to no regeneration. The injury could cause sustained cartilage degeneration from imposing detrimental stresses on the defect or following fibrocartilage formation and eventually lead to osteoarthritis.

The poor regenerative properties of articular cartilage emphasize the significance of minimizing the loss of viable chondrocytes during osteochondral allografting procedures. Given that these cells are responsible for producing and maintaining the extracellular matrix of articular cartilage, any damage done to them can jeopardize the success of a procedure that is intended to replace diseased cartilage. The relevance of chondrocytes in these procedures is further investigated in a study that explored the effect of donor chondrocyte viability in the overall success of osteochondral allografts performed in the femoral condyle of canines [7]. A successful procedure was defined as one that demonstrated graft integration and lacked cartilage disorder six months after implantation. The study found that successful grafts had at least 70% of viable donor chondrocytes at the time of transplantation. Grafts with less than 70% chondrocyte viability were unsuccessful following the six-month period and experienced infiltration from fibroblasts in the surrounding host tissue. Similarly, the quantity of viable chondrocytes in human osteochondral allografts has an integral role in the overall success of the procedure.

Product Design Specifications

This design must meet specific standards in order for it to be a valuable option for use in osteochondral allograft procedures. The most important criterion must be an increase in the percent chondrocyte viability from the current impaction method, which is approximately 70% mentioned by Cook et al. [7], as well as the client. This will prospectively be achieved through a reduction of mechanical forces on the articular cartilage during insertion. More specifically, the normal forces applied to the articular cartilage for proper insertion should not exceed 165 N, the force value measured by Dr. Walczak during impaction procedure. Another criterion is that the device will not cause any chipping or fragmentation of the bone plug during the procedure by remaining under the stress limitations of trabecular bone. These two specifications along with proper fitting of the graft into the recipient site will ensure success of the implantation with proper graft integration into native tissue, properly maintained hyaline cartilage, lack of

associated cartilage disorder, and lack of significant fissuring, fibrillation, or fibrous tissue infiltration [7]. These should improve upon the current success rate mentioned by the client of 75-80%.

Additionally, the device should be compatible for use in the operating room as well as easily sterilizable. Any necessary plastic or polymeric components used in the design will be designated for single use only, and all metals will be sterilizable and available for multiple uses if desired by the surgeon. The device should comply with FDA standards for surgical devices [9]. The sizing of the device will vary depending on the extent of the defect, but should be capable of creating a recipient site and a plug at mm increments ranging from 5mm-20mm. This device should also allow for easy insertion of the graft, and should not require more time in the operating room than the current standard of 5 hours mentioned by the client. Compliance of these specifications should be made with an estimated budget of $250. For the full project design specifications see Appendix I.

Preliminary Testing Designs

In order to accurately assess chondrocyte viability, three preliminary testing methods were considered. While the imaging techniques vary amongst these methods, they all use the same calcein acetoxymethyl (AM)/ ethidium homodimer stain to mark the live and dead cells, respectively. Calcein AM is permeable to the cell membrane and it is a precursor to fluorescent calcein. When the cell is alive this conversion will be made intracellularly by the enzymatic action of esterases. Calcein can then be a fluorescent marker as it is well retained in the cells, and it will illuminate as green. Dead cells cannot convert and contain Calcein acetyl acetoxymethyl (AM). Ethidium homodimer-1 does not enter intact cell membranes. However, if the membrane is damaged it can enter and bind to nucleic acids, which promotes its red fluorescence [10]. The approaches considered for imaging are fluorescence microscopy, confocal microscopy and flow cytometry.

Testing Alternative 1

Fluorescent microscopy shines ultraviolet light up to the specimen using a dichroic mirror, as pictured in *Figure 3*. Fluorescent microscopes use this ultraviolet light to display fluorescence within cells. For live cells stained with Calcein AM, the green fluorescence can be observed under the FITC channel. For dead cells stained with ethidium homodimer-1, the red fluorescence can be observed using the TRITC channel. These filters and stains can be utilized to determine the viability of chondrocytes. In order to adequately image cells at larger depths, tissue samples must be cryosectioned to view the horizontal plane at multiple depths. Transverse sections could also be made by sectioning perpendicular to the articular surface. This is required as only the superficial layer of the tissue can be imaged in this method.

