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14. ABSTRACT

Our proposal "Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma" addresses the current limitations in treating complex, high-energy musculoskeletal wounds incurred in active combat. High-energy blast-injuries produce immediate, short-term and long-term consequences such as acute limb loss, bone loss, cartilage loss, stiffness, limping, pain, arthritis, and permanent disability, often requiring multiple reconstructive surgeries and prolonged rehabilitation. These 'osteochondral health' issues ultimately affect a soldier's quality of life both during active service and after retirement. Tissue engineering technology is a rapidly evolving field and utilizes mesenchymal cells, tissue scaffolds and growth factors. However, there are no currently available tissue-engineering constructs exhibiting 'Ready-to-Use' functionality. The most significant barrier to the practical application of tissue engineering for combat-related bone and cartilage defects is the time- and labor-intensive process of mesenchymal stem cell expansion. The goal of this proposal is to introduce a new tissue engineering paradigm to the Defense Health Program (DHP) by utilizing a biomechanically competent and anatomically matched tissue construct without resorting to the cumbersome process of mesenchymal stem cell expansion.

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Ready to use tissue construct, biomechanically competent anatomically matched tissue, construct

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1. INTRODUCTION (excerpted from grant)

Our study "Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma" addresses current limitations in treating complex, high-energy musculoskeletal wounds incurred in active combat. High-energy blast-injuries produce immediate, short-term and long-term consequences such as acute limb loss, bone loss, cartilage loss, stiffness, limping, pain, arthritis, and permanent disability, often requiring multiple reconstructive surgeries and prolonged rehabilitation. These 'osteochondral health' issues ultimately affect a soldier's quality of life both during active service and after retirement. Tissue engineering technology is a rapidly evolving field and utilizes mesenchymal cells, tissue scaffolds and growth factors. However, there are no currently available tissue-engineering constructs exhibiting 'Ready-to-Use' functionality. The most significant barrier to the practical application of tissue engineering for combat-related bone and cartilage defects is the *time- and labor-intensive process of mesenchymal stem cell expansion*. The goal of the current study is to introduce a new tissue engineering paradigm to the Defense Health Program (DHP) by utilizing a biomechanically competent and anatomically matched tissue construct without resorting to the cumbersome process of mesenchymal stem cell expansion. Our project utilizes a series of *in vivo* large animal translational experiments that will hopefully lead to the development of new military technology products and utilities for the definitive and preventive orthopaedic care of military personnel and retirees. The project has 3 major aims, excerpted from the revised Statement of Work and listed below:

Aim 1. To examine whether our prefabricated constructs can reconstitute osteochondral defects of critical-size in a canine distal femoral condyle defect model simulating high-energy blast-injury. Osteochondral injuries of any size require anatomically perfect reconstruction to prevent pain and post-traumatic arthritis. We hypothesize that anatomically-conforming osteochondral constructs with controlled release of TGF-β3 can reconstitute physiologic *hyaline cartilage*-osseous transition in massive osteochondral defects in large animals. We will conduct functional outcome analysis, X-ray/MRI examination and histologic analysis.

Aim 2. To examine whether our prefabricated construct can reconstitute critical size segmental defects in canine tibiae. Critical-size segmental defects in long bone diaphyses require extensive reconstructive procedures and prolonged rehabilitation times. We hypothesize that our *Ready-to-Use* constructs can successfully restore 3 cm critical size segmental defects in dog tibiae. We will examine the incorporation and regeneration of the biogenic implant with host bone by conducting functional outcome assessments, radiography, biomechanical torsional testing and histologic examination.

Aim 3. To examine biomechanical suitability of ready-to-use constructs in massive osteochondral defects and segmental bone defects in human cadaveric femora. We have successfully developed anatomically conforming bone and cartilage constructs for rats and rabbits. Early joint motion and ambulation are important in human patients. We hypothesize that our *ready-to-use* construct can maintain the biomechanical and functional properties in human cadaveric bones under simulated physiologic load. We intend to optimize and adapt our "ready-to-use" scaffold construct for humans. We will verify the biomechanical competence in a critical size defect in human femora and knee joints by simulating loads seen during ambulation and knee range of motion.

Our central hypothesis is that an anatomically and biomechanically compatible scaffold with TGF-β3 and BMP-2 can reconstitute massive cartilage/bone defects without exogenous MSCs. The goal of this Technology Development Project is to simplify the current paradigm of tissue engineering by 1) eliminating the need for time- and labor-intensive stem cell harvesting and expansion and 2) adopting anatomically conforming constructs which promote incorporation, remodeling, early joint motion, partial weight bearing, and ambulation. Our hypothesis is based on compelling preliminary data in small animal models, such as mice, rats and rabbits. The current protocol will take another step towards military application by verifying successful regeneration of cartilage (**Aim 1)** and bone (**Aim 2**) in massive canine bone defects and by confirming biomechanical and functional suitability in human cadaveric knee and tibia defect models (**Aim 3**). **Aim 1** and **Aim 2** are significant in that they will introduce a simpler, more cost-effective approach to tissue engineering that obviates the need for extensive cell culturing and laboratory support. **Aim 3** is significant in that the injured soldiers can start early rehabilitation and ambulation following reconstructive surgeries using Ready-to-Use anatomically and biomechanically conforming biogenic scaffolds.

2. BODY

2.0 Overview

Our current Columbia and USAMRMC Animal Care and Use Review Office (ACURO) approved IACUC protocol has two components, reflecting Aim 1 (osteochondral defects) and Aim 2 (segmental defects) of the grant. The approved IACUC protocol permits 3 pilot dogs for Aim 1 and 3 pilot dogs for Aim 2. Several minor modifications to the original IACUC protocol were required which are provided in the Appendix. All the pilot dogs were to receive scaffolds composed of 90% poly-caprolactone (PCL) and 10% hydroxyapatite (HA) by weight (PCL+HA) without any seeding with either canine MSC or biologic agents (TGF-β3 for Aim 1 or BMP for Aim 2). The purpose of the pilot surgeries was twofold, a) to demonstrate the ability of the surgical team to perform the surgery and that the surgery did not result in excessive pain and discomfort to the animal and b) to demonstrate that there was no immune response to the implanted scaffolds. All three surgeries were performed for Aim 2 in May and June of 2012. Two of the three animals were taken to the full 16 week duration of the experiment and then were humanely sacrificed. Biomechanical testing was performed on their 4 hind limbs. The third Aim 2 dog had an untoward event that required it to be humanely sacrificed approximately 1 week post-surgery.

Surgeries were performed on all three Aim 1 pilot dogs in September and October of 2012. To date, all three dogs are doing well and will be followed through to the end of their 16 week experimental time, at which time they will be sacrificed and biomechanical testing will be performed on their 6 hind limbs.

CT images were obtained of the hind limbs of the postmortem segmental defect dogs and three-dimensional models were created to assess bony ingrowth. The first of monthly post-op MRI images were obtained for the first osteochondral defect dog and a three-dimensional model was created to establish a chronologic history of bony ingrowth/articular cartilage growth in the osteochondral dogs. Radiographs were taken on a bi-weekly basis for segmental defect dogs to and are ongoing for the osteochondral dogs to document the progression of healing. Outcome measures were recorded throughout the 16 week experimental period for the two segmental defect dogs and are ongoing for the osteochondral defect dogs. Histology was performed on the one segmental defect dog that was sacrificed prematurely to establish histology protocols for our newly acquired hard-sectioning histology laboratory.

To prepare for canine stem cell harvesting and cell multiplication, practice bone marrow aspirations and cell culturing were successfully performed to refine both techniques prior to their use in the experimental animals.

As a prerequisite to the surgeries that were performed to create osteochondral and segmental defects, the architecture and fabrication of both scaffold designs were perfected. In addition, cadaveric dog limbs were used to perfect the surgical approaches and techniques for both types of defect creations and scaffold implantations.

In parallel with Aim 1 and Aim 2, the testing protocols for Aim 3, testing of the scaffold in human cadaveric specimens, were developed and trialed for the segmental defect scaffold. Special testing jigs were designed and manufactured to facilitate these tests.

The subsections to follow will elaborate on the above accomplishments and experimental results.

2.1 Aim 1 - Osteochondral Defects

The design of the osteochondral defect was finalized, surgical procedures were performed on all 3 approved osteochondral pilot dogs and initial radiological and MR images were obtained as well as outcome measures.

2.1.1 Osteochondral Defect Scaffold Design

The objective of the osteohcondral defect scaffold is to promote both bony ingrowth into the subchondral bone as well as to promote the development of articular cartilage at the articular surface of the implant. The scaffold architecture is based on the previous work of Lee et al (1), one of the co-investigators on this grant. The efficacy of the design was first demonstrated in rabbit femurs by removing most of the rabbit condyle and replacing it with an implant made from Polycarprolacton (PCL). A unique feature of this scaffold was that the scaffold was constructed with two layers, a top layer of 500μm with a pore size of 400 μm to promote articular cartilage growth and a second layer with a pore size of 200 μm to support bone ingrowth from the surrounding subcondral bone. This design philosophy was replicated for the

dog osteochondral scaffold with some modifications. First, the effective size of the scaffold was reduced to be more representative of a large defect in the medial condyle rather than replacing the entire condyle. Second, because the entire scaffold was not being replaced, it was decided to design the scaffold to be held in place as a press-fit as performed in the previous rabbit model. Accordingly, the scaffold was designed with a "hat" whose curvature approximately matched that of the canine medial condyle from the region shown in Figure 1, with a size of approximately 13 x 6 x 9 mm^3 and a tapered keel (9 mm by 4 mm by 4 mm) to provide stability to the implant and prevent any rotation of the implant. The location, and hence curvature of the implant was selected to place the implant in the load bearing region of the canine knee joint (2) while still allowing access to the joint without disrupting the main ligaments of the knee. Both the implant design and surgical approach were determined and refined by trialing on several cadaveric canine knees of approximately the same size as the experimental dogs were anticipated to be.

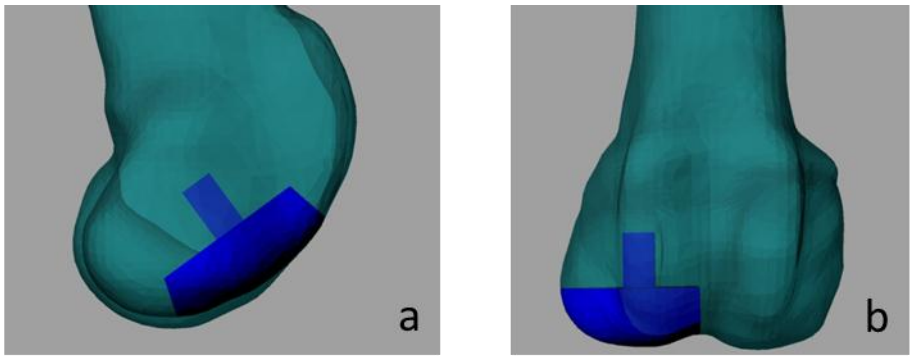

Figure 1: computer model of osteochondral scaffold virtually implanted in a 3-D computer model of the medial condyle of left canine knee, a) medial to lateral direction, b) anterior to posterior direction

The scaffolds were manufactured by first obtaining CTs of several of the cadaveric canine knees. The digital files from the CTs were then imported in a program (Mimics, Materialise) that combines the individual slices to create accurate three-dimensional models of the knee. This 3-D model was then exported as an STL file which was then converted into a DXF file to create the internal architecture of the scaffold, which in turn was used to create the commands to drive a 3D printer (Bioplotter™, EnvisionTec, Germany) which created the scaffold by laying down microstrands of scaffold material using a 27 G stainless steel needle for the articular layer pores and the subchondral bone pores. The resulting scaffold (Figure 2) had a pore size of 400 μm in the articular layer, which was approximately 5 mm deep. The underlying layer for subcondral bone growth had a pore size of 200 μm.

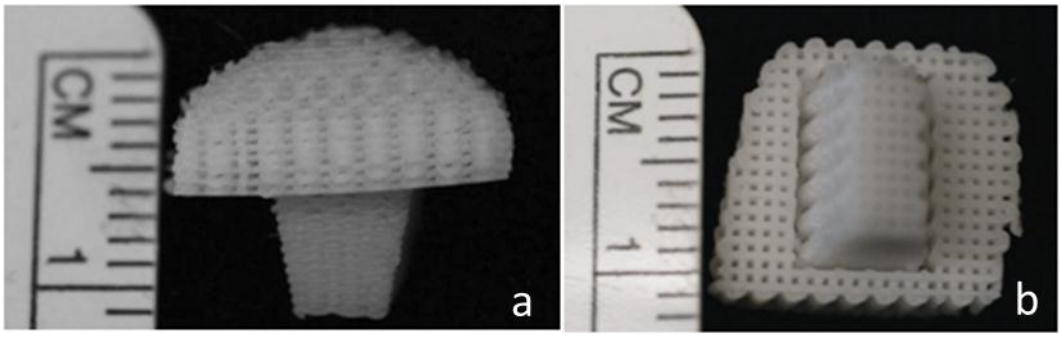

Figure 2: Osteochondral scaffold showing a) side view in superior-inferior direction, b) keel of scaffold

2.1.2 Osteochondral Defect Surgeries

After adequate anesthesia in accordance with the IACUC protocol, the dog was placed in a supine position. The leg was shaved and painted with Betadine® solution and the draping of the left leg to the groin was performed in a sterile fashion. The leg was flexed 90˚ and supported with a sterile cushion. A longitudinal midline incision (5 cm) was made on the left knee. Subcutaneous tissue and myofascial sheath were separated. A medial parapatellar approach was used by making an incision through the medial retinaculum and continued to the tendinous part of the vastus medialis muscle through which the joint capsule was entered and the medial femoral condyle identified. The medial condyle was cut enbloc, 1.4 x1.2 x0.5 cm, the same size as the scaffold using an electric mini saw. A trough of 1.0 x 0.8 x 0.5 cm was created in the medial condyle manually using osteotomes to accommodate to the keel of the scaffold. The scaffold was firmly placed in the space. Minimal adjustment was made to achieve pressed-fit settlement in the positions of full flexion, neutral flexion and full extension. The normal gliding of patella was checked prior to closure. Hemostasis was reassured and the joint capsule was closed. The medial retinaculum and quadriceps were reattached using 2-0 Vicryl (Ethicon, Inc.,

Somerville, NJ) sutures. The subcutaneous tissue was closed using 4-0 Vicryl sutures. The skin was closed using skin staples and a compression dressing was applied. Post-op radiographs were taken immediately following closure. Intraoperative photographs of the placed scaffold for all three osteochondral segmental defect dogs just prior to closure are provide in Figure 3.

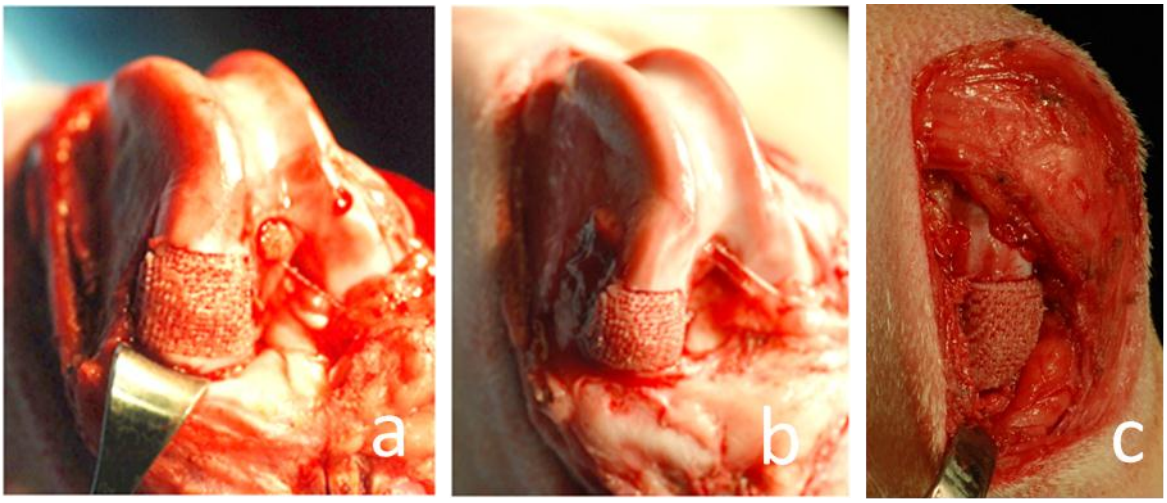

Figure 3: Osteochondral defect (Aim 1) surgeries showing scaffold implanted in medial condyle of left femur for a) Dog 1, b) Dog 2 and c) Dog 3. Note the minimally invasive surgical approach used for Dog 3.

2.1.3 Osteochondral Defect Outcome Measures

Outcome measures of gait, lameness, pain, knee motion and an aggregate of these measures were recorded for Osteochondral Defect Dog 1every weekday, excluding Saturdays and Sundays. However, because sling walking was prescribed, no outcome measures were able to be obtained until Day 11. As can be seen from Figure 4, Dog 1 showed a continued improvement with time until the last reportable day for this report, which was Day 35. The criteria used to determine the outcome measures are provided in Table 1.

Outcome	Criteria	Range
Gait	Non weight-bearing	0
	Partial weight-bearing	1
	Full weight-bearing	2
Lameness	does not use limb during walking	0
	partial use of affected limb, walks with noticeable limb	1
	no lameness when walking	2
Pain	severe reaction to touch, withdraws upon the slightest touch with guarding behavior and/or vocalization	0
	mild reaction to touch, withdraws limb upon touch	1
	no reaction to touch of affected limb	2
Knee Motion	significant reduction in range of motion (0-30%)	0
	moderate reduction in range of motion (30-60%)	1
	slightly reduced range of motion (60-80 %)	2
	Normal range of motion (90-100%, preoperative range)	3
Total		0-9

Table 1: Criteria used to grade Outcome Measures for both Osteochondral and Segmental Defect Dogs.

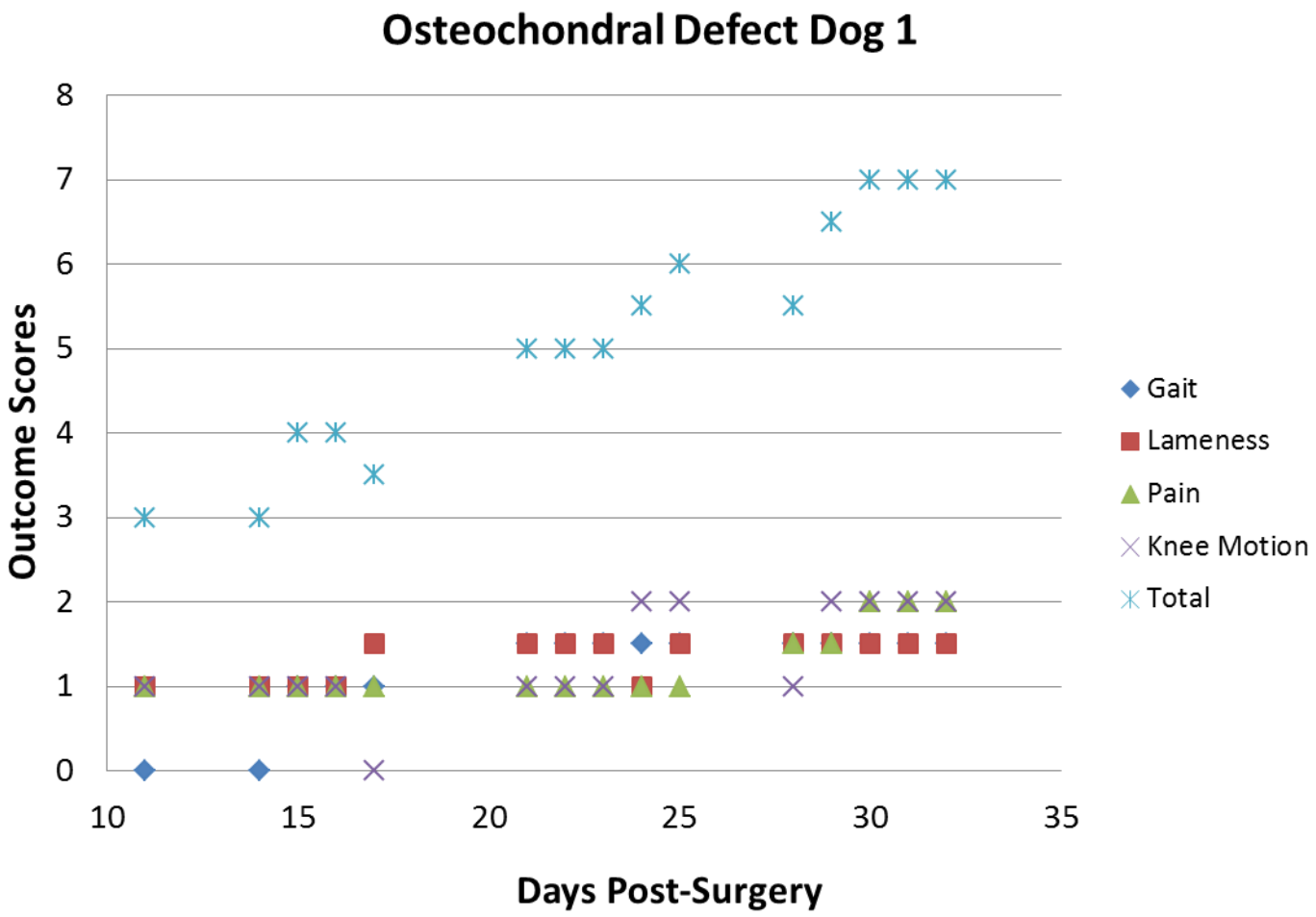

Figure 4: Outcome measures for Osteochondral Defect Dog 1. The last day for which outcome measure were for available for inclusion in this report was post-op day 35.

