Development of post-traumatic osteoarthritis (PTOA) is a common complication after an intra-articular fracture (IAF) in both combat and civilian settings. The broad goal of this expansion award is to determine if joint distraction can prevent the development of PTOA after an IAF. During this first project year, we obtained all of the animal protocol approvals required for the planned surgical series. We developed a new external fixator specifically for distracting Yucatan minipig hocks symmetrically and characterized the mechanical behavior of this device. We completed one of our two large surgical series of animals in which the new external fixator was applied at the time of fracture repair to prevent development of PTOA. Initial analysis of that first series indicated that delays in treatment of the fracture increase severity of cartilage degeneration. Our acute joint distraction treatment suffered some complications and did not appear to improve cartilage health when applied at this acute phase. We have encountered some significant delays and logistical challenges associated with animal availability as our planned vendor was forced to cull all immature animals from their herd as a result of PRRS (porcine respiratory and reproduction syndrome). This forced purchase of animals from alternative more expensive vendors and has significantly delayed the live animal work.
# Table of Contents

1. Introduction .............................................................................. 4
2. Keywords .................................................................................. 4
3. Accomplishments ..................................................................... 4-13
4. Impact ..................................................................................... 13
5. Changes/Problems ................................................................... 13-16
6. Products ................................................................................... 16
7. Participants & Other Collaborating Organizations ............... 16-22
8. Special Reporting Requirements ........................................... 22-23
9. Appendices .............................................................................. N/A
1. INTRODUCTION

Post-traumatic osteoarthritis (PTOA) is a severely debilitating degeneration of joints that frequently occurs after intra-articular fractures (IAFs) sustained both in military combat and training conditions and in active civilian populations. The purpose of this expansion project is to use the novel Yucatan minipig model of PTOA after IAF that was developed during our previous work (W81XWH-10-1-0864) to investigate the use of joint distraction to prevent development of PTOA subsequent to an IAF. Joint distraction has been found to be beneficial for treatment of late-stage osteoarthritis, when cartilage is all but absent in the joint. Our governing hypothesis is that distraction performed when cartilage is still present may have similarly positive effects for preventing joint degeneration. The effects of joint distraction applied acutely after IAF are the focus of Aim 1, and the effects of joint distraction applied shortly after the bone has healed are the focus of Aim 2. Of secondary interest are the effects of delayed fixation of a fracture (Aim 1), which is a common clinical practice in the treatment of IAF and almost universally a battlefield necessity. Also of secondary interest are the effects of articulated versus immobilized distraction (Aim 2) on both cartilage health and overall joint morbidity (arthrofibrosis, muscle weakness, etc.). Outcomes of both Aims investigating the effects of acutely applied and sub-acutely applied joint distraction will be a combination of macroscopic-level gait analysis for evaluating animal limb usage as a surrogate for function and pain, and microscopic/cellular levels of chondrocyte function and cartilage health. Upon conclusion of this project, we will have critically evaluated the use of joint distraction as a proactive treatment for PTOA.

2. KEYWORDS

Post-traumatic Osteoarthritis
Intra-articular Fracture
Yucatan Miniature Pig
Impact
Ankle
Cartilage
Gait Analysis
Joint Distraction
External Fixator
Histology
Mankin Score
Reactive Oxygen Species
Mitochondrial Metabolism
Western Blot
Inflammation
Micro CT

3. ACCOMPLISHMENTS

3.1 Major Goals

As outlined in the Statement of Work (SOW), the major goals for Project Year 1 were primarily associated with Specific Aim 1. Specific Aim 1 was to determine if treating animals with an IAF of the hock with joint distraction at the time of fracture repair would preserve cartilage structure and function. There were two hypothesis associated with this aim. The first hypothesis was that a delay between fracture creation and fracture fixation (such as would be incurred in almost any human IAF scenario) would lead to more severe PTOA after 12 weeks in our minipig model. The second hypothesis was the joint distraction at the time of fracture fixation would preserve the health of the cartilage better than treating the fracture alone.