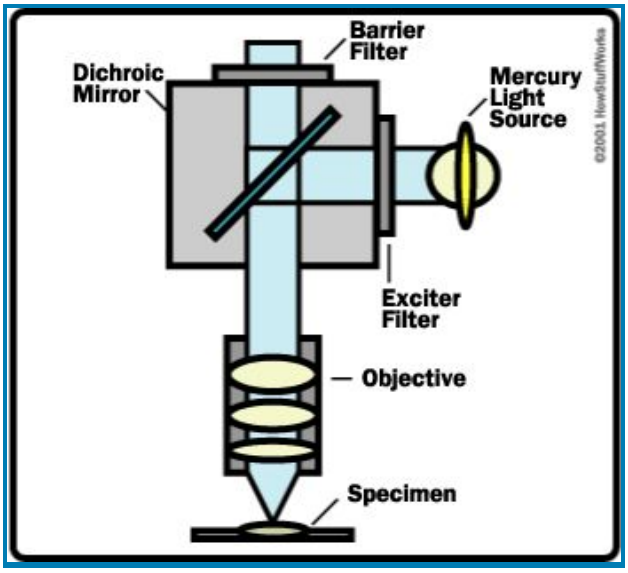

Figure 3: Light path of an epifluorescence microscope

Test Alternative 2

Confocal Laser Scanning Microscopy allows for imaging using fluorescence at different depths. This allows for viewing of structures that are difficult to section since the samples do not need to be relatively thin. The Confocal Laser Scanning Microscope (CLSM) functions by passing a light source through a slit which is then focused onto a small area of the sample by an objective lens (*Figure 4*) [11]. An image of the illuminated sample is then constructed through the collection of emitted photons in the sample. The CLSM is based on the conventional optical microscope, however it substitutes the lamp light source for a laser. Any light exposed to fluorescent areas is reflected back to the optical lens and is then displayed on the screen of a monitor after processing. Reflected light will have a low intensity and is amplified by the photomultiplier tube. The laser beam creates a standing image and as the laser moves, the system constantly detects this information and creates images as if it were a real-time image. This method allows viewing at multiple depths and allows for cross sectional images by solely changing depth or solely changing width, so as to view the entire sample at one depth. CLSM is paired with dies for staining live cells and dead cells, such as calcein-AM for live and ethidium homodimer-1 or CYTOX blue for dead. This allows the imaging software to output photos of the sample with the fluorescent regions clearly illuminated, indicating the live and dead cells based on the color of their fluorescence.

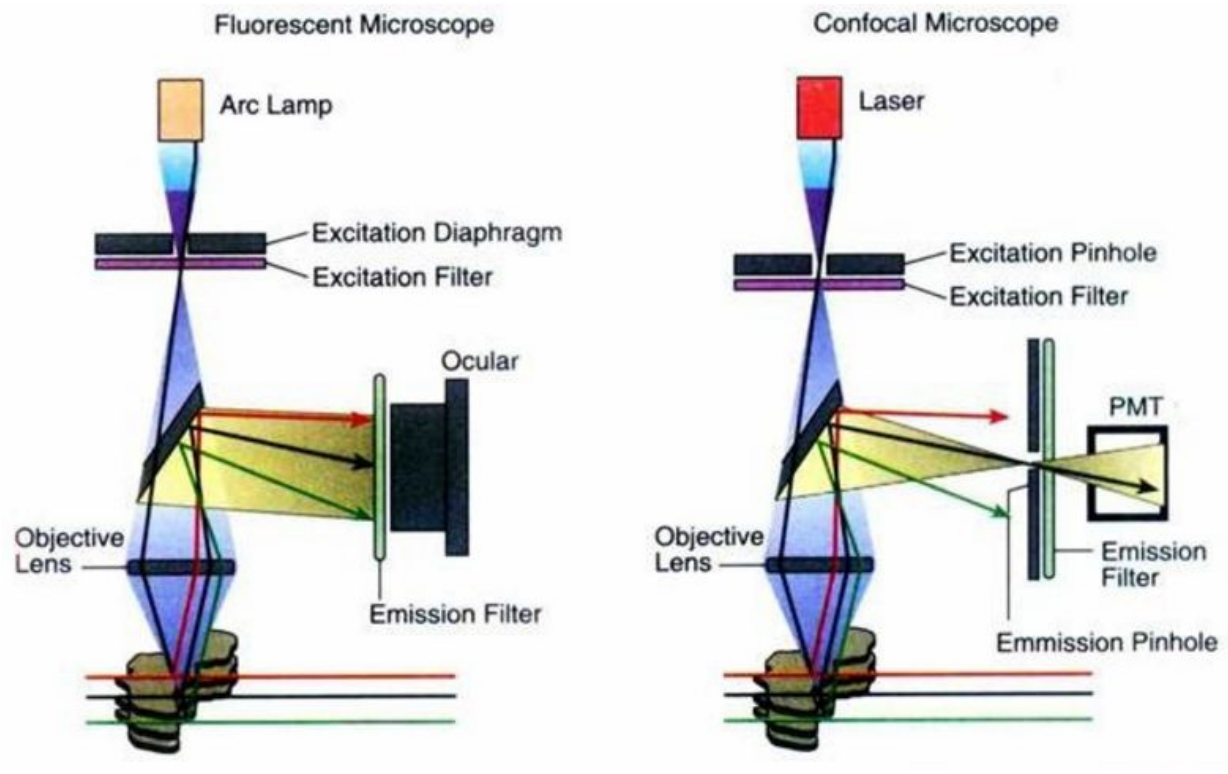

Figure 4: Fluorescent and Confocal Microscope - similarities and differences

Testing Alternative 3

Unlike the other two imaging approaches, flow cytometry requires the disintegration of the tissue sample so that the cells can freely flow through the optical instrument [12]. Typically a collagenase reagent is used because of its ability to break the peptide bonds present in collagen, the main structural protein found in the extracellular matrix of tissues. Without the presence of intact tissue sections, this imaging approach requires a fluid component for the transport of the cells. The fluid column allows for analysis of individual cells as they flow one at a time through the laser component of the instrument (*Figure 5*). The fluorescence and light scattering properties of each cell flowing through the optical component of the cytometer is then used to collect data regarding the size of the cell. Such a characteristic can allow for the separation of cells in a tissue sample that contain multiple types of cells. Further application can involve the use of stains with fluorochromes to assess cell viability. Typically, one stain is used to mark a dead cell and a different stain to mark a live cell. The stain used would cause the cell to emit a fluorescence intensity corresponding to the respective stain and this allows each cell to be registered as alive or dead. Flow cytometer technology also creates a visual representation of the data collected for the cell sample. Such a feature eliminates the factor of user error during data

analysis.