2.1.4 Osteochondral Defect Radiographs, MR Imaging and 3-D Models

Radiographs of Osteochondral Defect Dog 1 as a function of time are provided in Figure 5. An MR image of the knee and the 3-D model of the knee derived from the MR images are provided in Figure 6. The outline of the scaffold void can be vaguely seen in the radiographs. No obvious bony ingrowth has occurred by week 4, which, though early in the series, was the most recent radiograph available for this report. The dark region in the lower left hand part of the condyle in Figure 6a clearly outlines the void created in the condyle to receive the scaffold, which appears black in the MR image. The 3-D computer model is somewhat difficult to create with fluid perfusing the scaffold in places making it hard to properly outline the scaffold in each image necessary to create the 3-D model.

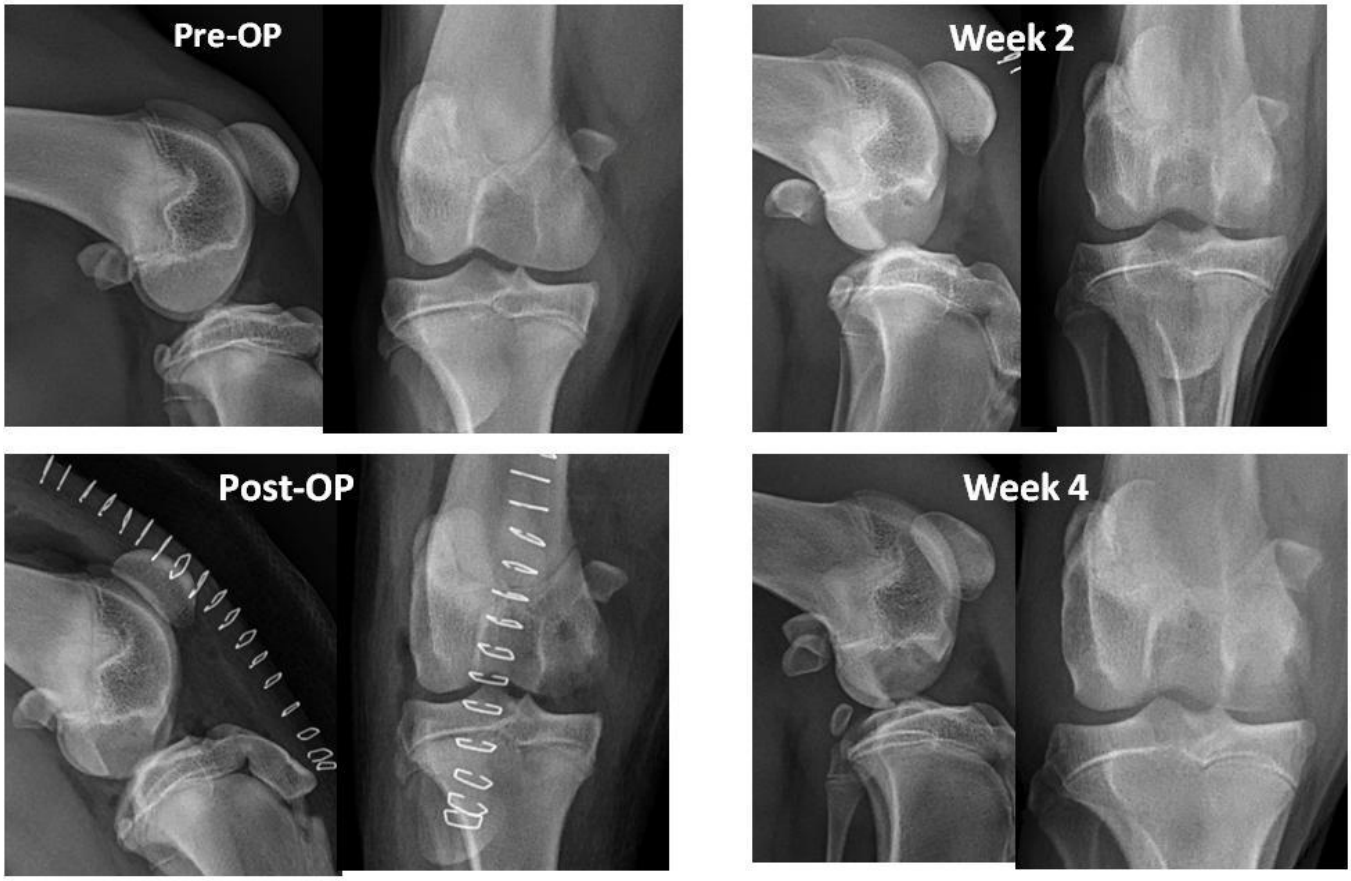

Figure 5: Medial-lateral and anterior-posterior radiographs of Osteochondral Defect Dog 1 pre-op, immediately post-op, 2 weeks post-op and 4 weeks post-op.

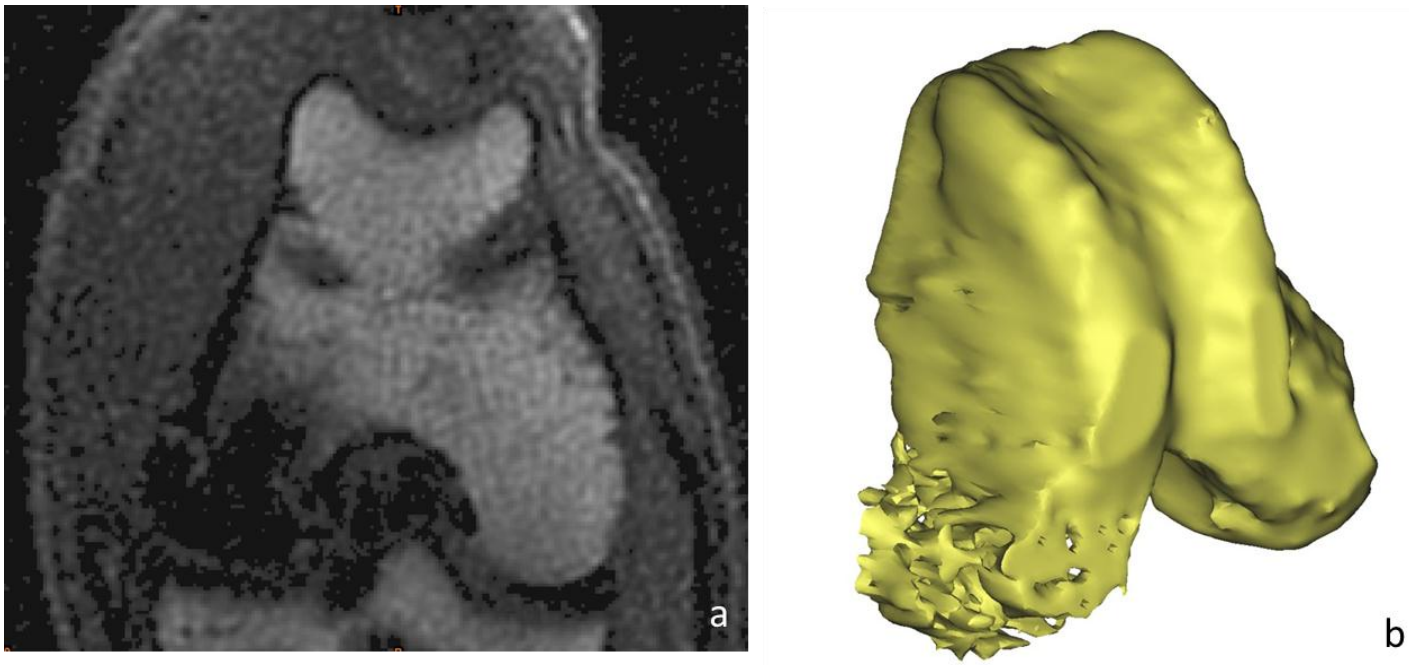

Figure 6: a) MR image of Dog1 after 4 weeks surgery, b) Computer 3-D model of femur from MR images.

2.1.5. Preliminary Bone Marrow Aspiration and Culturing

For the actual bone marrow aspirations the dogs will be given general anesthesia, as per the IACUC protocol. However, the practice bone marrow aspirations were done immediately following sacrifice. The dog was placed in prone position and a one centimeter incision was made on the right poster superior iliac crest. A bone marrow aspiration needle (Jamshidi's, gauge 16), was inserted into the pelvic bone, from which the bone marrow was aspirated. The sample was collected in the heparinized sterile tube. If the bone marrow could not be obtained in the first or second attempt, 5 mL of 0.9% normal saline was injected to help flush out bone marrow during the aspiration. The same procedure was repeated on the left posterior iliac crest. The cells were then transported immediately after the procedure to the laboratory for processing.

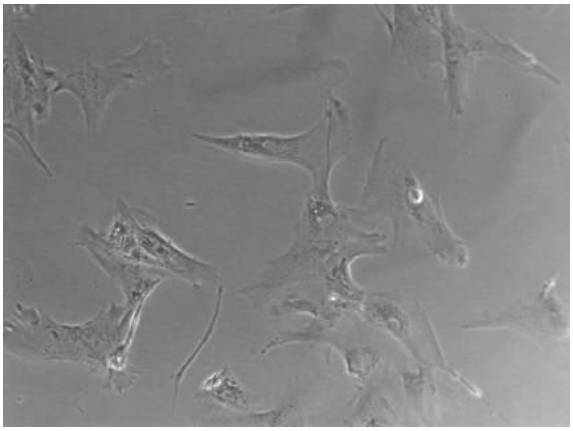

Figure 7: Isolated MSCs from canine bone marrow (P0). Mononucleus, adherent cells were isolated from hematopoietic cells using a density gradient method. Cells show spindle-shaped fibroblast-like morphology, consistent to previous reports.

Canine mesenchymal stem cells (cMSCs) were isolated from fresh whole bone marrow samples of hounds weighing from 235 to 250 N. Mononucleated and adherent cells were purified by centrifugation through a density gradient (Ficoll-Paque) using negative selection following manufacturer's protocols (RosetteSep, StemCell Technologies, Vancouver, Canada) to remove hematopoietic cells and other differentiated cells. Briefly, bone marrow was transferred to a 50 mL tube, and then 15mL PBS in 2% fetal bovine serum (FBS) and 1 mM ethylenediaminetetraacetic acid (EDTA) were added to a total volume of ~30 mL. The sample was layered on 15 mL Ficoll-Paque and centrifuged 25 min at 400×g with a break-off. The entire layer of enriched cells was removed from Ficoll-Paque interface. The cocktail was centrifuged at 400xg for 5 min. Collected cells were counted using trypan blue, plated at 0.5-1 million cells per 100 mm dish and allowed to attach for ~5 days, followed by regular medium change every two days. At 80-90% confluence, cells were trypsinized, centrifuged, resuspended in growth medium as passage 1 (P1) cells, and incubated in 5%CO_2 at 37°C, with fresh medium changes every 3-4 days. Growth medium was defined as Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG; Sigma, St. Louis, MO), 1% antibiotic (1× Antibiotic–Antimycotic, including 10 units/L Penicillin G sodium, 10 mg/mL Streptomycin sulfate and 0.25 µg/mL amphotericine B) (Gibco, Invitrogen, Carlsbad, CA) and 10% Fetal Bovine Serum (FBS; Atlanta Biologicals, Norcross, GA). The isolated MSCs will be further characterized for multi-lineage differentiation, flow cytometry, and real time RT-PCR (Figure 5).

2.2 Aim 2 - Segmental Defects

The segmental surgical procedures were performed on all 3 approved segmental defect pilot dogs. The first 2 dogs successfully went to the end of their 16 week recovery period and were sacrificed. The third segmental defect dog had an untoward event within the first week following surgery and was humanely sacrificed. An adverse event report (included in the Appendix) was generated and reviewed by the Columbia IACUC and the DOD ACURO. A modification to replace the dog was submitted and approved by both committees by September 2012. Radiologic and CT images of the hind limbs following sacrifice were obtained for both dogs. Outcome measures were recorded daily for both dogs through the 16 week recovery period.

2.2.1 Segmental Defect Scaffold Design

The objective of the segmental defect scaffold is to promote both bony ingrowth into the subchondral bone. As for the osteochondral defect scaffold design, the scaffold architecture is based on the previous work of Lee et al, (Lancet 2010) (1) one of the co-investigators on this grant. As compared to the osteochondral defect, the segmental defect scaffold has the same pore size throughout. It was decided to not have an outer cortex layer (effectively a closed surface with no pores) which might be considered to be representative of cortical bone to facilitate infiltration from the tissue surrounding the scaffold. In addition, the scaffold was constructed as an annulus, with an inner lumen of 8 mm and an outer diameter of 16 mm. The surface of the scaffold adjacent to the lumen also did not have a closed surface, rather it had pores that permitted the infusion of bone marrow material through the lumen and into the scaffold through the pores Figure 8. The scaffolds were made 20 mm long to represent a critical size defect in the dog (1).

The scaffolds were manufactured in the same manner as the osteochondral defect by first obtaining CTs of several of the cadaveric canine tibiae. The digital files from the CTs were then imported in a program (Mimics, Materialise,Plymouth, MI) that combines the individual slices to create accurate three-dimensional models of the tibia. This 3-D model was then exported as an STL file which was then imported into a DXF file to create the internal architecture of the scaffold, which in turn was used to devise the commands to drive a 3-printer (Bioplotter™, EnvisionTec, Germany) which manufactured the scaffold by laying down small droplets of the liquid 90% poly-caprolactone (PCL) and 10% hydroxyapatite (HA) by weight using a 25 G needle. The resulting scaffold (Figure 8) had a pore size of 400 μm. Manufacturing time was approximately 4 hours.

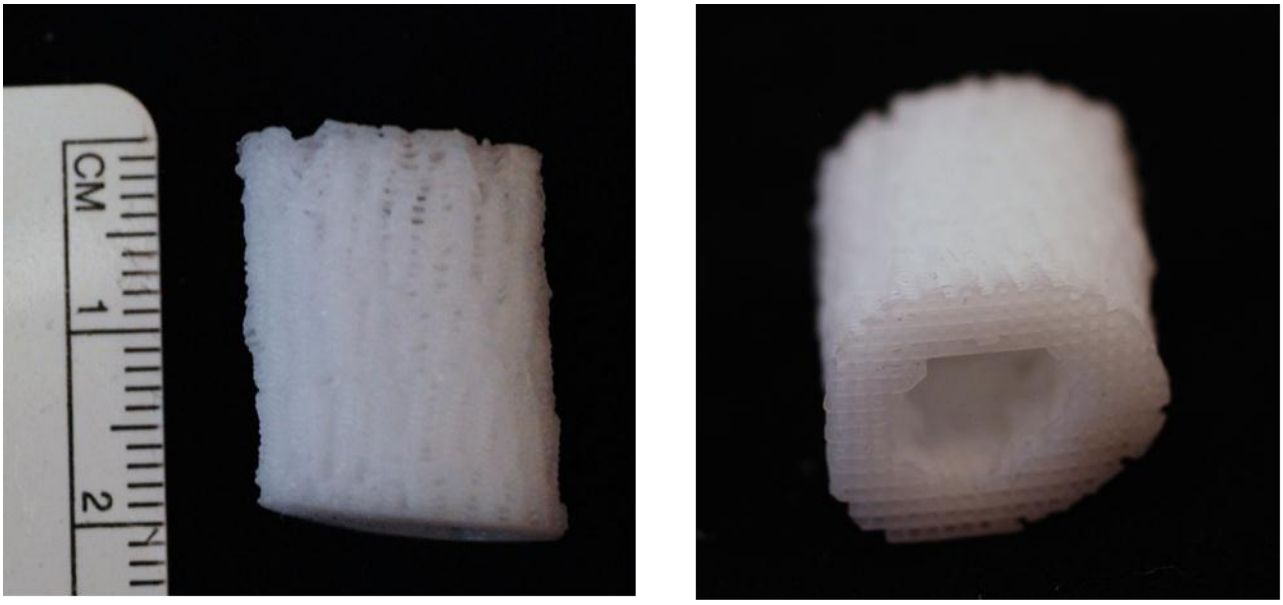

Figure 8: Photographs of two cm segmental defect scaffold showing no cortical shell.

2.2.2 Segmental Defect Surgeries

The surgical approach and procedure were done in accordance to the approved IACUC protocol. The small fragment locking compression plate system from the Synthes® veterinary division (West Chester, PA) was used. To facilitate stability of the implant in a repaired critical size tibial defect, a 3.5 mm 8 hole locking compression plate system was used.

The surgical procedure used to repair a segmental defect in the canine tibia was to perform a block excision of the middle 1/3 of the tibial shaft with an ORIF (open reduction, internal fixation) LCP (locking compression plated) and synthetic scaffold bone insertion. An anterolateral incision is made on the lateral side of the tibia, with the incision carried down to the periosteum, which was reflected back for later placement on top of the scaffold prior to closure. The long extensor digitorum are elevated laterally. The inferior check ligament was displaced laterally. A 2 cm tibial shaft cut was made half-way through the circumference of the medial side using an oscillating saw. An 8-hole (105 mm) locking compression plate was applied using 6 screws (3 superior, 3 inferior, all into native bone, none into the scaffold). All screws were sized to span both cortices. Locking screws are used where possible. A 2.8 mm drill bit was used for the 3.5 mm self-tapping locking screws. Once plate fixation was achieved, the superior and inferior cuts to the tibia were completed and a 2 cm bone piece was removed and replaced with a synthetic 2 cm bone graft (scaffold, Figure 9). One cortical screw was placed obliquely through the scaffold to maintain its stability. A 2.5 mm drill bit was used without a tap for the cortical screw. The incision was then closed and the wound bandaged with sterile dressing.