In support of this Aim and the two associated Hypotheses, several specific tasks/subtasks were outlined for Year 1 of the project in the SOW. In preparation for the live animal work, all animal approvals needed to be obtained, and the distraction device/methodology needed to be developed and tested (Task 1.1). Updates were to be ongoing to the automated histological analysis routines (Task 2.3) and development of analysis techniques for the gait analysis was to be ongoing (Task 2.5). Finally, 24 Yucatan minipigs were to be operated on for Aim 1 (Task 1.2), and all associated analysis that needed to occur after harvest of those joints was to be ongoing (Tasks 2.1, 2.2, 2.4). In preparation for articulated distractor work beginning in Year 2 of the project, articulated distractor design and testing (Task 3.1) was to be ongoing throughout Project Year 1.
3.2 Accomplishments

Animal Protocol Approvals
The original animal protocol was submitted to our institutional IACUC board on 27 May, 2015, prior to the beginning of funding. The protocol was approved 4 June, 2015, again prior to the beginning of funding. Since initial approval, there have been a total of 13 modifications made to the protocol, none of which relate to the approved study design. Eight of these modifications were personnel additions/deletions including the addition of a graduate research assistant, changes in Surgicenter personnel, and addition of summer students at the Surgicenter. The majority of those more temporary individuals were only responsible for animal transport for surgery and gait analysis, and are not listed on this project. One modification was for the addition of 2 pilot animals that were outside this study, but making use of similar techniques. A modification was made to add a harness for handing and training pigs for gait analysis, and two modifications were made to increase postoperative pain control drugs and add an anti-anxiety medication for sedation. The final modification was made at the request of our Office of Animal Resources (OAR) that allowed approved OAR employees to practice intubating on our animals while they were sedated prior to euthanasia. As none of these modifications impact study design, no modifications were made to the ACURO protocol which was approved 3 Sept 2015.

Distractor Design & Testing – Task 1.1
Postoperative joint distraction was originally planned to be performed using a commercially available monolateral external fixator. Our pilot work with that fixator indicated that while symmetric joint distraction could be achieved, the amount of fine adjustment required to do so was prohibitively time consuming for repeated intraoperative use of this device. Therefore, a custom joint distraction device was developed for the purposes of distracting Yucatan minipig hocks. The device was based on an Ilizarov fixator and a total of five separate iterations were built and tested before selecting the final version that was used in live animal surgery.

Version 1 was a gross proof of concept version made from combinations of human external fixator hardware and custom built U-rings (Figure 1). The device consists of a U-ring around the proximal tibia with the opening to the posterior so as not to be in contact with the soft tissue, and a U-ring around the distal tibia/hock joint with the opening to the anterior so as not to impede joint flexion in an articulated scenario. Half pins are inserted into the medial talar head, the medial and lateral calcaneus, and the tibial shaft. Shortcomings of this version included a proximal U that was too long and impinged upon the stomach, a distal U ring that was too short, and did not permit adequate fixation of the talus through the talar neck, and use of human device hardware that was ill fitting (screws are too long/too short, at odd angles, etc.) This device was able to achieve symmetric medial/lateral and symmetric anterior/posterior distraction with installation requiring a fraction of

Figure 1. Version 1 of the Yucatan minipig specific distraction device installed on a cadaveric pig leg. The original (top) and distracted (bottom) joint space can be seen on the fluoroscopic images on the right.
the time of the monolateral device. Therefore, the general approach was pursued through a series of additional iterations.

Version 2 was a similar combination of custom and commercially available human external fixator hardware. Key design features were lengthening the distal U-ring to provide more locations for fixation and shortening the proximal ring to decrease impingement of the device on the animal stomach. To achieve a more symmetric hold on the calcaneus, a tension wire was trialed, however it was determined not to be a sufficiently strong alternative. Version 3 was made entirely of custom hardware upon the identification of smaller diameter veterinary-grade transfixion pins. Again with the goal of symmetric joint distraction, this version had one transfixion pin distally through the calcaneus and one proximally through the proximal/middle tibial shaft. For added stability proximally, a half pin is needed into the tibia. However, once the device is positioned for the distal half pin into the talus, the transfixion pin through the calcaneus, and the transfixion pin into the tibia, it was not possible to insert a tibial pin through existing hardware that aligned directly with the frame. So for Version 4, a series of U-joints on a stabilizing crossbar was developed which allowed for holding a tibial half pin that was oriented in the tibia in a non-prescribed location and at an oblique angle. This provided the surgeon with total freedom to place the pin in the bone and grab it later with the hardware.

**Figure 2.** Versions 2-4 of the distraction device. The notable changes in design on Version 2 were the trial of the tension wire (arrow) and the shortening/lengthening of the proximal and distal U-ring, respectively (ellipses). The notable design changes on Version 3 were the addition of two small-diameter transfixion pins and the associated custom hardware (arrows). The notable design change on Version 4 was the addition of the linkage system for interfacing with the tibial half pin (ellipse).