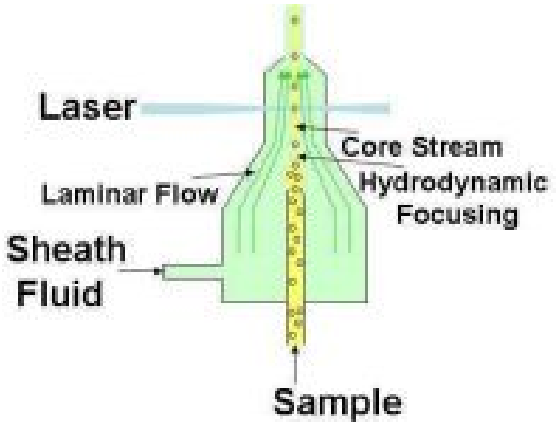

Figure 5: Structure and main components of a flow cytometer

Preliminary Design Evaluation

Criteria	Fluorescent Microscopy		Flow Cytometry		Confocal Microscopy	
Accuracy (35)	(3/5)	21	(4/5)	28	(5/5)	35
Cost (30)	(5/5)	30	(1/5)	6	(4/5)	24
Ease of Use (20)	(5/5)	20	(2/5)	8	(3/5)	12
Tissue Section Prep (10)	(3/5)	6	(5/5)	10	(4/5)	8
Procedure Length (5)	(3/5)	3	(2/5)	2	(5/5)	5
Total	80		54		84	

Table 1: Design matrix used to evaluate the three design considerations according the set criteria

The design matrix criteria (*Table 1)* were chosen and weighted based on importance from the criteria in the PDS. The goal was to determine the most efficient imaging approach to assess the viability of the cartilage samples after experimentation. Thus, the accuracy criteria was

weighted at 35. Given that confocal microscopy does not require the use of a potentially detrimental collagenase and its imaging is not limited to the superficial layer of a sample it was given the highest value for accuracy. Flow cytometry was given the second highest value for accuracy because of its requirement for the use of collagenase to disintegrate the tissue membrane. The collagenase may damage cells and result in altering the viability of the cells present in the tissue sample after experimentation. Since fluorescent microscopy is limited to collecting data of the cells on the superficial layers of a sample it was ranked the lowest with regards to the accuracy of the results that it would produce.

The cost of the imaging technique was weighted at 30 because the procedure must be affordable in order to image multiple times throughout the semester while staying within the $250 budget. Reagents required for the different imaging techniques in addition to the cost per use of the instrument contributed to the overall ranking of the considered designs. Flow cytometry was ranked the lowest because purchasing use on a flow cytometer is significantly above the indicated budget. Confocal microscopes are currently available for use with a fee of $30 per use. Fluorescent microscopes are available for use without any associated fees per use. Hence, fluorescence microscopy was ranked the highest.

Ease of use was weighted at 20, on account of the amount of experience and skill required to use the imaging instrument. The complex setup of optical filters and beam splitters required for flow cytometry it was ranked the lowest. In order to rent the confocal microscopes we must receive training, but we all have prior experience using a fluorescent microscope. The prerequisite for the use of the confocal microscope led to it being ranked below the fluorescent microscope in this category.

Tissue section preparation was rated at 10, as it is a relevant consideration because the constraints regarding the tissue sample vary amongst the three imaging approaches. Flow cytometry only requires the use of collagenase to disintegrate the extracellular matrix of the tissue to allow for the cells to flow in the cytometer. This procedure was given the highest value because it requires the least amount of skill to prepare the sample for imaging. While both fluorescence and confocal microscopy require that sectioning of the tissue samples into thinner slices, the use of fluorescence microscopes requires much thinner slices because they are only able to image cells at the superficial surfaces. Confocal microscopes, however, allow the user to image at various depths within a tissue section. Due to the imaging constraint of fluorescent microscopes, it was ranked the lowest since it would require a larger time and skill investment in obtaining the proper tissue sections.

Finally, procedure length was weighted at 10 on account of our time constraint throughout the semester. A tentative testing protocol calls for at least three tissue samples for each of the three conditions at different time points. This would result in a significant amount of time spent imaging to obtain the overall results of chondrocyte viability for each condition. Hence, confocal microscopy was ranked the highest in this category as it is expected to take the least amount of time invested in imaging each tissue sample. Our prior experience working with a fluorescence microscope and a speculation of the time required to set up a flow cytometer led to giving fluorescence microscopy a higher ranking in this category and flow cytometry the lowest rank.

Proposed Final Testing

Consideration of the aforementioned criteria for each of the imaging techniques resulted in a very close overall score for confocal and fluorescence microscopy with values of 84 and 80, respectively. We propose the use of confocal imaging methods for assessing chondrocyte viability after experimentation. The confocal microscope is the preferred imaging instrument because of its ability to image a various depths of a tissue section. It will also require no further sectioning of the tissue. Thus, allowing the cartilage to be maintained in its native state.

Fabrication/Development Process

Materials

Fabrication of the bone plugs and subsequent treatments will require a variety of materials from many different sources. Animal models will be utilized, and bovine tissue has been chosen due to the sizable knee area and ease of access. Fresh cow knuckles will be retrieved from a local butcher. For the creation of the recipient site and plugs, threading of the site and the plug, and sawing of the bone, tools will be obtained from the Student Shop. These will include a cordless power drill, drill bits, a hole saw, tap and die, hacksaw, a vice, and material to fabricate the guide for the hole saw. Stereologic surgical lube will be provided by the client for graft harvest.