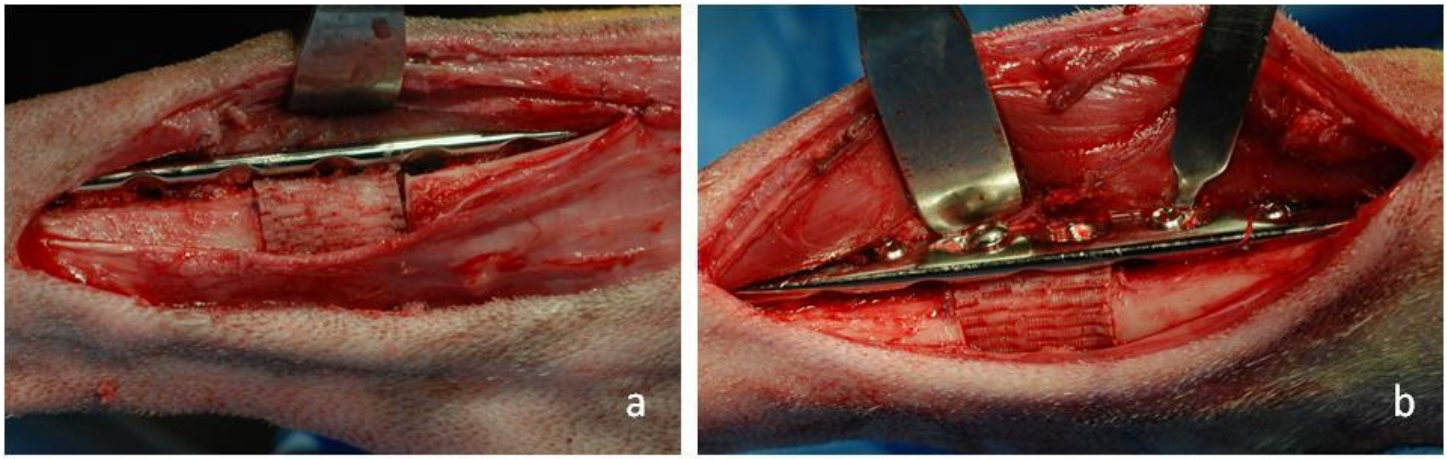

Figure 9: Operative photo showing sterile field, locking plate and placement of scaffold in segmental defect in the exposed lateral side of right tibia of Segmental Defect a) Dog 1 and b)Dog 2.

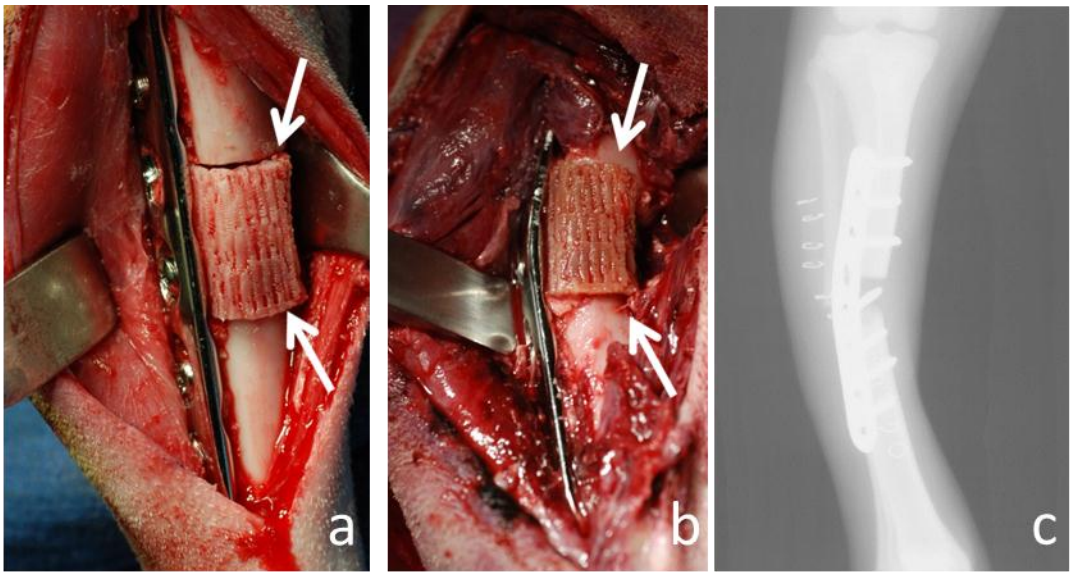

Figure 10: Segmental Defect Dog 3 right tibia a) scaffold placement during surgery, arrows denote misalignment of scaffold with transected bone ends, b)exposure following sacrifice showing bent plate with arrows denoting subsidence of proximal and distal bone edges into scaffold, c) radiograph of bent plate prior to dissection

Segmental defect Dogs 1 and 2 experienced no post-surgery problems. However, Dog 3 experienced an adverse event (see Appendix 7.4). The dog appeared to be recovering as expected after surgery, with moderate weight bearing on the operated limb. On post-op day 4, there was minimal right hind leg weight bearing, the incision site was erythematous, swollen, and hot to touch. The treatment with additional antibiotic was initiated. On the morning of post-op day 5, it was noticed that there was excessive flexion of the stifle, no weight bearing, and lateral deviation of the tibia. The dog was sedated, and radiographs of the right hind leg were taken. Radiographs showed a bent stabilization plate, fractured fibula, and deformed osteotomy site. The dog was euthanized with an overdose of euthanasia solution by the attending veterinarian. An immediate gross necropsy was performed. The complete Adverse Event Report is included in the Appendix.

After careful dissection of the affected limb and review of all surgery photographs and radiographs (Figure 10), it was concluded that the cause of the plate failure was malalignment of the scaffold with the transected bone ends, which in this dog had rougher edges than in the first two dogs due to the need for repeated cutting

of the bone ends to properly fit the scaffold. It is postulated that the combination of malalignment and rough bone edges caused the bone ends to effectively cut their way through the scaffold due to their sharpness and the fact that the stress was high because the annulus of the transected bone ends was not fully supported by the malaligned scaffold. As the bone ends subsided into the scaffold, they effectively pushed the scaffold into the plate causing it to bend. Once the effective length of the tibia was shortened because of the plate bending, the fibula received too great a percentage of the load supported by the limb, causing it to fracture, resulting in further deformation of the limb. Another possible confounding factor is that an increase in associated leg pain might have made it difficult for the dog to ambulate, increasing the likelihood of a fall, which would have further aggravated the problem.

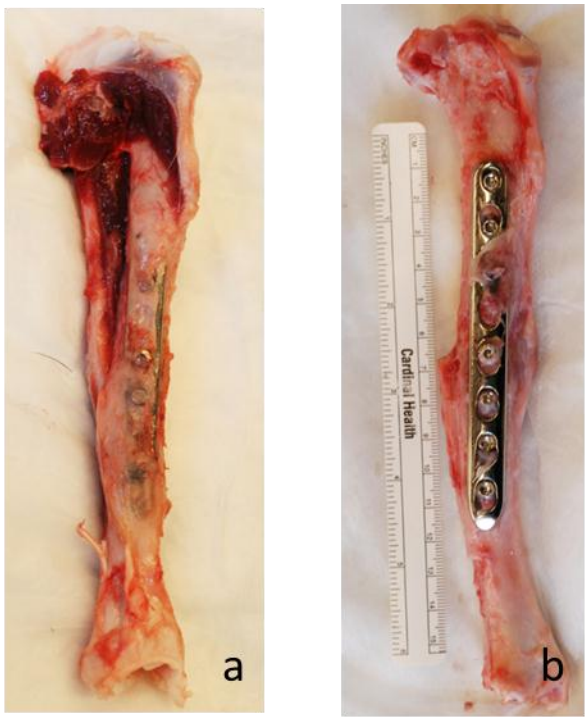

Figure 11: lateral view of excised tibiae of a)Segmental Defect Dog 1 with fibula still attached. Note the significant amount of bony callus surrounding the plate. b)Segmental Defect Dog 2 with part of the fibula near the middle of the plate not yet removed. Note significantly less bony callus surrounding the plate than for Dog 1.

To avoid this complication in future segmental defect surgeries, two primary corrective plans were proposed in a modification to the IACUC protocol, which has been approved. First and foremost, more care will be exercised during surgery to ensure that the scaffold is properly placed and aligned with the transected tibia. Secondly, the dog will be sling-walked immediately following surgery to preclude any possibility of a fall. Sling-walking will be continued until the attending veterinarian determines that the dog is properly and safely ambulating.

Sixteen weeks post-surgery, the animals were humanely sacrificed and both hind limbs removed by disarticulating at the hip. The tibiae were then carefully excised and all soft tissue removed in preparation for biomechanical testing. The fibulae of both experimental (right limbs) were found to be adhered to the tibia in the immediate vicinity of the plate via callus formation. Using sharp dissection and where necessary, a small oscillating power saw, the adhered fibulae were carefully removed without damaging or putting undue stress on the tibiae. There was callus formation around the edges of the plate and in the screw holes of the plate for both dogs, more so for Dog 1 than for Dog 2 (Figures 11a and b). All locking screws were found to be tight upon removal. The cortical screws in each of the scaffolds were not loose, but nor where they tight. They had not backed out, maintaining the same position on the plate as during initial insertion.

2.2.3 Segmental Defect Outcome Measures

Outcome measures of gait, lameness, pain, knee motion and an aggregate of these measures were recorded for each dog every weekday, excluding Saturdays and Sundays. As can be seen from Figure 12, Dog 1 maintained a high total score immediately following surgery and throughout the entire 16 week period. Dog 2 had a short initial period of a few days where more pain and lameness were experienced but these conditions improved by the second week and remained similar to those of Dog 1 for the duration of the experimental period. Criteria for the determination of the outcomes measures were the same as for the osteochondral dogs, and are given in Table 1.

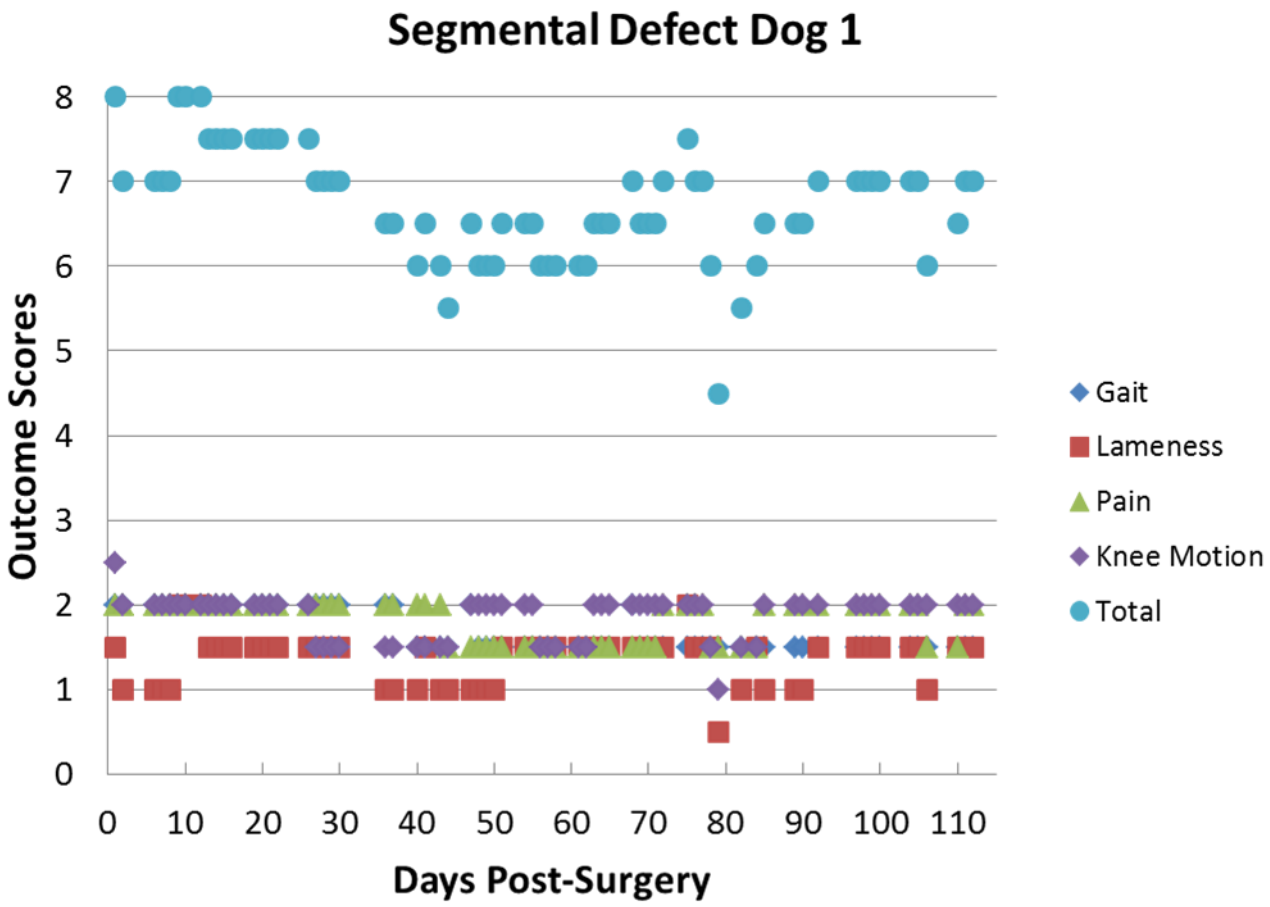

Figure 12: Outcome measures for Segmental Defect Dog 1.

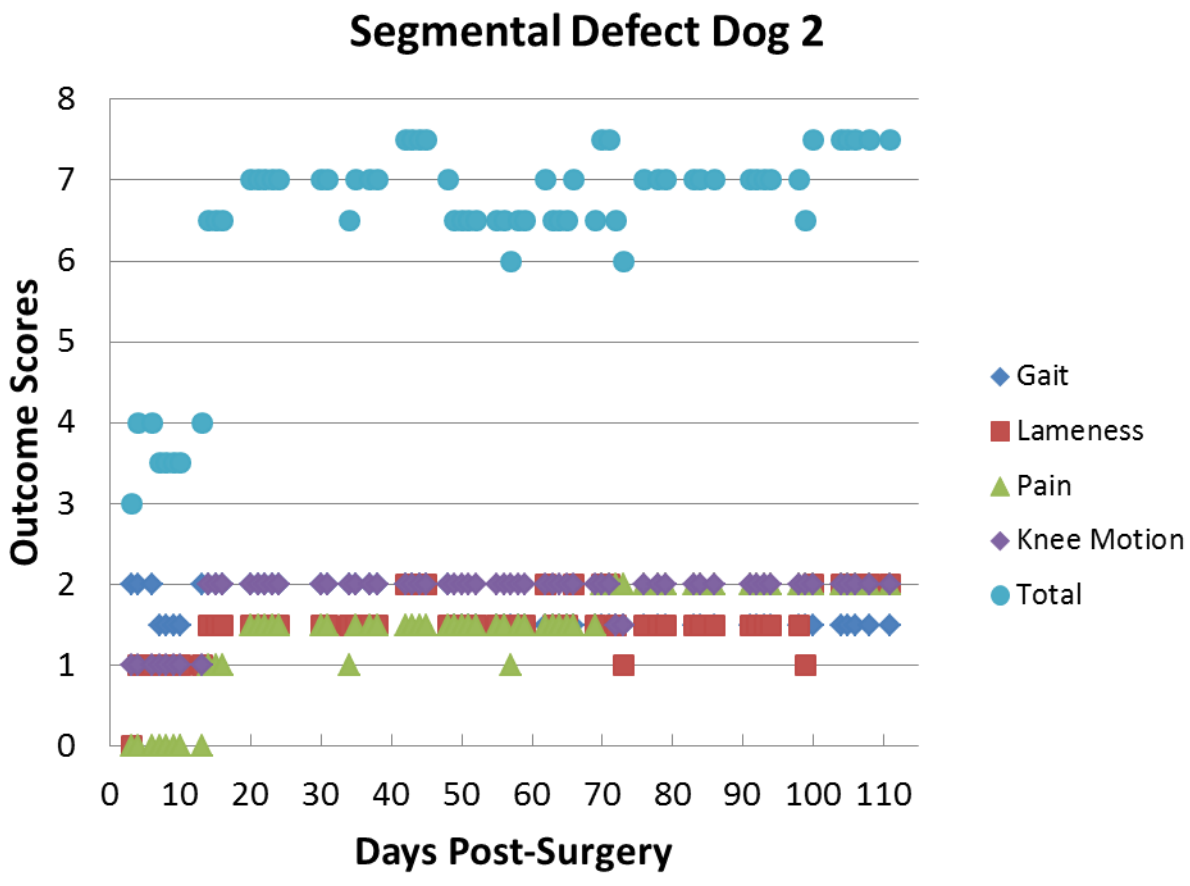

Figure 13 Outcome measures for Segmental Defect Dog 2

2.2.4 Segmental Defect Radiographs and3-D Models from CT Imaging

Plain film radiographs were taken of all segmental defect dogs every two to four weeks. Figure 14 shows the progression of healing for Segmental Defect Dog 1. Little callus formation is seen until week 8. By week 15, the bony ingrowth into the lumen of the scaffold is clearly seen, and is also demonstrated in the 3-D model from CT images of this dog, Figure 16. Figure 15 show the radiographic time history of Segmental Defect Dog 2. For this dog, slight callus formation can be seen by week 4 and progressing significantly until week 8, after which it remains fairly constant, with a slight increase in density by week 15. The 3-D computer model for CT images for Dog 2, Figure 17, has bony ingrowth into the lumen of the scaffold, but it is not as well-defined as it is for Figure 16.

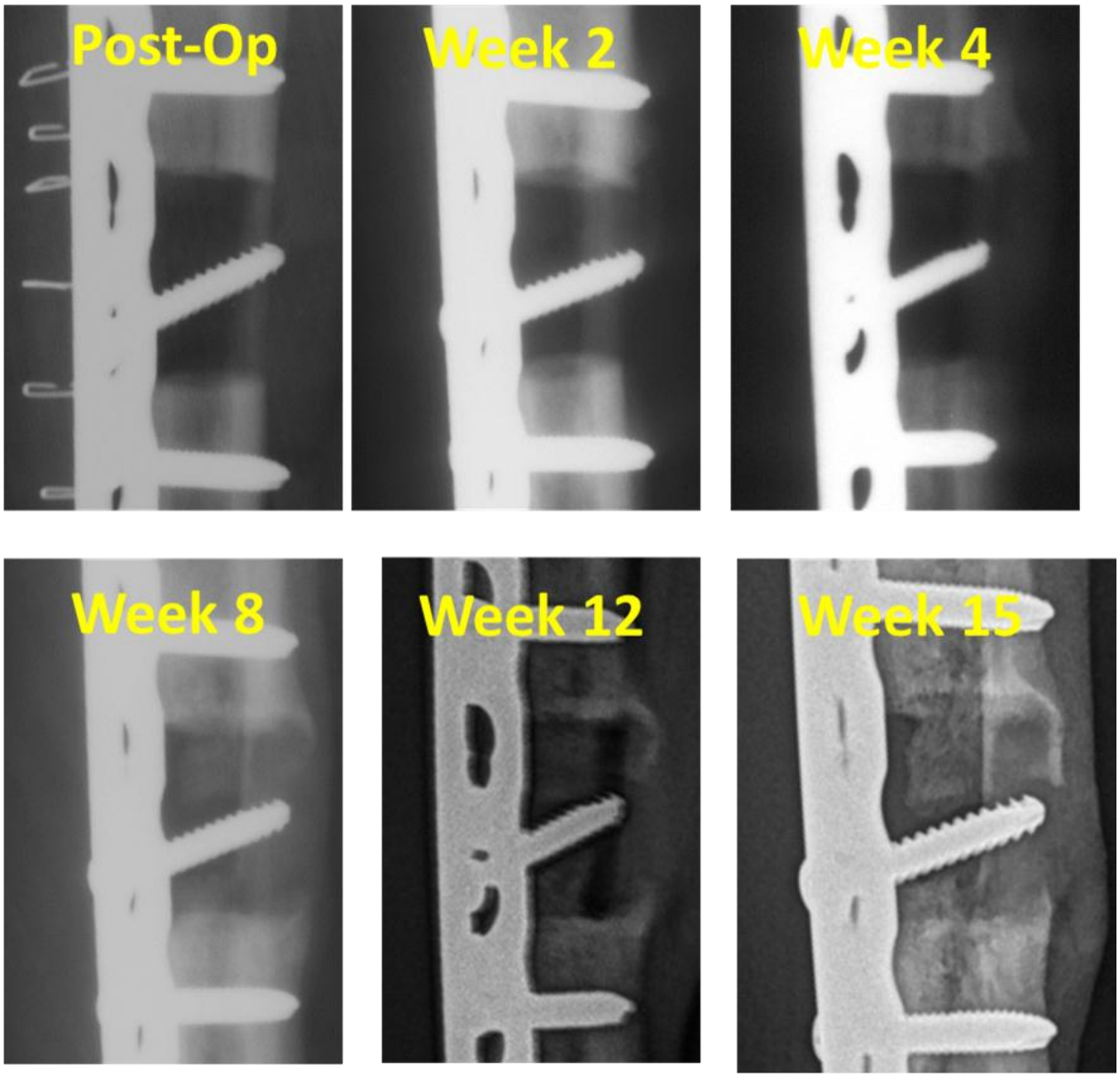

Figure 14: Radiographs for Segmental Defect Dog 1 as a function of time showing progression of callus formation in the vicinity of the scaffold (relatively dark area with oblique screw through it.

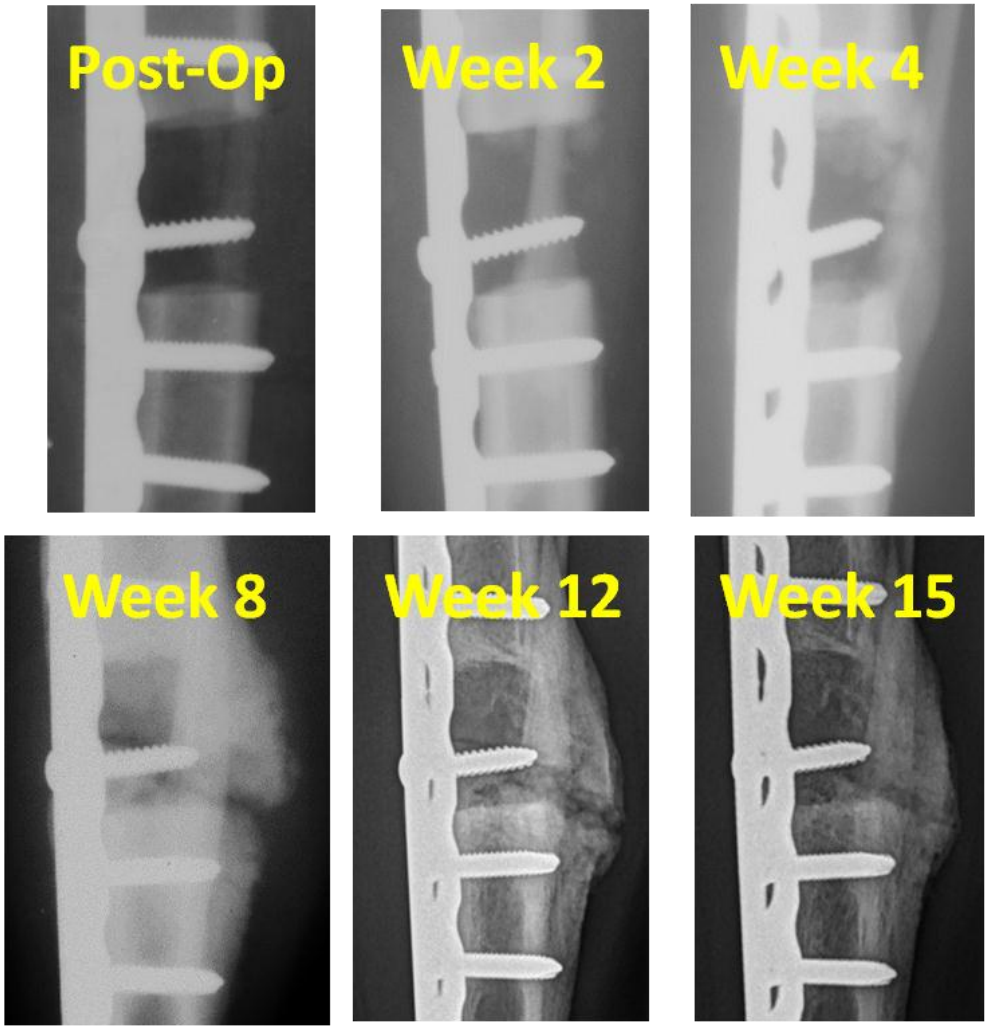

Figure 15: Radiographs for Segmental Defect Dog 2 as a function of time showing progression of formation in the vicinity of the scaffold (relatively dark area with oblique screw through it.

Three-dimensional computer models obtained from CT scans following sacrifice of Segmental Defect Dog 1 (Figure 16) and Segmental Defect Dog 2 (Figure 17). Note that the locking plates and screws were removed prior to imaging to eliminate any effects of metal artifact. Note that only the mid-diaphyseal region of Dog 2 is modeled. This is because the use of fixation plate and associated potting of the proximal and distal ends of the bone were instituted by Dog 2 (see section 2.2.5), which precluded CT imaging of these regions.

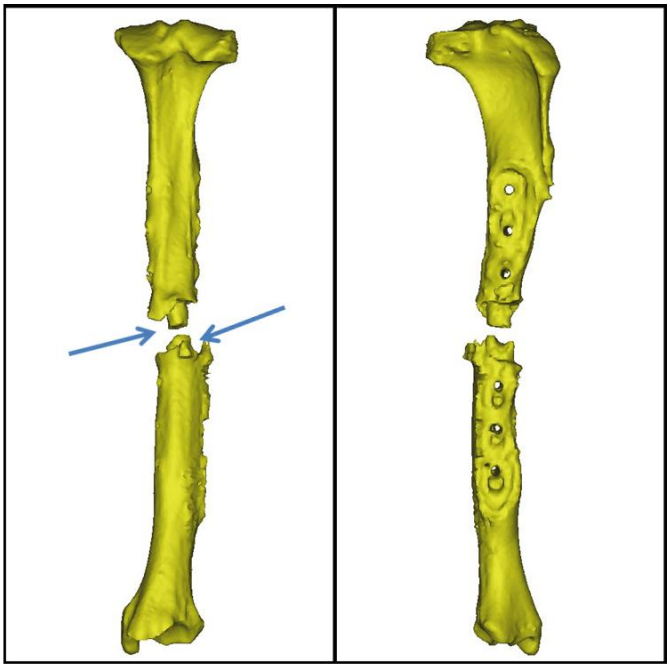

Figure16: 3-D computer model of experimental (right) tibia of Segmental Defect Dog 1. a)posterior-anterior view showing bony ingrowth into the lumen of the scaffold. The space between the two bony ingrowths is due to the cortical screw that secured the scaffold. b)Lateral-medial view of the tibia again showing bony ingrowth and bony callus formation around the border of the plate and into the unfilled holes in the plate.

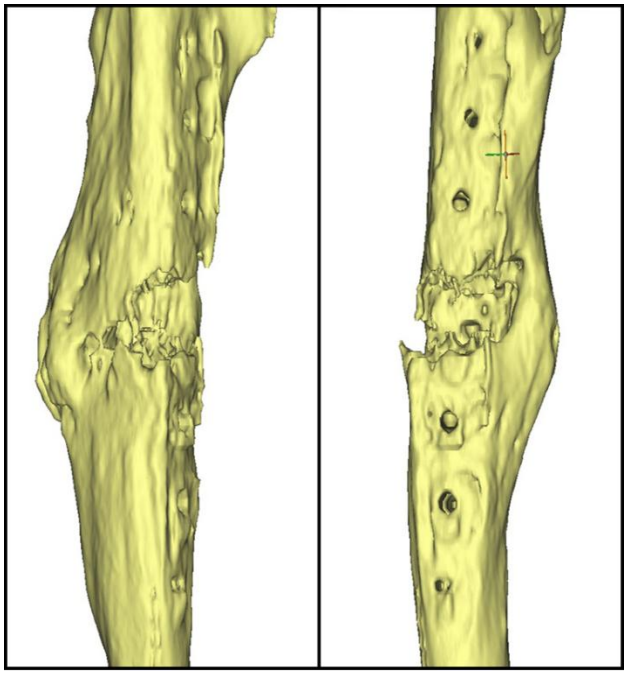

Figure 17: 3-D computer model from CT images of experimental (right) tibia of Segmental Defect Dog 2.

2.2.5 Biomechanical Testing of the Segmental Defect Dogs

Preparation

As stipulated in the grant, torsional testing to failure of tibiae from both the experimental hind limb (right) and contralateral control hind limb (left) was performed. Special torsional testing clamps and jigs to stabilize the joints during dissection were manufactured. In addition to the required torsional testing, non-destructive axial testing of the limbs was also performed prior to the torsional testing to compare the axial stiffness of the experimental limb with the locking plate in place to that of its contralateral control limb. Following sacrifice of the animal, both hind limbs were disarticulated at the hip and CT images obtained. The limbs were then dissected down to the tibia, wrapped in physiologic saline soaked gauze and frozen at -20˚C until the day of testing. On the day of testing, the limbs were thawed to room temperature and the proximal and distal ends of the tibia cleaned of any soft tissue, wiped clean with acetone and proximal and distal potting blocks applied. The potting blocks were 2" square by 1.5" high polycarbonate rectangular tubes. The proximal end of the tibia was placed in a vertical position in the tube, and a 1.6 mm diameter stainless steel Kirschner wire (Zimmer, Warsaw IN) was driven through the wall of the tube, through the bone and then out through the opposite wall of the tube. In a similar fashion, a second, similar wire was driven at right-angles to the first wire. Following wire insertion, the tube and K-wires were filled with PMMA to create a stable construct to resist torsion. A small depression was drilled in the center of the top of the proximal mounting block to accept a steel ball used during axial testing. Once the PMMA hardened in the proximal mounting block, the proximal block was placed in the material testing system and then carefully lowered into the distal mounting block to ensure that the tibia was centered and in a vertical position in the testing machine. K-wires and then PMMA were then applied to the distal mounting block in the same fashion as was done for the proximal mounting block. The tibia was kept wrapped in physiologic saline soaked gauze throughout the entire potting process to ensure that it did not dehydrate.

Mechanical testing was done in an MTS 858 Bionix material testing machine (MTS, Eden Prairie MN) with a biaxial (axial/torsional) load cell. The torsional range of the load cell is ± 50 N-m. The full axial range of the load cell is ± 5000 N. To ensure maximum accuracy of the axial loading, the gain of the axial range of the load cell was set to ± 500 N. Axial testing was done by applying a ramp load to 125 N, representing approximately one-half of the dog's body weight at a rate of 10 N/s (3-5). The compressive axial load was

applied by pressing down with a flat plate onto a large steel ball bearing (Figure 16) which was placed in a small depression that was drilled into the top of the PMMA filled proximal mounting block. The loading was repeated three times. Following the compressive testing, the proximal mounting block was placed into the square top clamp (Figure 18). The sides of the proximal mounting block were sanded prior to testing to ensure a close but not tight fit in the top mounting clamp. Grease was also placed around the sides of the proximal clamp prior to placing it in the top clamp. Fitting the proximal mounting block and applying grease to the block were done to allow for axial displacement of the proximal clamp to minimize any axial loading during the torsional testing. A rotation rate of 1 N/s was applied to the tibia. This rate was less than the 3 N/s rate stated in the original grant proposal because further review of the literature (3, 6, 7) indicated that this rate would lessen any viscoelastic effect and allow comparison to similar data in the literature which used the 1 N/s loading rate. A lateral-to-medial (internal rotation) direction of loading was used. The distal end of the tibia was fixed and the torsion was applied to the proximal end of the tibia. Time history data were collected at a frequency of 10 Hz for axial and 20 Hz for torsional testing.

Axial testing was done for the control (left) tibia and experimental limb (right). Axial testing was done with the stainless steel plate and screws intact for the experimental limb. Torsional testing for the experimental limb was performed following removal of the plate to properly measure the strength of the bone-scaffold interface that may have formed during the 16 weeks of healing. CT images were obtained prior to axial testing of the experimental limb with the plate intact, but the metal artifact made the resulting CT images of questionable value. Therefore, additional CT images were obtained following plate removal and prior to torsional testing so that metal artifact free CT images of the healed bone-scaffold-bone construct were recorded. To ensure that the bone-scaffold-bone construct was not damaged during plate removal and CT imaging, a special external fixation device was used. After axial testing of the experimental limb, 2 threaded screw holes were placed in both the proximal and distal mounting blocks while the tibia was still in the testing machine. A polycarbonate block was then attached to the proximal and distal mounting blocks using 4 thumb screws. This resulted in an extremely stable construct that allowed for removal of the locking plate without imposing loading on the healed bone, as well as transport to the CT while maintaining the rigidity of the clamped tibia. Following plate removal and CT imaging, the tibia was placed back in the testing machine and the polycarbonate support plate removed. Immediately following plate removal, and before torsional testing, the 6 screw holes in the bone (3 proximal to the segmental defect and 3 distal to the segmental defect) were filled with PMMA.

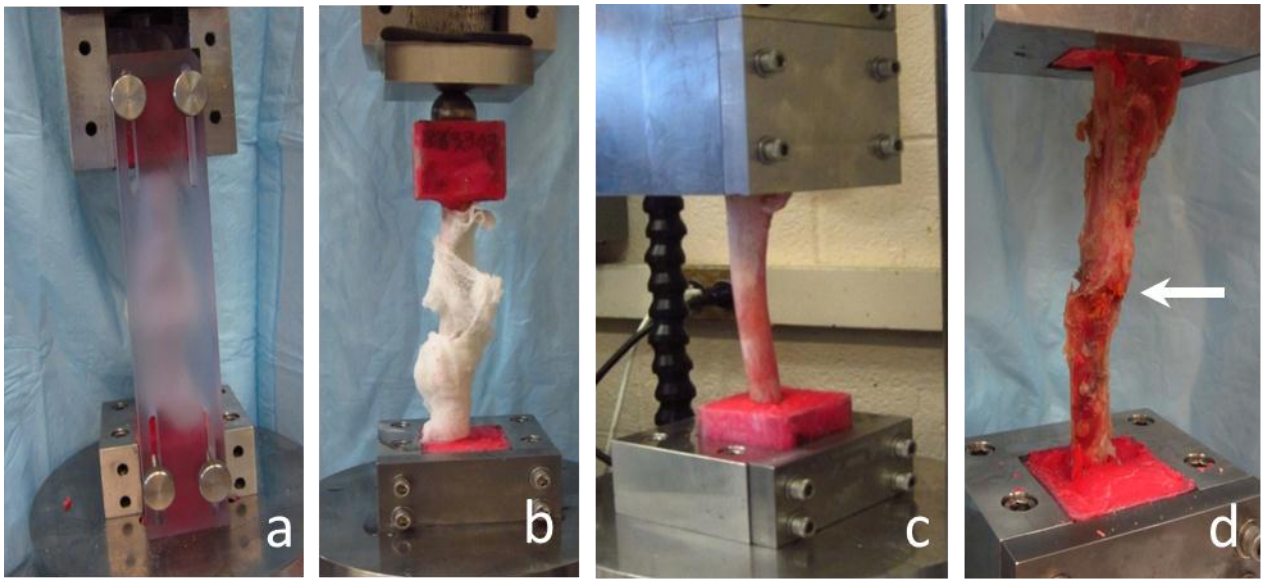

Figure 18: Mechanical testing of segmental defect canine tibia, a) support block maintain stability of experimental tibia during plate removal and subsequent CT imaging, b)axial testing of tibia with plate intact, showing saline-soaked gauze to maintain hydration during testing, c) torsional testing of control (left) tibia with gauge removed immediately prior to testing to prevent any effect on the torsional results, d) spiral fracture of control (left) tibia, denoted by arrow.

Results

Both limbs for segmental defect dogs 1 and 2 were tested. The load-displacement curves are given in Figure 19a and 19b and a summary of the measured parameters provided in Table 2. The failure torque and stiffness of the experimental leg for Dog 1 was significantly less than that of its contralateral control limb (10% and 7%, respectively), whereas these values for the experimental limb of Dog 2 were approximately 40% and 60%, respectively, of its control limb. The reason for this large discrepancy is attributed to damage that occurred to the experimental limb of Dog 1 during plate removal. The plate was removed from the experimental limb of Dog 1 without the benefit of the external fixation device described above to provide stability of bone-scaffold-bone construct during plate removal. It is for precisely this reason that this external fixation device was developed and used for Dog 2 and will be used in the testing of all subsequent segmental defect dogs. When comparing axial stiffness, it is seen that the axial stiffness of the experimental limb with the plate was approximately 48% stiffer than that of its contralateral control limb, whereas the axial stiffness of the experimental limb for Dog 1 was 72% less stiff than that of its contralateral control limb. The reason for the differences in axial stiffness between Dog 1 and Dog 2 is attributed to two factors. First, the plate was inadvertently removed prior to axial testing for Dog 1, whereas it was not removed for Dog 2 prior to testing. Second, as mentioned above, it is highly likely that the bone-scaffold-bone interface was disrupted during plate removal for Dog 1, which was not the case for Dog 2. Both of these factors are thought to have contributed to the much lower stiffness of the experimental limb compared to its control limb for Dog 1. However, now that the specimen preparation and testing techniques have been refined by testing Dog 1 and Dog 2, it is felt that these initial start-up problems have been resolved, as demonstrated by the mechanical results obtained for Dog 2.

Following mechanical testing, both tibae were placed in formaldehyde for subsequent histological analysis. Accurate cross-sectional areas of the proximal and distal ends of the bones adjacent to the scaffold will be obtained to permit the calculation of the shear modulus (modulus of rigidity) and resulting shear stress for the tibiae for both dogs, in addition to the structural properties presented at this time

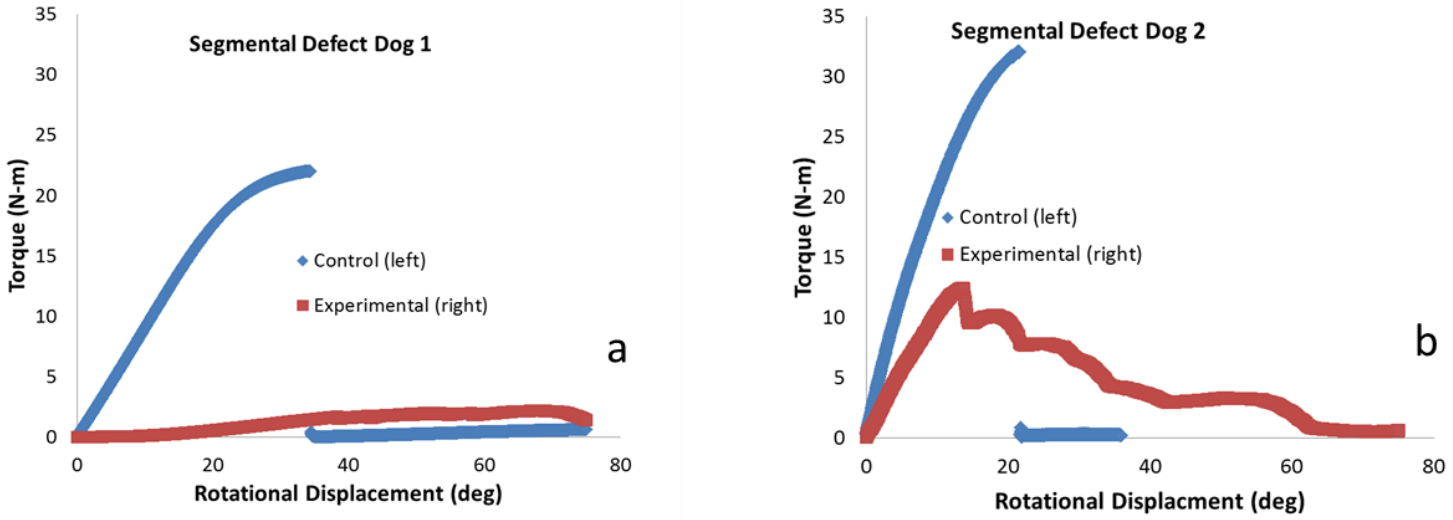

Figure 19: Torque versus rotational displacement for both control (left) and experimental (right) legs for Segmental Defect Dogs a) 1 and b) 2.