To test the aforementioned plugs for cell viability, materials will be obtained from the client and the BME department. To keep the femurs from the butcher fresh and moist, 1X PBS will be used. MEM-C media, a 24 well plate, and 2 μM Calcein AM and 4 μM Ethidium Homodimer will be used to culture and stain the cartilage disks from the bone plugs. Sterile scalpels used to remove cartilage from bone will be used. Training will be completed on the confocal microscope in WIMR and the materials science scope will be utilized thereafter. Detailed protocols can be found in Appendix III.

Methods

The bovine condyle will be acquired the the same day that the animal will be slaughtered. The lower extremity of the femur will be removed and placed into a Ziploc bag containing 1X PBS solution within a cooler containing an ice bath. The lower extremity of the femur will be transported to the Engineering Centers building where working surfaces and tools will be thoroughly washed with 70% ethanol. This will be the only form of sanitizing used for tools and working surfaces due to budget constraints and because many of the tools were incompatible to use with more efficient sterilizing techniques (e.g. autoclaves or ethylene oxide gas). Working surfaces will be covered with laboratory countertop paper and the condyle will be placed into an industrial vice, also covered with laboratory countertop paper. Using a hacksaw, the knuckle will be cut into two halves, separating the condyles from the patellar surface. This will allow for better access for graft retrieval and flatter surfaces for fixation within the vice.

To obtain the bone plugs, the condyle portion of bone will be placed into the vice with the knuckles facing upright. The hole saw guide will be fixed atop the knuckle with nails driven into the tissue. Stereological surgical lube will be used as a cutting fluid that will mitigate heat production from friction between the hole saw and the cutting guide as well as the tissue. After driving the hole saw to a sufficient depth into the bone, the cylindrical graft will be removed by

cutting perpendicular to the cylindrical cut at its bottom aspect (see *Figure 6 for an outline of this process*) . Additional plugs will be obtained in an identical fashion to obtain the necessary amount of samples. The plugs will be placed into media that will be placed into the incubator on Friday to keep until Monday when the confocal microscope will be available for imaging, which will an acceptable time period to keep cartilaginous tissue fresh [13].

Three randomly chosen plugs will be placed in a vice with the cartilaginous layer (lateral end) facing outwards and threading will start from the cartilage layer inwards using a die appropriately sized to the inner diameter of the hole saw. Recipient holes will be created using a drill bit, appropriately sized to the tap that matches the aforementioned die, attached to a cordless power drill. The tap will be used to create mating threads that will match the threads created on the plug. Additional threaded plugs and threaded recipient sites will be created for each of the plugs included in the threaded condition. The threaded bone plug will be manually screwed into the recipient site by turning the plug clockwise into the hole; slight pressure will be placed on the cartilage during the final turns when insufficient bone is available for grip. To test the impaction condition loads will be applied to extracted plugs using the device created by Dr. Walczak that supplies loads similar to those that would be used in the operating room. Using a sterilized scalpel, the cartilage will be removed from the threaded grafts following insertion, the impaction grafts after supplied loads, and a control condition in which plugs will be retrieved, but no further treatment will be made. These methods are detailed in Appendix III.

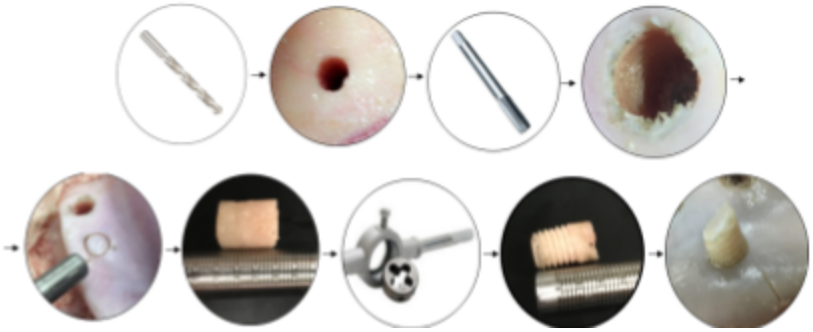

Figure 6: Flow chart outlining the steps in the proposed delivery method

Final Prototype

The final prototype includes the 10mm bone plug and recipient site. These are created using the methods outlined above and have been tested using the protocols below (*Figures 7-9*).

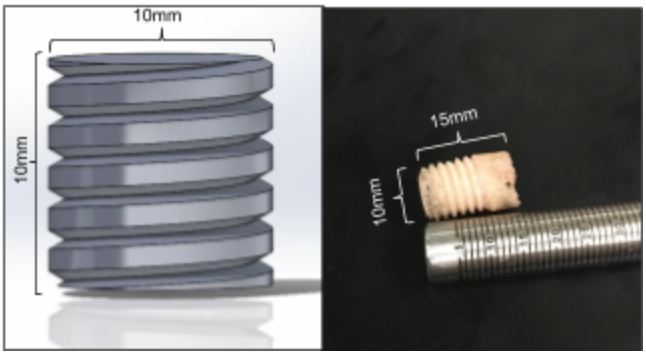

Figure 7: On the left, 10mm x 10mm plug representation modeled in Solidworks. On the right, a 10mm x 15mm bone plug created from a bovine condyle.