Animal	Hind Limb	Failure Torque (N-m)	Failure Rotation (deg)	Torsional Stiffness (N-m/deg)	Torsional Failure Energy (N-m-deg)	Axial Stiffness (N/m)
Dog 1	Control	22.0	34.3	0.91	424.6	770.8
	Experimental	2.2	67.8	0.06	81.4	211.5
	% Difference	90.0	97.8	-93.4	-80.8	-72.6
Dog 2	Control	32.1	21.6	1.85	398.9	653.2
	Experimental	12.4	13.6	1.05	96.5	968.1
	% Difference	-61.2	-36.9	-43.2	-75.8	48.2

Table 2: Summary of biomechanical test results for Segmental Defect Dogs 1 and 2 for both control (left) and experimental (right) limbs.

2.2.6 Preliminary Hard-Sectioning Histology

Immediately following mechanical testing, the proximal and distal tibial potting blocks were removed by sawing with a hand saw. The resulting diaphyseal portion of the tested bone was then placed in formalin for histological evaluation. Because the mechanical testing was completed in October, the tested specimens for Segmental Defect Dogs 1 and 2 have not yet completed their fixation. However, histology was performed on the experimental tibia from Segmental Defect Dog 3, which was sacrificed prematurely and did not undergo mechanical testing. Following fixation in formalin, bone-blocks were created that were embedded in one-component photo-curing resin (Exakt 7200 VLC, Oklahoma City, OK), and thin sections of the bone-blocks prepared using a precision micro-saw (Buehler, Lake Bluff, IL). Sections were progressively polished with 600 and 1200 grit paper (Buehler, Lake Bluff, IL) and adhered to glass slides using a methyl methacrylate resin (Surgipath Medical Ind., Richmond, IL). The sections where then stained for nuclei and cytoplasm with Haematoxylin & Eosin (H&E), Figure 20.

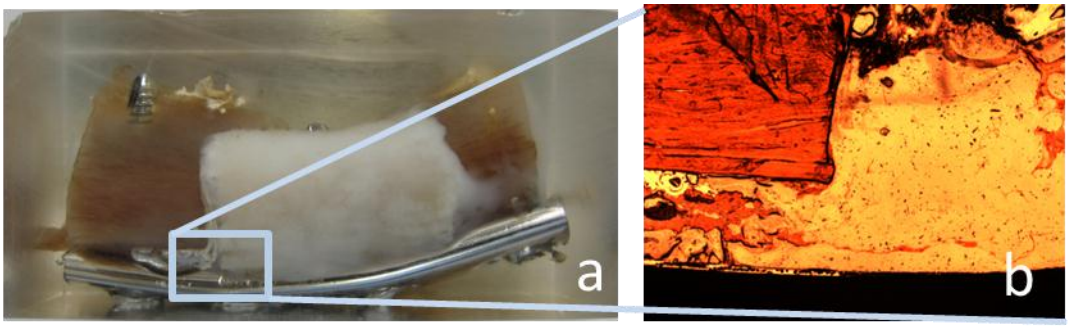

Figure 20: Hard sectioning histology of Segmental Defect Dog 3, a) post-sacrifice section of tibia with several mm of proximal and distal bone on either side of the segmental defect (white region) showing metal locking plate on the bottom with one of the tips of the locking screw protruding on the top left, b) H&E stain of region shown enclosed by light blue box, showing native cortical bone in darker red, scaffold in light orange and plate as black region at the bottom of the slide (2x magnification).

2.3 Aim 3 – Human Cadaveric Testing

Human cadaveric testing is provided for in the grant to assess the stability of scaffold at time zero for both Aim 1 (osteochondral defects) and Aim 2 (segmental defects). To date, efforts have concentrated on formulating and refining the testing protocol for the segmental defects.

2.3.1 Mounting of Plate in Human Cadaveric Tibia

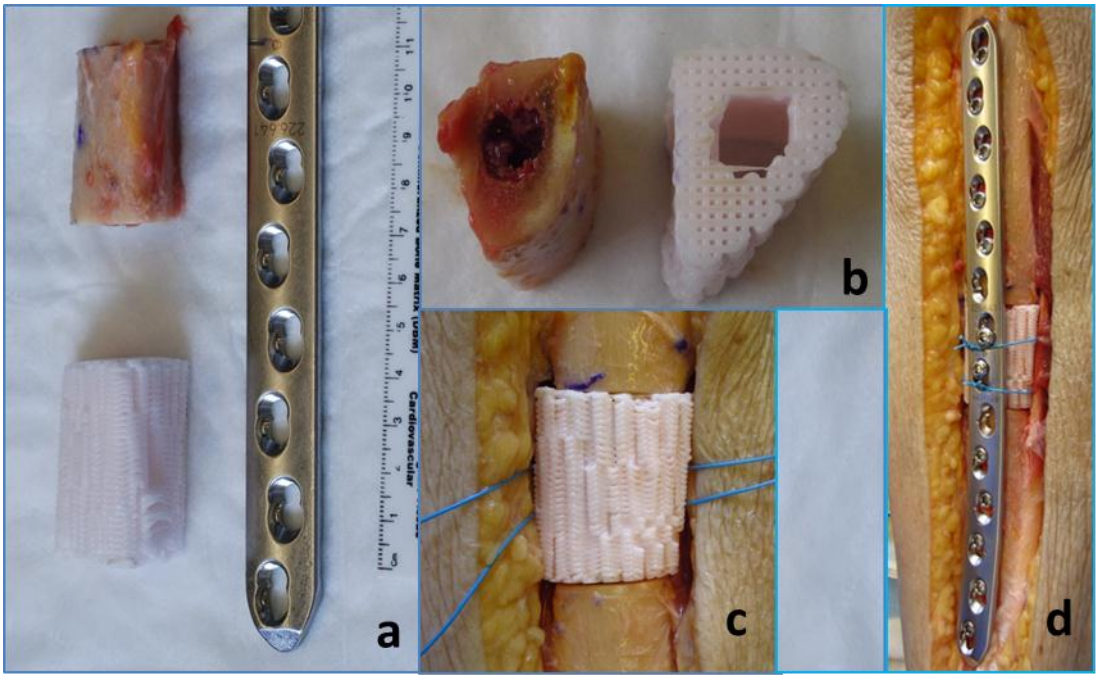

Figure 21: Human cadaveric tibial specimen showing a) excised segmental defect and scaffold to replace it, b) cross-sectional view showing human bone segment removed and scaffold, c) implanted scaffold showing tight-fit, d) locking plate, scaffold and bone construct.

A 72 year old female cadaveric specimen was used for pilot testing. A medial incision was made and the soft tissue was sharply dissected down to the bone. The surrounding soft tissue and fibula were maintained to ensure that when the approximately 5 cm segmental defect was created, the native length of the tibia would be maintained. A 14-hole 4.5 mm broad, 260 mm long locking compression plate from Synthes (Paoli, PA) was used with five 5.0 mm diameter locking screws placed both superior and inferior to the scaffold. Two 4.5 mm diameter cortical screws were placed into the scaffold to secure it. Prior to placement of the cortical screws, two sutures were place around the scaffold to hold it tightly to the plate as the screws were applied. All screws bridged both cortices. The scaffold was created using a mixture of 90% poly-caprolactone (PCL) and 10% hydroxyapatite (HA) by weight. The scaffold was created with 500 μm microchannels with 300 μm strands and did not have a cortex shell to replicate the scaffold architecture implanted in the dog. Figure 21 compares the implanted scaffold to the segment of bone removed from the tibia and its placement in the segmental defect. The plate was bent slightly at the distal end to conform to the distal tibia surface. In the future experimental cadaveric specimens, a lateral approach rather than the medial approach used for this pilot will be taken. This will mimic the lateral approach that was used for segmental defect surgeries in the dogs.

2.3.2 Mechanical Testing of Human Cadaveric Tibia

The tibial plateau was first prepared by cutting off the raised portions to create a flat surface. A Plexiglas® plate was then mounted on the resulting flat proximal surface of the tibia using bone cement and two long lag screws. The plate has both a slot and a spherical depression machined into its top surface. When axial compression loading was applied, a steel ball was placed into the spherical depression of the plate and the tibia was loaded via a flat platen (Figure 22a). When torsion loading was to be applied, the ball was removed and a steel key is placed into the slot (Figure 22b). This key protruded both distally into the medullary canal of the tibia and proximally above the plate. A slotted clamp was then lowered onto the key and torsion was applied to the tibia. The clamp allowed the key to slide in the axial direction to permit superior/inferior motion of the plate as the tibia was torqued to minimize any compressive force resulting from the

applied torque. The distal end of the tibia was potted in a 2" threaded steel pipe nipple with orthogonal pins placed through the nipple and tibia. The pipe nipple was filled with expansion cement to provide a rigid fixation.

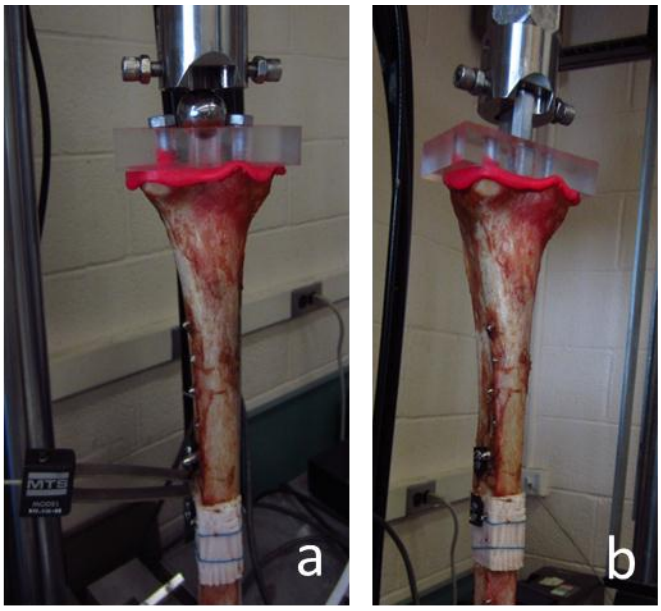

Figure 22: Mechanical testing of human cadaveric tibia with scaffold in segmental defect a) axial loading via ball bearing, b) torsional loading via rectangular shaft.

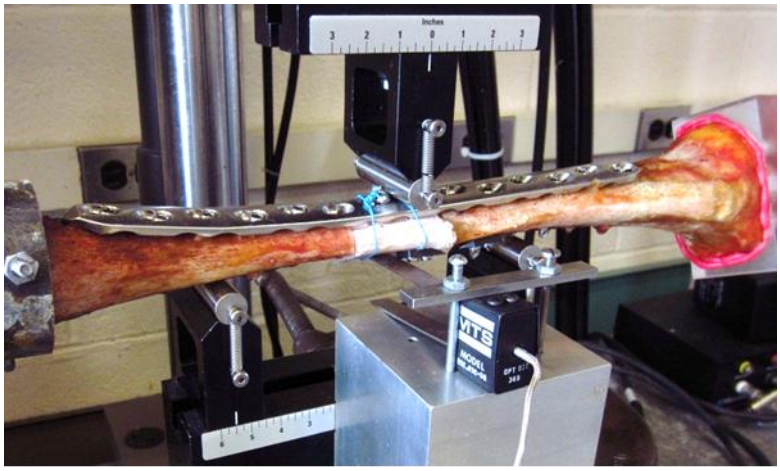

Figure 23: Three-point bending of tibia-plate-scaffold construct. Bending load is applied to the middle of the locking plate.

The loading was applied using with a MTS 858 Bionix (MTS Eden Prairie, MN) testing system. Axially loading was applied via a series of step loads of 50 N, 400 N, 500 N, 600 N and 700 N. At each step, the load was applied in a sinusoidal fashion for 50 cycles at a rate of 2 Hz. Data were collected at a rate of 10 Hz. Bending loads were applied to the middle of the plate also via a series of step loads of 50 N, 200 N, 250 N, 300 N and 350 N in the same manner as was done for the axial loading (Figure 23). Finally, torsional loading was applied via a series of step loads of ±3, ±6 and ±9 N-m for 20 sinusoidal cycles at a rate of 0.5 Hz. The loading protocol closely replicated the protocol used by Choi et al (8). Axial, bending and torsional stiffness values were obtained from the slope of the force versus axial displacement, bending moment versus mid-span displacement and torque versus angular displacement curves, respectively. Following testing with the scaffold in place, the scaffold was removed and the same series of tests repeated without the scaffold. This order was purposely not randomized because the main intent of the study was to determine the stiffness of the construct with the scaffold in place. Therefore, any confounding effects of screw loosening due to first testing it with the scaffold absent were avoided.

The results of the pilot human cadaveric tests are provided in Figure 24. The axial stiffness and bending stiffness were slightly higher for the bone-plate-scaffold construct as compare to just the bone-plate construct. This is as to be expected since the addition of the scaffold should provide some mechanical resistance to the overall construct. However, the torsional stiffness was found to be slightly higher without the scaffold. Though this might be considered contradictory, the scaffold provides little in the way of torsional resistance to the construct. Therefore, this slight difference in torsional stiffness may reflect the experimental variability of the experimental set-up because of the low torsional stiffness rather than representing a true effect of the scaffold being or not being present.

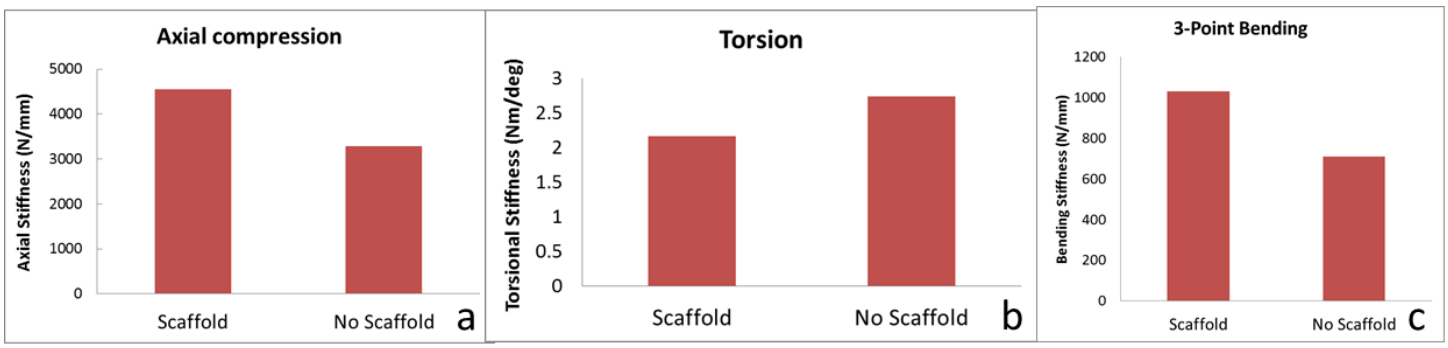

Figure 24: Mechanical testing results for right human cadaveric tibia with and without scaffold in segmental defect for a) axial compression, b) torsion, c) 3-point bending.

3. KEY RESEARCH ACCOMPLISHMENTS

3.1 The osteochondral defect scaffold design was perfected. All 3 pilot osteochondral defect (Aim 1) surgeries were performed and to date, the surgeries have been well-tolerated by the dogs.

3.2 Bone marrow aspirations were successfully performed and the cell culturing technique demonstrated effective cell proliferation (Aims 1 and 2).

3.3 All 3 segmental defect (Aim 2) pilot surgeries were performed and 2 of 3 animals successfully completed the full 16 weeks post-surgery recovery period.

3.4 Outcome measures for segmental defect dogs (Aim 2) demonstrated that the surgical procedure was well-tolerated by the dogs and did not impair their quality of life.

3.5 There was no immune response by any of the animals to the poly-caprolactone (PCL) and hydroxyapatite (HA) scaffolds (Aims 1 and 2).

3.6 An in-house hard-sectioning histology laboratory has been set-up and used to obtain histology for the Aim 2 dog that was sacrificed early.

3.7 The biomechanical testing protocol and methodology for segmental defect (Aim 2) limbs were established and refined.

3.8 Biomechanical testing demonstrated that the torsional strength of the bone-scaffold-bone segmental defect (Aim 2) construct alone was within 40% of the contralateral control limb, and within 60% of the contralateral torsional stiffness for one of the two tested dogs.

3.9 Based on the generally positive results for Aim 2 dogs, a modification of IACUC protocol requesting expansion of Aim 2 component of the protocol to all five arms of the segmental defect study (allografts, scaffold alone, scaffold + cMSCs, scaffold + BMP, scaffold + cMSCs + BMP) will be submitted.

3.10 The mechanical loading protocol was finalized and pilot tests performed to demonstrate segmental defect scaffold stability immediately post-op in human cadaveric specimens (Aim 3).

4. REPORTABLE OUTCOMES

The primary reportable outcomes based on the above key research accomplishments are that the use of the PCL+HA scaffold was well-tolerated by the animals and that the bone-scaffold alone-bone construct can result in a moderately stable structure. This result provides encouragement that a scaffold enhanced with the infusion of BMP, cMSCs or both may result in a construct that mimics the native strength of the pre-injured limb.

5. CONCLUSIONS

At the beginning of this reporting period, an IACUC protocol for surgeries for 6 pilot dogs (3 for Aim 1 and 3 for Aim 2) was in place. All three surgeries were performed for the segmental defect (Aim 2) dogs, two of the three being successful, with the animals taken to the end of the full 16 week recovery period. The third dog had an untoward event, which was reviewed and an IACUC protocol modification approved for a replacement dog. This surgery will be

performed within the next two weeks. In parallel, because of the good results for the two successful dogs, an IACUC modification is being submitted to permit the extension of the IACUC protocol for Aim 2 to expand it to the full Aim 2 experimental design. It is hoped that his protocol can be approved shortly and that the surgeries involving all arms of the Aim 2 experimental design can begin before the end of 2012. Biomechanical testing of the two successful segmental defect dogs demonstrated that it is possible to obtain moderate torsional strength in a bone-scaffold alone-bone construct. Furthermore, it was shown that the axial stiffness of the repaired segmental defect construct with a locking plate can be stiffer than that of the native bone.

All three surgeries have also been completed on the 3 osteochondral defect dogs (Aim 1). However, these surgeries occurred somewhat later than anticipated, being performed in early fall of 2012. Accordingly, these dogs are currently in their 16 week recovery period, scheduled to be sacrificed in January 2013. To date, all three of these dogs are doing well. The primary delay in performing the osteochondral defect surgeries was that more time was required than originally anticipated to perfect the design and manufacture of the osteochondral defect. A number of different designs and surgical approaches were considered and tested on cadaveric dog knees. The final design is a press-fit design that is ligament-sparing yet allows the scaffold to be placed in the load-bearing region of the medial condyle. Once the dogs undergo their full 16 week recovery period, they will be sacrificed, their knees biomechanically tested, and pending successful results an IACUC modification submitted requesting expansion of the IACUC protocol to include all arms of the osteochondral defect study design (allografts, TGF-β3, cMSCs). Hopefully these surgeries can start in the spring of 2013.

Bending, axial compression and torsional pilot tests of the stability of the scaffold design for the segmental defect were performed in human cadaveric specimens. Testing hardware and the testing protocol were refined. A sufficient number of the much larger human scaffolds were manufactured for human cadaveric testing. An implant manufacturer has provided on loan standard instrumentation trays to perform the plating of the human segmental defect as would be done in the operating room. With the pilot surgeries completed for both Aims 1 and 2, the segmental defect procedures will be performed in the 10 cadaveric specimens and mechanical testing completed in November and December of this year.

5.1 So What

Both osteochondral and segmental defect surgeries are well-tolerated and the animals in general demonstrated quick recovery from the surgeries. The response of the animals to the PCL+HA scaffold has been positive and the torsional strength that can be obtained from the scaffold alone in the segmental defect dogs provides preliminary support to the proposed aims of this study. Therefore, continued funding of this effort is justified to complete the full experimental design to demonstrate if *in vivo* large animal translational experiments will lead to the development of new military technology products and utilities for the definitive and preventive orthopaedic care of military personnel and retirees.