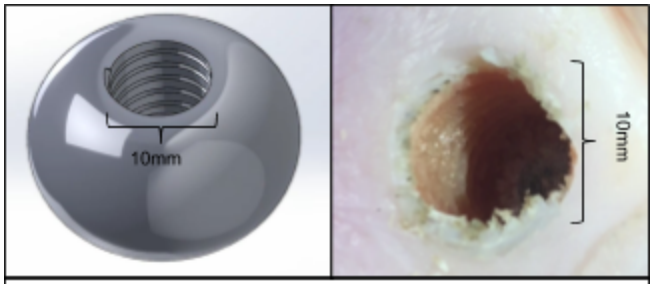

Figure 8: On the left is a Solidworks model of the threaded recipient site. On the right is a threaded recipient site on a condyle bovine femur

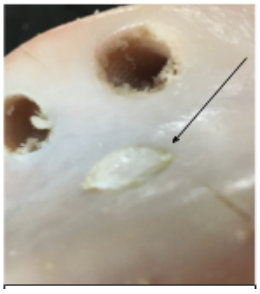

Figure 9: The threaded plug with intact cartilage properly screwed into the recipient site

Experimentation

The purpose of experimentation is to compare chondrocyte viability of the threading and impaction methods. This factor is prioritized due to its correlation to the overall success rate of the procedure. Although testing will be limited to a non-sterile environment, the described procedures will be conducted under the same conditions that are expected to impose a large variability for both methods. Hence, the maintenance of chondrocyte viability of the threading method greater than or similar to the impaction method will show promise for future testing and better results in an operating room setting.

Using the methods outlined above, three osteochondral plugs containing fresh cartilage

for each condition will be obtained from bovine condyles in the COE Student Shop area. The threading procedure will be conducted at this location, and the impaction procedure will be conducted at WIMR. The cartilage surfaces will be removed from each graft using a surgical scalpel and the cartilage disks will be placed into an appropriately labeled 24 well plate and cultured in MEM-C media. Disks will sectioned into three segments using a scalpel and will be incubated for 1 hour, 24 hours, and 48 hours to determine the long term success of the graft as a progression of greater cell death at later timepoints is noted in previous study [2]. Three biological replicates for the three conditions will be compared at the same time point to provide an indication of the error predicted by the sample. Additionally, it was found that >70% chondrocyte viability at the time of insertion was necessary for the success of the graft. Thus, measurement at one hour will provide useful representation of the success of our transplant method. The control condition will serve to demonstrate the extent to which treatment conditions caused cell death.

Testing will be completed using two stains to detect live and dead cells. Live cells will be stained green using calcein AM. Dead cells will be stained red with ethidium homodimer-1. The disks will be exposed to the stain for one hour using 1 mL of calcein AM/ethidium homodimer-1 stain solution per section at a concentration of 2μM calcein AM and 4μM ethidium homodimer in 1X PBS. Calcein AM/ethidium homodimer-1 was selected for Live/Dead assay on account of its visibility under the confocal microscope. An hour duration was selected for the staining to allow for complete penetration of the stain solution into our tissue slices. The recommended time for cells in culture was 30-45 minutes [10], but for larger tissue slices as long as 2 hours is necessary [14]. The sections will then be washed twice for five minutes in 1X PBS to allow for the removal excess stain.

The cartilage sections will then be imaged on a confocal microscope allowing visualization of the zones of the entire depth of the tissue. A 488 nm and 543 nm wavelength laser will be used to excite the Calcein AM and ethidium homodimer-1, respectively, and a 505-530 nm bandpass filter and a >560 nm laser will be used to capture the images, respectively [14]. These may be subject to change depending on the specific setting of the confocal microscope that we will gain access to. 10 μm segments will be imaged through the extent of the cartilage for representative images of all of the depth zones.

Analysis of the images will be completed using ImageJ. The detailed protocol can be found in Appendix IV. First, images will be placed in stacks according to their condition and wavelength of excitation. Next, subtract background will be used to remove residual staining from autofluorescence of the tissue. These will then be thresholded until only cells can be seen. Particles will then be separated using watershed. Analyze Particles will be used to count the cells and this data will be later used to assess cell viability.

Results

Using the cell count data obtained from ImageJ analysis of confocal images, averages will be obtained for percent viability from each graft. Percent viability will be obtained by dividing the number of live cells by total cells (i.e. the sum of live and dead cells). This value will be obtained for each image. Subsequently, all the values of percent viability from the images corresponding to one graft will averaged together to arrive at percent viability for each given plug. Then, an average will be obtained for all of the grafts within each condition. A standard

deviation will be calculated for the samples within each condition, and a standard error will be calculated for the mean estimate for each condition sampled by dividing the standard deviation value by the square root of the number of observations in the sample.

In order to test for a difference between the impacted and threaded conditions a null hypothesis has been established. The null hypothesis states that there is no difference in chondrocyte viability between the threaded and impacted samples. To test this null hypothesis a two sample t-test with unequal variances will be used. Given that both data samples are independent, an unequal variance was assumed. This will be compared to the standard significance level of $p \leq 0.05$. Results from this analysis performed on data from last semester is included in *Appendix IV*, which demonstrated a failure to reject the null hypothesis.

Discussion

The proposed experimentation aims to demonstrate the ability of the threaded delivery system to reduce the amount of compressive loads applied to the cartilage during allograft delivery and to test whether this would indeed minimize chondrocyte death after delivery. It is important to highlight the implications of successful allograft delivery through the proposed threaded mechanism. Current allograft delivery procedures that rely on impaction for proper delivery of the allograft have been shown to lose up to 21% of viable chondrocytes in the superficial 500 μm layer one hour after implantation [2]. The cylindrical allografts used in this study were 15 mm in diameter and required approximately 10 consecutive impacts, each generating an average 2.4 kN load, for delivery. Furthermore, the articular cartilage experienced a total loss of 47% of chondrocytes in the same superficial layer 48 hours after the procedure with observed chondrocyte death in deeper layers. This increase in cell death was noted as a result of induced apoptotic pathways from the forces applied to the cartilage [15]-[17]. Hence, it is essential to minimize the application of compressive loads to the articular cartilage during graft delivery to avoid triggering these apoptotic pathways. The proposed delivery system proved to accomplish this by eliminating the need for large compressive loads to insert the graft into the recipient site. Rather, the threads on the allograft allowed for manual insertion into the delivery site through the application of relatively small torsional forces. Given previous and ongoing research, the reduction of force applied directly to the articular cartilage surface should in theory reduce loss of chondrocytes through necrosis as well as apoptotic pathways.

The proposed tests aim to reduce the error experienced in previous testing. This will be done by greater reliability in graft harvest as a guide in conjunction with a hole saw will be utilized instead of a manual autograft tool that was extremely susceptible to failure and plastic deformations with repeated use. The implementation of the new graft harvesting method should result in similar grafts with each iteration. The confocal imaging technique should improve upon the images received as this will eliminate the need for cryo freezing and sectioning of the tissue, which could reduce signal clarity. Confocal imaging will also allow for the acquisition of a greater amount of data as this will allow imaging of the horizontal planes at different depths in the native state of the tissue, where only one layer of cells could be viewed from transverse and horizontal sections in fluorescent microscopy.

Ethical Considerations

Osteochondral allografts were introduced decades ago and the safety of the procedure is well documented. Multiple clinical studies have shown positive results from fresh osteochondral allograft transplantation [18]. Viral and bacterial infections are rare, but potentially fatal. Prior to the implementation of a screening process, allografts were inserted within 24 hours of harvest and in 2002, the American Association of Immunologists reported 37 bacterial infections associated with allograft procedures. Infection most commonly occurs with donor allografts as bacteria can lie in the donor tissue or bacterial contamination can occur during the procurement and/or processing of condyles. Currently, meticulous screening of the donor is required before the operation. Fresh transplants are no longer used in the USA and the FDA has banned any procedures without proper screening. Most commercial grafts in the USA are used between 15 and 35 days; and are rarely implanted before a 7 to 10-day screening process. Studies have shown good clinical results up to 42 days after harvest although chondrocyte viability decreases with storage time. While the screening process prevents many infectious diseases, clostridium contamination risk increases with time after donor death [19, 20]. Thus, the screening process must be kept short while remaining effective. In order to keep donor tissue free of disease, there must be emphasis on aseptic harvesting, aseptic processing, and use of antimicrobials at every stage. Safety guidelines established by the American Association of Tissue Banks (AATB) advocate donor screening, serologic, bacterial, and viral testing as well as procurement and storage requirements are required until negative testing results have been received.

A difficulty associated with controlling disease is that sterilizing techniques are often cytotoxic. Every process that the donor tissue undergoes must avoid contamination while also keeping the desired cartilage alive. The procedure is performed to replace damaged cartilage with healthy, mature hyaline cartilage from the donor, so maximizing the cell viability of the donor is important.

Another important aspect of the surgery is the efficacy of the transplant. The patient must report improvements postoperatively and currently osteochondral transplants are effective more often than not. Clinical studies published as far back as 1997 reported an overall success rate as high as 85%. Proper healing in 108/126 knees in 123 patients were successful after a mean check-up time of 7.5 years. The check up times ranged from 2 to 20 years, indicating that these grafts are successful long term. In this case, failure was related ($p \leq 0.05$) with age over 50 years and bone instability. Collapse of the graft by more than 3mm was seen frequently in the failed grafts [21] . This highlights the need for a more stable graft, which may be possible with a threaded technique provided that future testing corroborates this hypothesis. Failure in the osseous portion of the allograft is most common, where subchondral collapse may occur. Some patients also reported low grade pain, which may be attributed to chronic inflammation surrounding the graft. Despite these problems, the International Knee Documentation Committee (IKDC) subjective knee force scores show that the operations are effective overall. On a 0-100 point system, the preoperative mean was 27 and the postoperative mean was 79 [22]. While 100% satisfaction is nearly impossible to attain using threaded techniques may have a positive impact on current procedures. The methods for producing threaded allograft donors have the capability of meeting all standards and can be screened using current techniques. After extensive future testing, threaded techniques may be able to be implemented in clinical studies.

The possible benefits in the operating room of threaded techniques could be greater

stability due to the thread engagement, quicker operation times due to threading technique, and overall improved chondrocyte viability because of the lack of impaction required. These benefits must be studied further to have any validity. In addition, MRI techniques are improving, as stated by our client, Dr. Walczak. The possibility of sizing a defect prior to operation is now possible and could prove to be extremely beneficial. This would allow the allograft to be created and threaded prior to surgery. The patient could then be operated upon, the defect drilled out and tapped, and the allograft could be inserted without the defect having to be manually sized. Furthermore, this would decrease procedure length as according to our client, the sizing and depth matching is the most time intensive part of the operation. Creating the plug before the operation would inevitably reduce operating time and limit the open wound exposure to contaminants in the operating room, ultimately increasing the safety of the procedure.