6. REFERENCES

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7. APPENDICES

Appendix 1 – Year 2 Continuation of IACUC Protocol for Live Dogs

Summary: No additional animals or modifications to the protocol were requested for this Year 2 continuation

Columbia University Animal Care Protocol Continuation Data Sheet

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Protocol: AC-AAAB7357 (Y2 M00)		Protocol Status: Approved (Done)	
		Approval Date: 02/08/2012 at 00:00	
Name: Francis Lee (fl127)		Faculty Title:	Associate Professor
Title: Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma			
Species:	Dog	Effective Date:	02/11/2012
Number of Animals:	6	Expiration Date:	02/10/2013
Pain Levels:	D	Inspection Date:	
Originating Department:	ORTHOPEDIC SURGERY	Previous Protocol Number:	AC-n/a
Protocol Type:	Research	Protocol Submitted to:	Health Sciences

Modifications

None

Funding

Has ARRA Funding:	N	Funded by Department:	N	Funded Through Private Gift: N

Awarded Institution	External Funding Type	Source Identifier	External Funding Agency	Proposal Number
	Government Grant	OR090175	Department of Defense	PT-AAAH0521

Protocol Staff

Personnel	Role	1+ yrs Exp W/ Species	Proc. Exp	Species Specific Training.	Wet Lab Date	OHP Exp Date	Regulatory Lecture Date
Lee, Francis (fl127) phone: 212-305-3293 cell: 201-248-1630 pager:	Principal Investigator	yes	yes	07/07/2010		06/28/2010	
Procedure Experience:							
		1) 25years of experience as an orthopaedic and oncology surgeon, and more than 5 years of experience with survival surgeries in dogs. Dr.Lee will be the surgeon in charge on this protocol performing both control and experimental procedures. - 2) - Orthopedic surgery					
Nizami, Saqib (sn2452)	Staff Researcher	no	yes	07/07/2010		11/04/2010	
Procedure Experience:							
		1) Saqib Nizami is a technician and will be trained to handle dogs. - Orthopedic surgery					
Akelina, Yelena (ya67)	Staff Researcher	yes	yes	05/17/2007		05/16/1997	
Procedure Experience:							
		1) Dr. Akelina has approx 4 years experience in chronic studies in dogs involving orthopedic procedures. She will coordinate all surgeries, post-op care and all data collection and analysis. Dr. Akelina is an Associate Research Scientist - Orthopedic Surgery					
Gardner, Thomas (trg1)	Lead Researcher	yes	yes	07/07/2010		05/26/2010	
Procedure							

Experience:							
		1) Mr. Gardner is an Engineer and Manager of the Biomechanic lab of the Dept. He will be responsible for helping with the coordination of project, participate in post-op care, oversee all biomechanical testing and data analysis. - Orthopedic Surgery					
Training Courses:		TC0506 - Macacine Herpes Virus-1 (aka Herpes B Virus) Training			05/22/2012		
		TC0650 - The Dog: Computer Based Training Program			02/15/2012		
		TC1000 - The Ruminant: Computer Based Training			03/08/2012		
Mao, Jeremy (jm2654)	Lead Researcher	yes	yes	01/05/2010	03/04/2011	12/06/2011	
Procedure Experience:							
		1) Dr Mao has extensive experience in small animal handling and will be trained to handle dogs. Dr. Mao will be responsible and provide overall supervision. He will assist in surgery, post surg. monitoring and tissue harvesting. - Orthopedic Surgery					

Administrative Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Emergency Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu

Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757	kmk2148@columbia.edu

Locations

Housing Location	# of Animals
BB 18	6

Satellite Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Exp.Procedure Location	Proc. Type	Duration (hrs)	Responsible Person
Black Building, 18, CSS, -	Survival Surgery	4	Francis Lee (fl127)
Neurological Institute, Basement, Hatch Center	No Surgery	2	Francis Lee (fl127)

Post Op Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Drugs

Anesthetic or Analgesic	Dosage	Route	Responsible Person
CARPOFEN	4 MG/KG	PI, IM, SC	ICM (Institute of Comparative Medicine)
MORPHINE	0.1 MG/KG	EPIDURAL	ICM (Institute of Comparative Medicine)
DIAZAEPAM	0.2-0.5 MG/KG	Intravenous	ICM (Institute of Comparative Medicine)
Propofol	2-5mg/kg	Intravenous	ICM (Institute of Comparative Medicine)

Hydromorphone	0.025-0.05 mg/kg PRE-SURGERY & EVERY 4-8 hrs POST-SURGERY	IM, SC, IV	ICM (Institute of Comparative Medicine)
Fentanyl	50 mcg/hr FOR 10-20KG DOGS OR 75 MCG/HR FOR 20-30 KG DOGS	transdermal patch	ICM (Institute of Comparative Medicine)
Pentobarbital	100mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Acepromazine	0.025-0.05 MG/KG	IV, IM, SQ	ICM (Institute of Comparative Medicine)
MARCAINE	0.25%	Subcutaneous	ICM (Institute of Comparative Medicine)
Isoflurane	1-5%	Inhalation	ICM (Institute of Comparative Medicine)
METHOHEXITAL	4 11 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)

Other Drug or Substances	Dosage	Route	Responsible Person
CEPHALEXIN	30 MG/KG TWICE DAILY	Oral	ICM (Institute of Comparative Medicine)
Cefazolin	10-30 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
TGF-b3	10 nanograms/ml	osteochondral scaffold will be infused/coated with this material	Mao, Jeremy (jm2654)
BMP-2	160 micrograms per scaffold	segmental defect scaffold will be infused with this material	Mao, Jeremy (jm2654)
CEFPODIXIME (AS AN ALTERNATIVE TO CEPHAFEXIN)	5-10 MG/KG	PO SID	ICM (Institute of Comparative Medicine)

Hazardous Materials

No hazardous materials used.

Scientific Questions

Continuation
Questions

1. Describe in non-technical terms what was learned during the last year

Because of unforeseen delays due to a variety of factors, no animals were purchased during the 1st year of this protocol. Hence no surgeries were performed and nothing was learned.

2. Describe any adverse events which have affected animal use, welfare, morbidity, or mortality.

As explained in the response to Question 1, no animals were purchased or surgeries performed in year 1 of this protocol, hence this question is not applicable.

3. Discuss any changes to the planned use of animals and/or objectives.

There are no changes to the planned use of animals or objectives. There are no changes to personnel, number of animals requested, protocol, drugs, or any other component of this protocol

4. Justify the need for the number of animals requested

Since no work has progressed on this protocol in the first year of the protocol, no animals were ordered or used. Hence there is no change in the number of animals requested. We are requesting the same number of dogs, 6, that were approved for year 1 but not used.

5. Since the last IACUC approval have alternatives to the use of animals become available that could be substituted to achieve your specific project aims? If yes, explain why the alternative to animal use is not feasible for your project.

An Ovid MEDLINE (1946 to January, Week 2, 2012) search was preformed on January 23, 2012. The full search is provided as an attachment. The literature search did not provide any feasible alternatives or alterations to the current procedure. Our current model is in keeping with other bone defect studies. We have attached the search strategy below:

Search Strategy:
---
1 exp Animal Experimentation/ (5125)
2 exp Animal Welfare/ (9177)
3 exp Osteotomy/ (22977)

4 1 or 2 (12418)
5 3 and 4 (0)
6 exp Bone Regeneration/ (14729)
7 exp Fracture Healing/ (7786)
8 Tibia/ (24081)
9 6 or 7 (22079)
10 8 and 9 (1353)
11 Tibial Fractures/su, th [Surgery, Therapy] (8020)
12 10 or 11 (9218)
13 4 and 12 (2)
14 Dogs/ (271218)
15 12 and 14 (329)
16 3 and 15 (46)
17 Tissue Engineering/ (14821)
18 Bone Transplantation/ (23430)
19 exp Biocompatible Materials/ (60214)
20 Tissue Scaffolds/ (4230)
21 Mesenchymal Stem Cells/ (9092)
22 exp Transforming Growth Factor beta/ (37624)
23 critical size defect*.mp. (279)
24 segmental bone defect*.mp. (289)
25 osteochondral defect*.mp. (733)
26 Cartilage, Articular/in, su, tr [Injuries, Surgery, Transplantation] (4445)
27 Transplantation, Homologous/ (69825)
28 or/17-27 (204825)
29 12 and 28 (1288)
30 14 and 29 (71)
31 4 and 30 (0)
32 osteotomy.mp. (27732)
33 30 and 32 (8)
34 8 or 11 (31317)
35 28 and 34 (3062)
36 32 and 35 (295)
37 14 and 36 (12)
38 14 and 28 and 34 (148)
39 4 and 38 (0)
40 4 and 28 and 34 (1)
41 limit 38 to yr="2010 - 2012" (10)
42 13 or 16 or 30 or 33 or 37 or 38 (190)
43 limit 42 to yr="2010 -Current" (12)

****

6. Surgical procedures on the non-rodent mammals covered by this protocol have been discussed with

Dr. Alexander Romanov

Attachments

Document	File Name	Date Attached
UPDATED LITERATURE SEARCH	Animal_Protocol_Search_Jan_2012.txt	01/27/2012 at 10:50
PREVIOUS LITERATURE SEARCH	Animal_Protocol_Search_Sept23_2010.pdf	12/12/2011 at 11:32
Ms Jo Ann Henry approval of animal transport	Re_ Fw_ Protocol AAAB7357_Jo_Ann_Henry.txt	12/12/2011 at 11:32

Signature Approval

I am aware of, understand and will follow the ILAR Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Regulations administered by the United States Department of Agriculture.

I understand that these laws and regulations are applicable to all biomedical research projects using animals that are funded through and administered by Columbia University Health Sciences. As required by the Animal Welfare Act regulations, I hereby assure the IACUC that this experiment does not unnecessarily duplicate previous experiments.

Furthermore, I will obtain the approval of the IACUC for any significant changes in the experiment before they are implemented. I certify that the statements herein are true, complete and accurate to the best of my knowledge.

I am aware that any false statements or departures from the approved procedures may subject me to administrative penalties that include suspension of my animal-based research (AWA 9CFR, ch.1, sect. 2.31, par.8 and PHS Policy, document 94-2).

I also certify that the experiments described in this protocol faithfully reflect the work proposed in the sponsored project(s) identified in this application. I have given each person listed in this protocol a copy of the protocol to read.

Electronically Signed and Submitted by Francis Lee (fl127) on 01/27/2012.

Appendix 2 – Modification 1 of Year 2 Protocol

Summary: no animals or changes to the experimental protocol were requested, only personnel were added to the protocol

Columbia University Animal Care Protocol Modification Data Sheet

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Protocol: AC-AAAB7357 (Y2 M01)

Protocol Status: Approved (Done)

Approval Date: 03/23/2012 at 00:00

Name: Francis Lee (fl127)

Faculty Title: Associate Professor

Title: Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma

Species:	Dog	Effective Date:	03/23/2012
Number of Animals (Previous):	6 (6)	Expiration Date:	02/10/2013
Pain Levels:	D	Inspection Date:	

Originating Department:	ORTHOPEDIC SURGERY	Previous Protocol Number:	AC-n/a
Protocol Type:	Research	Protocol Submitted to:	Health Sciences

Modifications

Staff/Personnel

Funding

Has ARRA Funding:	N	Funded by Department:	N	Funded Through Private Gift: N

Awarded Institution	External Funding Type	Source Identifier	External Funding Agency	Proposal Number
	Government Grant	OR090175	Department of Defense	PT-AAAH0521

Protocol Staff

Personnel	Role	1+ yrs Exp W/ Species	Proc. Exp	Species Specific Training.	Wet Lab Date	OHP Exp Date	Regulatory Lecture Date
Lee, Francis (fl127) phone: 212-305-3293 cell: 201-248-1630	Principal Investigator	yes	yes	07/07/2010		06/28/2010	

Procedure Experience:

1) - Orthopedic surgery

2) 25years of experience as an orthopaedic and oncology surgeon, and more than 5

		years of experience with survival surgeries in dogs. Dr.Lee will be the surgeon in charge on this protocol performing both control and experimental procedures. -			
Akelina, Yelena (ya67)	Staff Researcher	yes	yes	05/17/2007	05/16/1997
Procedure Experience:					
		1) Dr. Akelina has approx 4 years experience in chronic studies in dogs involving orthopedic procedures. She will coordinate all surgeries, post-op care and all data collection and analysis. Dr. Akelina is an Associate Research Scientist - Orthopedic Surgery			
Nizami, Saqib (sn2452)	Staff Researcher	no	yes	07/07/2010	11/04/2010
Procedure Experience:					
		1) Saqib Nizami is a technician and will be trained to handle dogs. - Orthopedic surgery			
Oh, Daniel (dso2113)	Lead Researcher	yes	yes	02/16/2012	02/22/2012
Procedure Experience:					
		1) Dr. Oh has more than 10 years experience in scaffold research and design. He has performed similar experiments in dogs at his previous institution. - Dr. Oh is an assistant professor of orthopaedic surgery who has a Ph.D. in materials science. He will assist with the scaffold design			
Training Courses:		TC0650 - The Dog: Computer Based Training Program 02/16/2012			
		TC0800 - The Mouse and Rat: Computer Based Training 02/16/2012			
		TC0850 - The Rabbit: Computer Based Training Program 02/16/2012			
Bai, Hanying (hb2375)	Staff Researcher	no	yes	02/16/2012	08/05/2011
Procedure Experience:					
		1) Dr. Bai will be training in the biomechanical testing procedures by Mr. Gardner,			

		who will oversee all biomechanical testing. - Dr. Bai is a post-doctoral research fellow who will perform the biomechanical testing of the canine limbs following sacrifice under the supervision of Mr. Gardner			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			02/16/2012
Chandhanayingyong, Chandhanarat (cc3391)	Staff Researcher	no	yes	12/07/2011	10/19/2011
Procedure Experience:					
		1) Dr. Chanhanayingyong has extensive experience with human orthopaedic surgies. Dr. Francis Lee will oversee and instruct Dr. Chanhanayingyong in the canine orthopaedic procedures. - Dr. Chandhanayingyong is a visiting orthopaedic surgeon from Thailand. She will assist Dr. Francis Lee in all the canine surgeries.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/07/2011
Vorys, George (gcv2101)	Staff Researcher	no	yes	02/23/2011	02/17/2011
Procedure Experience:					
		1) Dr. Vorys is a PGY3 orthopaedic resident who has some experience with human orthopaedic procedures. He will be overseen and instructed in the canine orthopaedic procedures by Dr. Lee. - Dr. Vorys is an orthopaedic surgery resident who will assist Dr. Lee in the canine segmental defect and osteochondral repairs.			
Kweon, Suc (sk3573)	Staff Researcher	no	yes	12/13/2011	10/14/2011
Procedure Experience:					
		1) Dr. Kweon has extensive experience in tibial and segmental defects in humans. He will assist and be overseen during the surgeries by Dr. Lee. - Dr. Kweon is a visiting orthopaedic surgeon from Korea. He will assist Dr. Lee in the canine segmental and tibial defect repairs.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/13/2011

Song, Wongseok (ws2353)	Staff Researcher	no	yes	12/13/2011	10/27/2011	
Procedure Experience:						
		1) Dr. Song has extensive experience in segmental and tibial defect repairs in humans. He will assist and be overseen by Dr. Lee during the canine orthopaedic procedures. - Dr. Song is a visiting orthoapedic surgeon from Korea. He will assist Dr. Lee with the canine segmental and tibial defect repairs.				
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/13/2011	
Mao, Jeremy (jm2654)	Lead Researcher	yes	yes	01/05/2010	03/04/2011	12/06/2011
Procedure Experience:						
		1) Dr Mao has extensive experience in small animal handling and will be trained to handle dogs. Dr. Mao will be responsible and provide overall supervision. He will assist in surgery, post surg. monitoring and tissue harvesting. - Orthopedic Surgery				
Gardner, Thomas (trg1)	Lead Researcher	yes	yes	02/15/2012	05/26/2010	
Procedure Experience:						
		1) Mr. Gardner is an Engineer and Assoc. Director of the Biomechanic lab. He will be responsible for helping with the coordination of project, participate in post-op care, oversee all biomechanical testing and data analysis. - Orthopedic Surgery and biomechanical testing.				
Training Courses:		TC0506 - Macacine Herpes Virus-1 (aka Herpes B Virus) Training			05/22/2012	
		TC0650 - The Dog: Computer Based Training Program			02/15/2012	
		TC1000 - The Ruminant: Computer Based Training			03/08/2012	

Administrative Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-	201-248-		fl127@columbia.edu

	3293	1630		
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Emergency Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Locations

Housing Location	# of Animals
BB 18	6

Satellite Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Exp.Procedure Location	Proc. Type	Duration (hrs)	Responsible Person
Black Building, 18, CSS, -	Survival Surgery	4	Francis Lee (fl127)
Neurological Institute, Basement, Hatch Center	No Surgery	2	Francis Lee (fl127)

Post Op Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Drugs

Anesthetic or	Dosage	Route	Responsible Person

Analgesic

CARPOFEN	4 MG/KG	PI, IM, SC	ICM (Institute of Comparative Medicine)
MORPHINE	0.1 MG/KG	EPIDURAL	ICM (Institute of Comparative Medicine)
DIAZAEPAM	0.2-0.5 MG/KG	Intravenous	ICM (Institute of Comparative Medicine)
Propofol	2-5mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Hydromorphone	0.025-0.05 mg/kg PRE-SURGERY & EVERY 4-8 hrs POST-SURGERY	IM, SC, IV	ICM (Institute of Comparative Medicine)
Fentanyl	50 mcg/hr FOR 10-20KG DOGS OR 75 MCG/HR FOR 20-30 KG DOGS	transdermal patch	ICM (Institute of Comparative Medicine)
Pentobarbital	100mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Acepromazine	0.025-0.05 MG/KG	IV, IM, SQ	ICM (Institute of Comparative Medicine)
MARCAINE	0.25%	Subcutaneous	ICM (Institute of Comparative Medicine)
Isoflurane	1-5%	Inhalation	ICM (Institute of Comparative Medicine)
METHOHEXITAL	4 11 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)

Other Drug or Substances	Dosage	Route	Responsible Person
CEPHALEXIN	30 MG/KG TWICE DAILY	Oral	ICM (Institute of Comparative Medicine)
Cefazolin	10-30 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)

TGF-b3	10 nanograms/ml	osteochondral scaffold will be infused/coated with this material	Mao, Jeremy (jm2654)
BMP-2	160 micrograms per scaffold	segmental defect scaffold will be infused with this material	Mao, Jeremy (jm2654)
CEFPODIXIME (AS AN ALTERNATIVE TO CEPHAFEXIN)	5-10 MG/KG	PO SID	ICM (Institute of Comparative Medicine)

Hazardous Materials

No hazardous materials used.

Scientific Questions

Modification Questions - Increase in the Number of Animals Only

A1. Brief Progress Report.

No live surgeries have yet been performed. Dr. Romanov of the ICM was visited to work out proper procedures pre, during the operation and post-op for the dogs. Practice surgies have been performed on SawBones models of the dog leg.

A2. Justification for Additional Animals

n/a

Change in Planned Use of the Animals

B1. Description of Changes to Protocol

The only requested modification to this protocol is to add the following researchers: Dr. Daniel Oh, Dr. Hanying Bai, Dr. George Vorys, Dr. C. Chanhanayingyong, Dr. S. Kweon, Dr. S. Song.

B2. Number and Justification for Additional Animals Requested

n/a

B3. Number of Previously Approved Animals in New Procedures 6

B4. Discussion of Less Stressful Alternatives

The protocol was just renewed and included an updated literature review, which found that there are no less stressful alternatives to accomplish the scientific aims of this study.

Attachments

Document	File Name	Date Attached
PREVIOUS LITERATURE SEARCH	Animal_Protocol_Search_Sept23_2010.pdf	03/22/2012 at 10:52
UPDATED LITERATURE SEARCH	Animal_Protocol_Search_Jan_2012.txt	03/22/2012 at 10:52
Ms Jo Ann Henry approval of animal transport	Re_ Fw_ Protocol AAAB7357_Jo_Ann_Henry.txt	03/22/2012 at 10:52

Signature Approval

I am aware of, understand and will follow the ILAR Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Regulations administered by the United States Department of Agriculture.