Conclusions

Current surgical procedure for knee grafts is rudimentary and archaic as it requires brute force to implant living tissue that has poor regenerative abilities. The extent of compressive loads applied to the cartilage throughout the procedure ultimately results in necrotic and apoptotic chondrocyte death that is detrimental to the overall success of the procedure. A novel technique is required to reduce these compressive forces used for implantation of the grafts that in return increases long-term surgical success. To prevent the loss of chondrocytes after implantation, a new method has been devised that relies on threading the allograft to create a bone screw from donor tissue that can then be implanted into a threaded recipient hole. The delivery of the allograft is accomplished through successful clockwise rotations by hand until it is appropriately flushed. From inspection, delivery through rotational means greatly reduces the amount of compressive loads applied to the cartilage surface. The new delivery system will be further assessed through live/dead staining to determine if it successfully increases postoperative cell viability. This method has demonstrated the possibility of creating implantable bone screws and shows promise for healing bone defects through similar means in other parts of the body. Furthermore, bone screws created and used in this study allow for retrieval of the allograft through counterclockwise retraction. Such a feat was noted to be of particular significance by the client given his emphasis on the current delivery system's inability to retrieve the bone plug without first damaging the articular cartilage surface. The ability to easily retract the allograft is of convenience for the surgeon in the case that the plug is pushed beyond the desired depth.

Given that the results from initial testing did not show any comparable difference in viability between the existing delivery method and the proposed delivery methods, future efforts will focus on the use of more advanced imaging technology to assess tissue samples. A confocal microscope will be used to analyze cells at various depths within each specimen. In addition to observing cell death at multiple layers of each tissue sample, the confocal microscope will also offer better resolution than the previously used fluorescent microscope.

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Appendix

I. PDS

Osteochondral Graft Delivery System

Team:
Rodrigo Umanzor (Team Leader)
Nicholas Zacharias (BSAC & BWIG)
Eduardo Enriquez (Communicator)
Bilin Loi (BPAG)

Date: February 22, 2017

Function: Osteochondral allografting is a common procedure performed on patients that require replacement of diseased bone. Current methods of implantation require the application of mechanical forces that have a detrimental effect on the live chondrocytes present on the implant. Maximizing the amount of viable tissue during and after the surgery is a crucial factor for the success of the procedure. Hence, the client requests a delivery system that will reduce the amount of mechanical forces required to securely place the implant into the donor site.

Client Requirements:

- Budget: $250
- Donor tissue must be placed into donor site with less than 165 N of force applied to the articular cartilage layer (the average impaction force for current procedures)
- Must securely fit into donor site while keeping chondrocyte cell viability >70% during and after the procedure.
- Delivery system must only require the use of sterile tools available in a surgery room, or must be made to to fit surgical standards.

Design Requirements:

- *Performance Requirements:*
    - Application of bone graft should result in approximately 70 - 90% viability of chondrocytes on donor graft.
    - Procedure must be simple enough to be done in operating room, within 5 hours (the time period of a surgery).
    - Bone graft and vice should be positioned perpendicular (relative to each other) so screws on bone graft remain straight.
    - System must be capable of decreasing the 165 N used to insert the bone graft.
    - Forces exerted on the bone by the device should not cause any bone chipping, or fragmentation and minimal damage to the articular cartilage.

- *Safety:*
    - The delivery system should not increase the chances of infection, graft dislocation, or create complications post-op.
    - Our device has failed if, postoperatively, the graft does not exhibit proper integration into the native tissue, if the hyaline cartilage is not properly maintained, if an associated cartilage disorder develops, or if significant fissuring, fibrillation, or fibrous tissue infiltration occurs.
- *Accuracy and Reliability:*
    - The delivery system should have a success rate that exceeds that of current devices (75-80% success rate)
    - The delivery system should also allow for successful graft implantation with no greater risk for post-op complications than standard practice
- *Life in Service*:
    - Expensive or specialized components should be reusable and easily manufactured components should be one time use. Both should be sterilizable. Length of time to be determined with materials chosen
    - If plastic or biodegradable materials are included in the delivery system, then these components may be one-use only
- *Shelf Life:*
    - The delivery system should be capable of storage at room temperature for 9 months unless sterility is compromised before then. No corrosion should be observed on the delivery system during its life of service and must be compliant with hospital regulations
- *Operating Environment:*
    - The delivery system has to be sterile while in use
    - Should operate in temperatures typical of an operating room (20-23 °C), with humidity of 20-60%
    - All pieces will have to withstand the forces exerted on them during operation (tension, torsion, shear, and compression).
    - Must be able to be used in conjunction with other orthopedic tools, including supports, water, and bone glue.
    - Must be able to be utilized by an orthopedic surgeon
- *Ergonomics:*
    - The delivery system should be able to be used easily by surgeon without damage during operation. Forces placed on the cancellous bone of the graft should not exceed 6.6 MPa from torsional stress, 3-20 MPa in tension, and 1.5-50 MPa in compression. Cortical bone is stronger than cancellous, so force limitations inherently include the cancellous bone. The forces applied to the articular cartilage should not exceed those at which there is less than 70% viability.
- *Size:*
    - The delivery system will be sized appropriately based on the size of the defect.
    - Range of 5mm-20mm diameter for threading bone graft

    - Height of graft must be at least 10 mm
- *Weight:*
    - Components of the delivery system are appropriately weighted for use by an orthopedic surgeon
- *Materials:*
    - Materials used in the delivery system should comply with medical standards set out by the FDA
    - Reusable materials must be sterilizable
- *Aesthetics, Appearance, and Finish:*
    - No color or aesthetics
    - Function over form

Production Characteristics:

- *Quantity:*
    - 1 final delivery system, preferably multiple testing using delivery system
- *Target Product Cost:*
    - TBD
- *Standards and Specifications:*
    - Implanted allograft should be in compliance with the FDA regulations under Section 361 of the Public Health Service Act as monitored by the Tissue Reference Group . All surgical tools should comply with the code of federal regulations under Title 21 with the FDA.