I understand that these laws and regulations are applicable to all biomedical research projects using animals that are funded through and administered by Columbia University Health Sciences. As required by the Animal Welfare Act regulations, I hereby assure the IACUC that this experiment does not unnecessarily duplicate previous experiments.

Furthermore, I will obtain the approval of the IACUC for any significant changes in the experiment before they are implemented. I certify that the statements herein are true, complete and accurate to the best of my knowledge.

I am aware that any false statements or departures from the approved procedures may subject me to administrative penalties that include suspension of my animal-based research (AWA 9CFR, ch.1, sect. 2.31, par.8 and PHS Policy, document 94-2).

I also certify that the experiments described in this protocol faithfully reflect the work proposed in the

sponsored project(s) identified in this application. I have given each person listed in this protocol a copy of the protocol to read.

Electronically Signed and Submitted by Francis Lee (fl127) on 03/22/2012.

Appendix 3 – Modification 2 of Year 2 Protocol

Summary: This modification requested an additional dog to replace Segmental Defect Dog 3 which was sacrificed prematurely due to an adverse event (see Appendix 4) which was due to the bending of the steel locking plate and a fractured fibula. In addition, the use of sling-walking for the first 3 days post-op on a non-slippery floor was also added to the protocol. In addition, the use of fluoroscopy and the taking of intraoperative photographs was also formally requested.

Columbia University Animal Care Protocol Modification Data Sheet

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Protocol: AC-AAAB7357 (Y2 M02)

Protocol Status: Approved (Done)

Approval Date: 08/20/2012 at 00:00

Name: Francis Lee (fl127)

Faculty Title: Associate Professor

Title: Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma

Species:	Dog	Effective Date:	08/20/2012
Number of Animals (Previous):	7 (6)	Expiration Date:	02/10/2013
Pain Levels:	D	Inspection Date:	
Originating Department:	ORT Ctr for Orthopaedic Rsch	Previous Protocol Number:	AC-n/a
Protocol Type:	Research	Protocol Submitted to:	Health Sciences

Modifications

Animal Numbers

Procedures

Staff/Personnel

Hazardous Materials

Funding

Has ARRA Funding:	N	Funded by Department:	N	Funded Through Private Gift:	N

Awarded Institution	External Funding Type	Source Identifier	External Funding Agency	Proposal Number
	Government Grant	OR090175	Department of Defense	PT-AAAH0521

Protocol Staff

Personnel	Role	1+ yrs Exp W/ Species	Proc. Exp	Species Specific Training.	Wet Lab Date	Introduction to ICM	OHP Exp Date	Regulatory Lecture Date
Lee, Francis (fl127) phone: 212-305-3293 cell: 201-248-1630 pager:	Principal Investigator	yes	yes	07/07/2010			06/28/2010	

Procedure Experience:

1) 25years of experience as an orthopaedic and oncology surgeon, and more than 5 years of experience with survival surgeries in dogs. Dr.Lee will be the surgeon in charge on this protocol performing both control and experimental procedures. -

2) - Orthopedic surgery

Gardner, Thomas (trg1)	Lead Researcher	yes	yes	02/15/2012			05/26/2010	

Procedure Experience:

1) Mr. Gardner is an Engineer and Assoc. Director of the Biomechanic lab. He will be responsible for helping with the coordination of project, participate in post-op care, oversee all biomechanical testing and data analysis. - Orthopedic Surgery and biomechanical testing.

Training Courses:

TC0506 - Macacine Herpes Virus-1 (aka Herpes B Virus) Training	05/22/2012
TC0650 - The Dog: Computer Based Training Program	02/15/2012
TC1000 - The Ruminant: Computer Based Training	03/08/2012

Mao, Jeremy (jm2654)	Lead Researcher	yes	yes	01/05/2010	03/04/2011	12/06/2011

Procedure Experience:

1) Dr Mao has extensive experience in small animal handling and will be trained to handle dogs. Dr. Mao will be responsible and provide overall supervision. He will assist in surgery, post surg. monitoring and tissue harvesting. - Orthopedic Surgery

Chandhanayingyong, Chandhanarat (cc3391)	Staff Researcher	no	yes	12/07/2011	10/19/2011	

Procedure Experience:

1) Dr. Chanhanayingyong has extensive experience with human orthopaedic surgies. Dr. Francis Lee will oversee and instruct Dr. Chanhanayingyong in the canine orthopaedic procedures. - Dr. Chandhanayingyong is a visiting orthopaedic surgeon from Thailand. She will assist Dr. Francis Lee in all the canine surgeries.

Training Courses:

TC0650 - The Dog: Computer Based Training Program	12/07/2011

Bai, Hanying (hb2375)	Staff Researcher	no	yes	02/16/2012	08/05/2011	

Procedure Experience:

1) Dr. Bai will be training in the biomechanical testing procedures by Mr. Gardner, who will oversee all biomechanical testing. - Dr. Bai is a post-doctoral research fellow who will perform the biomechanical testing of the canine limbs following sacrifice under the supervision of Mr. Gardner

Training Courses:		TC0650 - The Dog: Computer Based Training Program			02/16/2012
Kweon, Suc (sk3573)	Staff Researcher	no	yes	12/13/2011	10/14/2011
Procedure Experience:					
		1) Dr. Kweon has extensive experience in tibial and segmental defects in humans. He will assist and be overseen during the surgeries by Dr. Lee. - Dr. Kweon is a visiting orthopaedic surgeon from Korea. He will assist Dr. Lee in the canine segmental and tibial defect repairs.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/13/2011
Nizami, Saqib (sn2452)	Staff Researcher	no	yes	07/07/2010	11/04/2010
Procedure Experience:					
		1) Saqib Nizami is a technician and will be trained to handle dogs. - Orthopedic surgery			
Vorys, George (gcv2101)	Staff Researcher	no	yes	02/23/2011	02/17/2011
Procedure Experience:					
		1) Dr. Vorys is a PGY3 orthopaedic resident who has some experience with human orthopaedic procedures. He will be overseen and instructed in the canine orthopaedic procedures by Dr. Lee. - Dr. Vorys is an orthopaedic surgery resident who will assist Dr. Lee in the canine segmental defect and osteochondral repairs.			
Oh, Daniel (dso2113)	Lead Researcher	yes	yes	02/16/2012	02/22/2012
Procedure Experience:					
		1) Dr. Oh has more than 10 years experience in scaffold research and design. He has performed similar experiments in dogs at his previous institution. - Dr. Oh is an assistant professor of orthopaedic surgery who has a Ph.D. in materials science. He will assist with the scaffold design			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			02/16/2012
		TC0800 - The Mouse and Rat: Computer			02/16/2012

		Based Training				
		TC0850 - The Rabbit: Computer Based Training Program				02/16/2012
Akelina, Yelena (ya67)	Staff Researcher	yes	yes	05/17/2007		05/16/1997

Procedure Experience:

1) Dr. Akelina has approx 4 years experience in chronic studies in dogs involving orthopedic procedures. She will coordinate all surgeries, post-op care and all data collection and analysis. Dr. Akelina is an Associate Research Scientist - Orthopedic Surgery

Administrative Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Emergency Contacts	Phone	Cell Phone	Pager	Email
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Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Locations

Housing Location	# of Animals
BB 18	6

Satellite Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Exp.Procedure Location	Proc. Type	Duration (hrs)	Responsible Person
Black Building, 18, CSS, -	Survival Surgery	4	Francis Lee (fl127)
Neurological Institute, Basement, Hatch Center	No Surgery	2	Francis Lee (fl127)

Post Op Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Drugs

Anesthetic or Analgesic	Dosage	Route	Responsible Person
Fentanyl	50 mcg/hr FOR 10-20KG DOGS OR 75 MCG/HR FOR 20-30 KG DOGS	transdermal patch	ICM (Institute of Comparative Medicine)
Pentobarbital	100mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Acepromazine	0.025-0.05 MG/KG	IV, IM, SQ	ICM (Institute of Comparative Medicine)
MARCAINE	0.25%	Subcutaneous	ICM (Institute of Comparative Medicine)
Isoflurane	1-5%	Inhalation	ICM (Institute of Comparative Medicine)
METHOHEXITAL	4 11 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
CARPOFEN	4 MG/KG	PI, IM, SC	ICM (Institute of Comparative Medicine)

MORPHINE	0.1 MG/KG	EPIDURAL	ICM (Institute of Comparative Medicine)
DIAZAEPAM	0.2-0.5 MG/KG	Intravenous	ICM (Institute of Comparative Medicine)
Propofol	2-5mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Hydromorphone	0.025-0.05 mg/kg PRE-SURGERY & EVERY 4-8 hrs POST-SURGERY	IM, SC, IV	ICM (Institute of Comparative Medicine)

Other Drug or Substances	Dosage	Route	Responsible Person
Cefazolin	10-30 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
TGF-b3	10 nanograms/ml	osteochondral scaffold will be infused/coated with this material	Mao, Jeremy (jm2654)
BMP-2	160 micrograms per scaffold	segmental defect scaffold will be infused with this material	Mao, Jeremy (jm2654)
CEFPODIXIME (AS AN ALTERNATIVE TO CEPHAFEXIN)	5-10 MG/KG	PO SID	ICM (Institute of Comparative Medicine)
CEPHALEXIN	30 MG/KG TWICE DAILY	Oral	ICM (Institute of Comparative Medicine)

Hazardous Materials

X-Ray, Fluoroscope: Y

Scientific Questions

Modification Questions -

Increase in the Number of Animals Only

A1. Brief Progress Report.

Three dogs were operated on for tibial segmental defects. A critical size defect was created, a scaffold implant was placed in the defect, and a Synthes veterinary LCP plate was used to support the defect in each of the dogs. The 1st two dogs on which the procedure was performed are doing well. They are ambulating in their cages and can stand up on their hind legs. Unfortunately, the 3rd dog had complications and was sacrificed 5 days following surgery. The Adverse Event report from ICM WAS SUBMITTED VIA IACUC AS AN ADVERSE EVENT. The purpose of this modification is to request an addition dog to replace the 3rd segmental defect dog. The review of the adverse event and plans to avoid this problem in the subsequent dogs is given in the response to the animal number justification question below.
This protocol originally approved 6 dogs, 3 for segmental defect repair and 3 for osteochondral defect repairs. The experimental approach and design of the osteochondgral defect implant is underway, utilizing cadaveric dog limbs obtained under a different protocol that also permitted imaging of the cadaveric dog limbs. Once the implant design and surgical approach is finalized, we plan to perform the osteochondral defect procedures with the live dogs, probably sometime in August, 2012.

A2. Justification for Additional Animals

See responses to questions below.

Change in Planned Use of the Animals

B1. Description of Changes to Protocol

Staff - Dr. Wongseok Song, a visiting orthopaedic surgeon from Korea has been removed from the protocol since he as left the lab and returned to Korea. Drs. Kweon and Chandhanayingyong, both also orthopaedic surgeons, remain on the protocol to assist Dr. Lee with the dog surgeries.
Hazardous Materials - in addition to the X-rays and MR images that are already approved in the protocol, it is requested that the use of fluoroscopy during the surgical procedures in the ICM operating room be permitted.
Procedures - we would permission to take still photos of the operative field only during the surgical procedures to better document what was actually done. All photographs will be limited to the operative field only, with no pictures of the entire dog, or of any personnel or of the operative room.
Surgical Procedure - 2 problems were encountered with the 3 segmental defect dog; a)a localized infection at the surgery site and, most importantly b)bending of the fixation device. It cannot be determined from the Adverse Event report what was the cause of the infection. The source of the infection

could have been manyfold. No modification of the surgical procedure is planned for this problem. The operative will continue to practice sterile technique and be ever more vigilant about all details related to maintaining a sterile field. The reason for early termination of the animal was the bending of the fixation plate. After examination of the immediate post-op x-rays, and following dissection of the dog limb, as well as comparison to the same data from the first 2 dogs which had no complications from the same procedure and are doing fine, several reasons are postulated for the bending of the plate and resultant fracture of the fibula. Compared to the first 2 dogs, the placement of the 3rd implant was not ideal. There was more difficulty in implant placement during the surgery, AND from PHOTOS OF THE FULLY EXPOSED surgical site OF THE FAILED REPAIR WHICH WERE TAKEN DURING THE NECROPSY, it appears that the cortical area of the implant was not precisely aligned with the bone. This resulted in only part of the implant proximal and distal ends supporting the adjacent bone. It is postulated that this less than perfect alignment of the implant with the adjacent bone resulted in greater stress on the implant because less surface area of the implant was supporting the bone than what it was designed for. The resultant higher localized stress caused the adjacent bone edges to "cut" or subside into the implant. This subsidence produced 2 effects, first, it shortened the distance between the bone edges opposite the plate, causing the plate to bend; secondly, it effectively pushed the implant towards the plate, creating a 3-point bending condition on the plate, exacerbating the bending condition. As the tibia+plate construct bent, it overloaded the fibula, resulting it is fracture. It should be noted that the implant (scaffold) itself showed no signs of bending or collapse. Rather, the bone "cut" its way into the scaffold, with the scaffold maintaining its original height.

AS PER IACUC REQUIREMETNS, THIS MODIFICATION WAS SUBMITTED FOR REVIEW TO THE ICM ON JULY 23, 2012 AND REVIEWED BY DR. ANDREA SLATE. HER SUGGESTED MODIFICATIONS ARE INCORPORATED BELOW IN CAPS.

Proposed changes to surgical procedure/protocol to prevent this problem from reoccuring - the following changes are planned based on discussion with the ICM staff and the PI's team of orthopaedic surgeons and engineers:
a)more care will be taken during the procedure to ensure that the scaffold and native bone are precisely aligned;
b) FOR A MINIMUM OF 3 DAYS POST OPERATIVELY, THE DOG WILL BE WALKED WITH THE SUPPORT OF A SLING AND/OR WILL BE CONFINED TO A SMALL AREA (SUCH AS A CUBICLE LOCATED IN THE ANIMAL ICU) WITH NON-SLIPPERY FOOTING. THE DOG WILL BE OBSERVED AT ALL TIMES WHEN OUT OF ITS CAGE FOR AT LEAST THE FIRST 7 DAYS AND WILL BE PROVIDED NON-SLIPPERY FOOTING DURING THIS TIME. THE DOG WILL BE

RETURNED TO THE STANDARD EXERCISE PROGRAM AFTER A DISCUSSION BETWEEN THE ICM VETERINARIAN AND THE RESEARCHERS.

c)light-weight splintING MATERIAL MAY be incorporated into the post-surgical wrapping. This splint will be removed, at the latest, prior to the 2 week x-ray point so that it will not degrade the 2nd week x-rays.

B2. Number and Justification for Additional Animals Requested

Note: 6 dogs were originally approved; 3 for critical size defect repairs, and 3 for the osteochondral repairs. 3 of the critical size defect dogs have been used. None of the osteochondral dogs have been used. We are asking to increase the total number of dogs from 6 to 7 so that the additional dog can be used to replace the 3rd critical size defect dog, which needed to be sacrificed early. This will allow us to have the 3 critical size segmental defect dogs that were originally required as pilots before the full study number would be approved.

B3. Number of Previously Approved Animals in New Procedures 7

B4. Discussion of Less Stressful Alternatives

An updated review of the literature was performed for the recent continuation of this protocol (February, 2012), which found that no less stressful alternatives have been reported in the literature.

Signature Approval

I am aware of, understand and will follow the ILAR Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Regulations administered by the United States Department of Agriculture.

I understand that these laws and regulations are applicable to all biomedical research projects using animals that are funded through and administered by Columbia University Health Sciences. As required by the Animal Welfare Act regulations, I hereby assure the IACUC that this experiment does not unnecessarily duplicate previous experiments.

Furthermore, I will obtain the approval of the IACUC for any significant changes in the experiment before they are implemented. I certify that the statements herein are true, complete and accurate to the best of my knowledge.

I am aware that any false statements or departures from the approved procedures may subject me to administrative penalties that include suspension of my animal-based research (AWA 9CFR, ch.1, sect. 2.31, par.8 and PHS Policy, document 94-2).

I also certify that the experiments described in this protocol faithfully reflect the work proposed in the

sponsored project(s) identified in this application. I have given each person listed in this protocol a copy of the protocol to read.

Electronically Signed and Submitted by Francis Lee (fl127) on 08/20/2012.

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Appendix 4 – Adverse Event Report for Protocol AAAB7357

Summary: This is the adverse event report generated for the early sacrifice for Segmental Defect Dog 3 due to a bent internal fixation plate and fracture fibula. Modification 2 of the protocol was requested based on this report (see Appendix 3).

Columbia University IACUC Adverse Event Report

Requested Effective date: 09/21/2012

Date created: 07/23/2012

Principal Investigator: Lee,Francis

Initiator: Gardner,Thomas

Protocol Number: IC-AAAB7357

Species: Dog

Number of Animals Approved: 7

Protocol Year: 2

Title: Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma

USDA Animal Number: 869155

Date of event: 06/08/2012

Date reported: 07/27/2012

Status of Animal Subject: Was Euthanized

Description of event, treatment and outcome:

Adverse Event Report

ICM Clinicians: Drs. Romanov/Baker

Animal #: 869155

Species: dog

Sex: intact male

Investigator: Lee

Protocol: AAAB7357

Date: 6/8/12

Clinical and experimental history:

The dog #869155 arrived to ICM from Marshall Farms animal facility on 5/30/12. The initial physical exam was normal, and no abnormalities were found on CBC and chemistry blood test.

On June 4, 2012, dog #860155 underwent a right tibial osteotomy and graft implantation, as per protocol. The anesthesia induction, surgery, and immediate post-operative recovery were uneventful. The dog continued to receive an intensive post-operative care in ICU overnight.

This included observations every two hours, intensive pain management regimen, antibiotic, gastrointestinal support medications, intravenous fluid infusion, and daily bandage changes.

The dog appeared to be recovering as expected after surgery, with moderate weight bearing on the operated limb. However, he had been lacking appetite after surgery and vomited small amounts of bile several times. Additional GI support medications and hand feeding were administered. On 6/6/12, the incision site was found moderately swollen during the bandage change. Wound infection and cellulitis were suspected by attending veterinarian. Additional pain medication and fluid support were administered, and compressing leg bandage applied. On post-op day 4, there was a minimal right hind leg weight bearing, the incision site was eryhtematous, swollen, and hot to touch.

The treatment with additional antibiotic was initiated, and the animal's condition discussed at length with the lab. On the morning of 6/8/12, it was noticed that there was excessive flexion of the stifle, no weight bearing, and lateral deviation of the tibia. The dog was sedated, and radiographs of the right hind leg were taken. Radiographs showed the bend stabilization plate, fractured fibula, and deformed osteotomy site. After consultation with the lab, the dog was euthanized with overdose of euthanasia solution by the attending veterinarian. Immediate gross necropsy was performed (findings listed below). The operated leg was collected by the lab for further ex-vivo examination. Representative samples of GI tract were submitted to outside laboratory for histological evaluation.

Gross necropsy findings:

Post mortem interval: immediate

Autolysis: none

General: the body was in good condition with some body fat

Integument: the area around incision on right hind leg was erythematous and diffusely thickened. The incision was still closed and there was no discharge observed.

Cardiovascular: no abnormalities

Respiratory: no abnormalities

Gastrointestinal: large volume (approx. 200ml) of green fluid emitted from mouth following euthanasia. Stomach was empty. Multiple small (approx 2mm diameter) erythematous foci were found on glandular part of gastric mucosa. Duodenal mucosa was diffusely erythematous. Jejunum and colon were within normal limits. Small amount of partially formed feces were found in distal colon and rectum.

Liver: no abnormalities

Endocrine system: no abnormalities

Urinary system: no abnormalities

Spleen: no abnormalities

Necropsy Performed: Y

Results of clinical tests are pending and an additional report to the IACUC will follow:N

Is there a proposed change to the protocol as a result of the adverse event?: Y

(If a change is proposed, submit a modification to the protocol through RASCAL).

Signature

Electronic Signature: Francis Lee (fl127) - Principal Investigator Date: 07/27/2012

Appendix 5 – Modification 3 of Year 2 Protocol

Summary: The only changes to the protocol for this modification were the addition of the following medications: Dexmedetomidine, Butorphanol, Atipemazole. This modification was made at the request of Columbia University's Institute for Comparative Medicine, which is responsible for the care of all research animals at Columbia University.

Columbia University Animal Care Protocol Modification Data Sheet

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Protocol: AC-AAAB7357 (Y2 M03)

Protocol Status: Approved (Done)

Approval Date: 09/21/2012 at 00:00

Name: Francis Lee (fl127)

Faculty Title: Associate Professor

Title: Ready-to-Use Tissue Construct for Military Bone and Cartilage Trauma

Species:	Dog	Effective Date:	09/21/2012
Number of Animals (Previous):	7 (7)	Expiration Date:	02/10/2013
Pain Levels:	D	Inspection Date:	
Originating Department:	ORT Ctr for Orthopaedic Rsch	Previous Protocol Number:	AC-n/a
Protocol Type:	Research	Protocol Submitted to:	Health Sciences

Modifications

Drugs

Funding

Has ARRA Funding: N		Funded by Department: N		Funded Through Private Gift: N
Awarded Institution	External Funding Type	Source Identifier	External Funding Agency	Proposal Number
	Government Grant	OR090175	Department of Defense	PT-AAAH0521

Protocol Staff

Personnel	Role	1+ yrs Exp W/ Species	Proc. Exp	Species Specific Training.	Wet Lab Date	Introduction to ICM	OHP Exp Date	Regulatory Lecture Date
Lee, Francis (fl127) phone: 212-305-3293 cell: 201-248-1630 pager:	Principal Investigator	yes	yes	07/07/2010			06/28/2010	

Procedure Experience:

1) - Orthopedic surgery

2) 25years of experience as an orthopaedic and oncology surgeon, and more than 5 years of experience with survival surgeries in dogs. Dr.Lee will be the surgeon in charge on this protocol performing both control and experimental procedures. -

Akelina, Yelena (ya67)	Staff Researcher	yes	yes	05/17/2007			05/16/1997	

Procedure Experience:

1) Dr. Akelina has approx 4 years experience in chronic studies in dogs involving orthopedic procedures. She will coordinate all surgeries, post-op care and all data collection and analysis. Dr. Akelina is an Associate Research Scientist - Orthopedic Surgery

Nizami, Saqib (sn2452)	Staff Researcher	no	yes	07/07/2010			11/04/2010	

Procedure Experience:

1) Saqib Nizami is a technician and will be trained to handle dogs. -

		Orthopedic surgery			
Oh, Daniel (dso2113)	Lead Researcher	yes	yes	02/16/2012	02/22/2012
Procedure Experience:					
		1) Dr. Oh has more than 10 years experience in scaffold research and design. He has performed similar experiments in dogs at his previous institution. - Dr. Oh is an assistant professor of orthopaedic surgery who has a Ph.D. in materials science. He will assist with the scaffold design			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			02/16/2012
		TC0800 - The Mouse and Rat: Computer Based Training			02/16/2012
		TC0850 - The Rabbit: Computer Based Training Program			02/16/2012
Bai, Hanying (hb2375)	Staff Researcher	no	yes	02/16/2012	08/05/2011
Procedure Experience:					
		1) Dr. Bai will be training in the biomechanical testing procedures by Mr. Gardner, who will oversee all biomechanical testing. - Dr. Bai is a post-doctoral research fellow who will perform the biomechanical testing of the canine limbs following sacrifice under the supervision of Mr. Gardner			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			02/16/2012
Chandhanayingyong, Chandhanarat (cc3391)	Staff Researcher	no	yes	12/07/2011	10/19/2011
Procedure Experience:					
		1) Dr. Chanhanayingyong has extensive experience with human orthopaedic surgies. Dr. Francis Lee will oversee and instruct Dr. Chanhanayingyong in the canine orthopaedic procedures. - Dr. Chandhanayingyong is a visiting orthopaedic surgeon from Thailand. She will assist Dr. Francis Lee in all the canine surgeries.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/07/2011

Vorys, George (gcv2101)	Staff Researcher	no	yes	02/23/2011		02/17/2011
Procedure Experience:						
		1) Dr. Vorys is a PGY3 orthopaedic resident who has some experience with human orthopaedic procedures. He will be overseen and instructed in the canine orthopaedic procedures by Dr. Lee. - Dr. Vorys is an orthopaedic surgery resident who will assist Dr. Lee in the canine segmental defect and osteochondral repairs.				
Kweon, Suc (sk3573)	Staff Researcher	no	yes	12/13/2011		10/14/2011
Procedure Experience:						
		1) Dr. Kweon has extensive experience in tibial and segmental defects in humans. He will assist and be overseen during the surgeries by Dr. Lee. - Dr. Kweon is a visiting orthopaedic surgeon from Korea. He will assist Dr. Lee in the canine segmental and tibial defect repairs.				
Training Courses:		TC0650 - The Dog: Computer Based Training Program				12/13/2011
Mao, Jeremy (jm2654)	Lead Researcher	yes	yes	01/05/2010	03/04/2011	12/06/2011
Procedure Experience:						
		1) Dr Mao has extensive experience in small animal handling and will be trained to handle dogs. Dr. Mao will be responsible and provide overall supervision. He will assist in surgery, post surg. monitoring and tissue harvesting. - Orthopedic Surgery				
Gardner, Thomas (trg1)	Lead Researcher	yes	yes	02/15/2012		05/26/2010
Procedure Experience:						
		1) Mr. Gardner is an Engineer and Assoc. Director of the Biomechanic lab. He will be responsible for helping with the coordination of project, participate in post-op care, oversee all biomechanical testing and data analysis. - Orthopedic Surgery and biomechanical testing.				
Training Courses:		TC0506 - Macacine Herpes Virus-1 (aka Herpes B Virus) Training				05/22/2012
		TC0650 - The Dog: Computer Based				02/15/2012

Training Program

TC1000 - The Ruminant: Computer Based Training 03/08/2012

Administrative Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Emergency Contacts	Phone	Cell Phone	Pager	Email
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Akelina, Yelena (ya67)	212-305-0992	845-536-2737	845-536-2737	ya67@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965	201-541-9757		kmk2148@columbia.edu

Locations

Housing Location	# of Animals
BB 18	6

Satellite Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Exp.Procedure Location	Proc. Type	Duration (hrs)	Responsible Person

Black Building, 18, CSS, -	Survival Surgery	4	Francis Lee (fl127)
Neurological Institute, Basement, Hatch Center	No Surgery	2	Francis Lee (fl127)

Post Op Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Drugs

Anesthetic or Analgesic	Dosage	Route	Responsible Person
Fentanyl	50 mcg/hr FOR 10-20KG DOGS OR 75 MCG/HR FOR 20-30 KG DOGS	transdermal patch	ICM (Institute of Comparative Medicine)
Pentobarbital	100mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
Acepromazine	0.025-0.05 MG/KG	IV, IM, SQ	ICM (Institute of Comparative Medicine)
MARCAINE	0.25%	Subcutaneous	ICM (Institute of Comparative Medicine)
Isoflurane	1-5%	Inhalation	ICM (Institute of Comparative Medicine)
METHOHEXITAL	4 - 11 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
CARPOFEN	4 MG/KG	PI, IM, SC	ICM (Institute of Comparative Medicine)
MORPHINE	0.1 MG/KG	EPIDURAL	ICM (Institute of Comparative Medicine)
DIAZAEPAM	0.2-0.5 MG/KG	Intravenous	ICM (Institute of Comparative Medicine)
Propofol	2-5mg/kg	Intravenous	ICM (Institute of Comparative Medicine)

Hydromorphone	0.025-0.05 mg/kg PRE-SURGERY & EVERY 4-8 hrs POST-SURGERY	IM, SC, IV	ICM (Institute of Comparative Medicine)
Butorphanol	0.2 mg/kg	Intramuscular	ICM (Institute of Comparative Medicine)
Dexmedetomidine	0.01-0.05 mg/kg	Intramuscular	ICM (Institute of Comparative Medicine)
Atipemazole	0.2 mg/kg	Intramuscular	ICM (Institute of Comparative Medicine)

Other Drug or Substances	Dosage	Route	Responsible Person
Cefazolin	10-30 mg/kg	Intravenous	ICM (Institute of Comparative Medicine)
TGF-b3	10 nanograms/ml	osteochondral scaffold will be infused/coated with this material	Mao, Jeremy (jm2654)
BMP-2	160 micrograms per scaffold	segmental defect scaffold will be infused with this material	Mao, Jeremy (jm2654)
CEFPODIXIME (AS AN ALTERNATIVE TO CEPHAFEXIN)	5-10 MG/KG	PO SID	ICM (Institute of Comparative Medicine)
CEPHALEXIN	30 MG/KG TWICE DAILY	Oral	ICM (Institute of Comparative Medicine)

Hazardous Materials

X-Ray, Fluoroscope: Y

Scientific Questions

Modification Questions -

Increase in the Number of Animals Only

A1. Brief Progress Report.

Three pilot segmental defect dogs were operated on in June 2012. Two are doing very well and will be sacrificed this month as per the protocol. One was sacrificed earlier due to problems with the repair, which was reported in the previous modification

A2. Justification for Additional Animals

n/a

Change in Planned Use of the Animals

B1. Description of Changes to Protocol

The following medications are being added to the protocol at the request of ICM:
Dexmedetomidine, Butorphanol, Atipemazole.

ICM requested the addition of these drugs because they feel that the medications that are currently approved do not provide a level of sedation sufficient enough for safe transportation or x-raying of the dog.

B2. Number and Justification for Additional Animals Requested

n/a

B3. Number of Previously Approved Animals in New Procedures 7

B4. Discussion of Less Stressful Alternatives

There no less stressful alternatives to the procedures currently in the protocol.

Signature Approval

I am aware of, understand and will follow the ILAR Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Regulations administered by the United States Department of Agriculture.

I understand that these laws and regulations are applicable to all biomedical research projects using animals that are funded through and administered by Columbia University Health Sciences. As required by the Animal Welfare Act regulations, I hereby assure the IACUC that this experiment does not unnecessarily duplicate previous experiments.

Furthermore, I will obtain the approval of the IACUC for any significant changes in the experiment before they

are implemented. I certify that the statements herein are true, complete and accurate to the best of my knowledge.

I am aware that any false statements or departures from the approved procedures may subject me to administrative penalties that include suspension of my animal-based research (AWA 9CFR, ch.1, sect. 2.31, par.8 and PHS Policy, document 94-2).

I also certify that the experiments described in this protocol faithfully reflect the work proposed in the sponsored project(s) identified in this application. I have given each person listed in this protocol a copy of the protocol to read.

Electronically Signed and Submitted by Francis Lee (fl127) on 09/18/2012.

Appendix 6 – Year 2 Continuation of IACUC Protocol for CT Imaging Cadaveric Dog Limbs

Summary: This is a continuation of the ancillary IACUC protocol to permit the CT imaging of cadaveric dog limbs. No changes were requested to the original protocol.

IMAGING

Columbia University Animal Care Protocol Continuation Data Sheet

Protocol: AC-AAAD0334 (Y2 M00)	Protocol Status: Approved (Done) Approval Date: 03/21/2012 at 00:00
Name: Francis Lee (fl127)	Faculty Title: Associate Professor
Title: Imaging of Canine Limbs for Military	

Trauma Research

Species:	Dog	Effective Date:	03/31/2012
Number of Animals:	0	Expiration Date:	03/30/2013
Pain Levels:	C	Inspection Date:	
Originating Department:	ORTHOPEDIC SURGERY	Previous Protocol Number:	AC-N/A
Protocol Type:	Research	Protocol Submitted to:	Health Sciences

Modifications

- Staff/Personnel
- Procedures

Funding

Has ARRA Funding:	N	Funded by Department:	Y	Funded Through Private Gift:	N

Awarded Institution	External Funding Type	Source Identifier	External Funding Agency	Proposal Number
	N.A.		N.A.	

Protocol Staff

Personnel	Role	1+ yrs Exp W/ Species	Proc. Exp	Species Specific Training.	Wet Lab Date	Introduction to ICM	OHP Exp Date	Regulatory Lecture Date
Lee, Francis (fl127) phone: 212-305-3293 cell: 201-248-1630 pager:	Principal Investigator	yes	yes	07/07/2010			06/28/2010	
Procedure Experience:		1) Dr. Lee is a fellowship trained orthopaedic surgeon. However, there are no procedures involved with this tissue use protocol - No procedures involved with this cadaveric tissue use protocol.						
Chandhanayingyong, Chandhanarat (cc3391)	Staff Researcher	no	yes	12/07/2011			10/19/2011	
Procedure Experience:		1) Dr. Chandhanayingyong does not have experience with canine orthopaedic surgies, but has extensive experience with human orthopaedic procedures as a practicing orthopaedic surgeon in Thailand. - Dr. Chandhanayingyong is an orthopaedic surgeon from Thailand with a subspecialty in orthopaedic tumor. She will be involved with the live dog surgeries, and will use the cadaveric limbs associated with this protocol for practice surgery						
Training Courses:		TC0650 - The Dog: Computer Based Training Program					12/07/2011	
Vorys, George	Staff Researcher	no	yes	02/23/2011			02/17/2011	

(gcv2101)					
Procedure Experience:					
		1) Dr. Vorys has an undergraduate degree in biomedical engineering and is well-versed with computer imaging programs. - Dr. Vorys is an orthopaedic surgery resident who will assist with the creation of the computer models from the images of the cadaveric canine limbs.			
Song, Wongseok (ws2353)	Staff Researcher	no	yes	12/13/2011	10/27/2011
Procedure Experience:					
		1) Dr. Song does not have experience with canine orthopaedic surgies, but has extensive experience with human orthopaedic procedures as a practicing orthopaedic surgeon in Korea. - Dr. Song is an orthopaedic surgeon from Korea with a subspecialty in orthopaedic tumors. He will be involved with the live dog surgeries, and will use the cadaveric limbs associated with this tissue-use protocol as practice for the live animal surgery.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/13/2011
Kweon, Suc (sk3573)	Staff Researcher	no	yes	12/13/2011	10/14/2011
Procedure Experience:					
		1) Dr. Kweon does not have experience with canine orthopaedic surgies, but has extensive experience with human orthopaedic procedures as a practicing orthopaedic surgeon in Korea. - Dr. Kweon is an orthopaedic surgeon from Korea with a subspecialty in orthopaedic trauma. He will be involved with the live dog surgeries, and will use the cadaveric limbs associated with this tissue-use protocol as practice for the live animal surgery.			
Training Courses:		TC0650 - The Dog: Computer Based Training Program			12/13/2011
Gardner, Thomas (trg1)	Lead Researcher	yes	yes	02/15/2012	05/26/2010
Procedure Experience:					
		1) Mr. Gardner is an Engineer and Head of the Biomechanics Lab of the Dept. He will be responsible for helping with the coordination of project and oversee the creation of the 3-D computer models obtained from the MR images of the cadaveric canine tissue. - This is a tissue use protocol. No procedures are associated with this protocol.			
Training Courses:		TC0506 - Macacine Herpes Virus-1 (aka Herpes B Virus) Training			05/22/2012
		TC0650 - The Dog: Computer Based Training Program			02/15/2012
		TC1000 - The Ruminant: Computer Based Training			03/08/2012
Nizami, Saqib (sn2452)	Staff Researcher	no	yes	07/07/2010	11/04/2010
Procedure Experience:					
		1) Saqib Nizami is a technician who will assist with the creation of the 3-D computer models created from the images of the cadaveric canine tissue to be obtained under this protocol - No procedures			

		associated with this tissue use protocol.				
Mao, Jeremy (jm2654)	Lead Researcher	yes	yes	01/05/2010	03/04/2011	12/06/2011

Procedure Experience:

1) Dr. Mao will supervise the creation of the scaffolds using the 3-D computer models developed from the MR images of cadaveric canine limbs. - No procedures associated with this protocol.

Administrative Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu
Kennedy, Kathryn (kmk2148)	212-305-7965			kmk2148@columbia.edu

Emergency Contacts	Phone	Cell Phone	Pager	Email
Lee, Francis (fl127)	212-305-3293	201-248-1630		fl127@columbia.edu

Locations

Housing Location	# of Animals
BB 18	0

Satellite Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Exp.Procedure Location	Proc. Type	Duration (hrs)	Responsible Person
Neurological Institute, BASEMENT, Hatch Center	No Surgery	2	Thomas Gardner (trg1)
Black Building, 14, 1408	No Surgery	6	Thomas Gardner (trg1)
Allan Rosenfield Building, R!, CT/PET suite	No Surgery	12	Francis Lee (fl127)

Post Op Location	# of Animals	Duration (hrs)	Responsible Person
N.A.			

Drugs

Anesthetic or Analgesic	Dosage	Route	Responsible Person
N.A.			

Other Drug or Substances	Dosage	Route	Responsible Person
N.A.			

Hazardous Materials

No hazardous materials used.

Scientific Questions

Continuation
Questions

1. Describe in non-technical terms what was learned during the last year
The initial protocol requested 8 cadaveric canine limbs for imaging. To date, 6 cadaveric canine hind limbs have been purchased. The original intent of the protocol was to image these limbs to create 3-D computer models from which scaffolds would be manufactured for use in our live animal protocol [AC-AAAB7357]. Of the 6 cadaveric limbs purchased, 2 limbs underwent CT & MR imaging from which the computer models were generated. After imaging, it was decided to practice the anticpated orthopaedic procedures for the live animals in protocol AC-AAAB7357 on the cadaveric specimens. To date, 1 segmental defect repair was performed, and the approach for the osteochondral defect was practiced on a different limb.
2. Describe any adverse events which have affected animal use, welfare, morbidity, or mortality.
This is a tissue-use protocol using cadaveric dog limbs obtained from an animal tissue bank, so there is no morbidity or mortality associated with this protocol.
3. Discuss any changes to the planned use of animals and/or objectives.
The original use of the cadaveric canine limbs was to create 3-D computer models for use in AC-AAAB7357. It was decided that to maximize the use of these cadaveric specimens, after imaging, the same limbs would be used to practice the orthopaedic procedures planned for live animals in a separate protocol, AC-AAAB7357. The two procedures to be practiced are segmental defect repair of the tibia and an osteochondral repair of the femur. The benefit of using these cadaveric limbs for practicing the live dog surgeries is two-fold: a)practicing the procedure beforehand will refine the surgical skills of the surgeons prior to the actual surgery; and b)utilizing the tibial and osteochondral scaffolds in the cadaveric limbs will help perfect the design of these scaffolds prior to their use in the live animals. It is hoped that the above two benefits will improve the outcome of the live surgeries in the associated protocol AC-AAAB7357.
4. Justify the need for the number of animals requested
No live animals are being requested for this protocol. This is a tissue-use protocol using cadaveric limbs from an animal tissue bank.
5. Since the last IACUC approval have alternatives to the use of animals become available that could be substituted to achieve your specific project aims? If yes, explain why the alternative to animal use is not feasible for your project.
This is a tissue-use protocol utilizing the hind limbs from canines from an animal tissue bank. These animals were sacrificed for other reasons and this protocol is only using the hind limbs from these animals. The are no alternative options to using these cadaveric limbs.
6. Surgical procedures on the non-rodent mammals covered by this protocol have been discussed with
n/a

Signature Approval

I am aware of, understand and will follow the ILAR Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act Regulations administered by the United States Department of Agriculture.

I understand that these laws and regulations are applicable to all biomedical research projects using animals that are funded through and administered by Columbia University Health Sciences. As required by the Animal Welfare Act regulations, I hereby assure the IACUC that this experiment does not unnecessarily duplicate previous experiments.

Furthermore, I will obtain the approval of the IACUC for any significant changes in the experiment before they are implemented. I certify that the statements herein are true, complete and accurate to the best of my knowledge.

I am aware that any false statements or departures from the approved procedures may subject me to administrative penalties that include suspension of my animal-based research (AWA 9CFR, ch.1, sect. 2.31, par.8 and PHS Policy, document 94-2).

I also certify that the experiments described in this protocol faithfully reflect the work proposed in the sponsored project(s) identified in this application. I have given each person listed in this protocol a copy of the protocol to read.

Electronically Signed and Submitted by Francis Lee (fl127) on 03/15/2012.