Characteristics:

- The delivery system should consist of a tap, die, vices and a bone screwdriver
- Must be made of surgery-grade material
- Various devices to stabilize tools will be necessary
- *Patient-related concerns:*
    - Completed bone graft must not cause pain
    - Allergies, immune response hemocompatibility, and biocompatibility
    - Allograft should not release unwanted fragments of bone
    - Required surgery with the delivery system should not be more invasive than current procedures
- *Customer:*
    - Orthopedic surgeons implanting an osteochondral graft will be the intended user

    o

II. Graft Retrieval and Threading Protocol

Preparations Before Threading Procedure (not for viability testing):

Materials:

- Large plastic bin
- Kitchen knife
- 1 cow knuckle
- Hand saw
- Plastic bag

Procedure:

1. Two bone samples were obtained from a butchery and stored in a kitchen freezer
2. 24 hours prior to testing, one of the knuckles was removed from the freezer, placed in a plastic bin and maintained at room temperature
3. After the sample was thawed for 24 hours, excess fat was removed from the surrounding bone using a kitchen knife and a hand saw was used to remove the ligaments of the knee
4. The sample was then placed in a plastic bag and transported for further testing

.

Threading Protocol

Materials:

- 1 cow knuckle
- Hole saw and guide
- Surgical lube
- Drill and bit
- Tap and die
- 70% Ethanol
- Impaction device
- Hacksaw

Procedure:

1. On a relatively flat surface on the knuckle, a drill with an appropriately sized bit to the tap and die will be used to ream the recipient site.
2. Bone shavings and all other residue will be removed to clear out the cylindrical hole that is meant to serve as the recipient site.
3. The tap was used to thread the recipient site.
4. On another flat surface, to retrieve a bone plug the guide will be fixed atop the distal femur by driving nails into the tissue.
5. A hole saw will cut a cylindrical graft with the guide preventing running of the hole saw.
6. The hole saw will be removed, and a hacksaw will be used to cut perpendicular to the

cylindrical cut underneath the graft to remove it.
7. The plug will be threaded.
8. The threaded plug will be placed into the recipient site by turning it similar to a thumb screw.

III. Image Analysis Protocol

Protocol for Creating Stacked Images

Materials:

- Images of tissue named in order by tissue sample and picture number, specifying FITC or TRITC channel
- ImageJ

Procedure:

1. Open two stacks of images on ImageJ, one of FITC and one of TRITC for one condition (i.e. threaded condition)
2. Process - Enhance Contrast - Equalize histogram + process all 17 slices - OK
3. Process - Subtract Background - Rolling ball radius: 30 pixels - OK
4. Image - Color - Merge Channels - Red: TRITC, Green : FITC - save as .tif composite
5. Image - Color - Split Channels
6. (For each channel) Image - Adjust - Brightness/Contrast. Put Contrast almost all the way up, and adjust brightness until you see only cells and remove most auto-fluorescence across slices.
1. Image - Color - Merge Channels - Red: TRITC, Green: FITC - save as .tif composite

Protocol for Calculating Cell Viability

1. Using ImageJ, press File-Import-Image Sequence -(Choose Image Sequence) - (Name either FITC or TRITC to separate filters) - make it 8 bit greyscale
2. Once you have image sequence, go to Process - Subtract Background - (Rolling Ball 100)
3. Go to Image - Adjust - Threshold - Stack Histogram - (Adjust until you see no background on layers with the most cells) - Don't press apply, just exit from the threshold screen
4. Process - Binary - Make Binary
5. Process - Binary - Watershed
6. Analyze Particles - Size (Pixel^2): 0-15.00 - (All other settings the same)
7. Copy and paste 'summary' info into an excel sheet

IV: Imaging Results

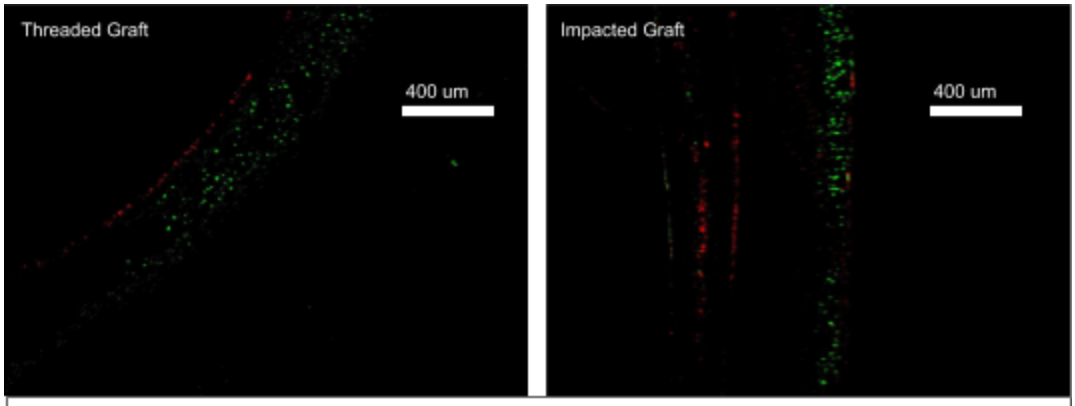

Figure 11: The left photo shows live/dead, 4X images of a threaded condition, and the left shows live/dead for an impacted condition

	Viability of Threaded Plugs	Viability of Impacted Plugs
1	59.56 %	63.50 %
2	96.73 %	83.43 %
3	80.54 %	84.41 %
Average	78.94 %	77.11 %
Standard Deviation	18. 64 %	11.80 %
Standard Error	10.76 %	6. 81 %

Table 2: Summary of raw cell count data collected from the threaded and impacted conditions