Version 4 of the distractor was tested in the operating room on cadaveric pig legs and under load in a Materials Testing System (MTS Inc, Eden Prairie, MN). Assuming a general quadruped weight distribution of 60/40 between the forelimbs and rear limbs, a 100kg animal would be expected to load the distractor with forces of approximately 200N while standing. It was found that when the AP and ML movement of the bones was constrained it required a 800N of load to close 2 mm of joint distraction applied using our device. Is this was a much higher load than the animals were expected to place on the limb, it was decided that the frame was sufficiently stiff to maintain distraction under load. However, application of 600-800N of load caused 1.8-2.2 mm of compressive displacement of the fixator and when the bones were permitted to move in the AP and ML directions, only 250N of load was required to induce displacement of 2.1mm. As all other joints were removed/potted for our testing, this displacement required flexion of the ankle joint, and upon further investigation, it was seen that this flexion
was permitted by bending of the transfixion pins. To stiffen the construct and decrease joint flexion, we developed Version 5 (Figure 3) of the distractor in which the calcaneal transfixion pin was replaced by two half pins inserted obliquely from posteriorly, one from the medial side, and one from the lateral side. With this alternative pin configuration stiffening the flexion of joint, the distractor began to rotate around the proximal tibial pin. To reduce this rotational degree of freedom and further stiffen the construct, a second tibial half pin was inserted out of plane from the transfixion pin and the original tibial half pin. This alternative also maintained similar distraction under similar loads, but was much stiffer. This version was ultimately applied to the live animals in Series #1 of Aim 1.

**Histology Analysis Updates – Task 2.3**

The updates to the automated histological analysis methods have been modest. We have implemented a new cell identification algorithm that better identifies cell nuclei in fibrotic repair tissue and fibrocartilage, which is a key step for developing the O'Driscoll score. Framework for that automated scoring tool has been laid out based upon the automated Mankin scoring program. Work will continue in this area during the upcoming project year.

**Gait Analysis – Task 2.5**

Since the date of the original project proposal, a new animal gait analysis lab has been opened on the University of Iowa campus. This laboratory has an 8-camera Vicon (Centennial, CO) motion capture system and an instrumented treadmill. (FitFurLife, GAIT4dog/CIR Systems Inc., Franklin NJ). These technologies replaced the originally proposed motion capture using a portable Qualisys motion capture system and limb loading using a static Tekscan Walkway pressure sensitive mat. A substantial effort went into developing a training protocol to acclimatize the minipigs to walking onto the treadmill. This included identification of an appropriate harness, acceptable positive reinforcement methods (food), and comfortable walking speeds. For acclimatization, animals arrived at the University two weeks prior to their scheduled surgery day. They were exposed to study staff in their pens. During this exposure, the animals were allowed to investigate the harness for the first time and received food reinforcement consisting of shredded wheat, marshmallows, or jelly. Animals wore the harness for a period of several hours in their pens on subsequent days. Approximately 4 days after arrival acclimation to the harness the animals were moved to a large open room and walked with the harness and a leash around the room. Around one week, animals were coaxed onto the treadmill. Progressively longer treadmill sessions, up to 20 minutes of walking, were the goal.

Two to three days prior to surgery, definitive preoperative data capture was performed at two speeds, “healthy” pig speed, and “postop” pig speed. It was determined that the normal healthy pigs preferred to walk between 1.8 and 2.2 km/hr on the treadmill. This was a

![Figure 3. Version 5 of the distractor selected for use in the live animal surgeries. The U-rings are as in Version 3, the proximal transfixion pin is as in Version 4, but attached distally to the upper U, and the two tibial half pins have multiple degrees of freedom for best bone purchase. The two calcaneal half pins angle superiorly and converge in the center of the calcaneus.]
comfortable speed they could maintain for a period of several minutes for the collection of several data captures of kinematics. At faster speeds, animals tended to only walk with a normal looking gait cycle for only a very few steps before transitioning to a run. At slower speeds they tended to ride the treadmill belt. Thus “healthy” speed was chosen as 1.8 km/hr. As these animals were also going to be evaluated early postoperatively, it was clear that a slower speed would also be needed. A velocity of 1.2 km/hr was found to be tolerated early postoperatively, and an achievable speed for at least one trial preoperatively that was not invalidated by riding the treadmill. Thus “post-operative” speed was 1.2 km/hr. Preoperatively, all animals performed at least 5 trials at each speed, although several of the slower trials were confounded by riding. Postoperatively, 3-5 trials were collected at the slower speed only. The number of trials was purely based on veterinary assessment of the comfort of the animal walking. Painful looking animals were stopped after 3 trials. With increasing postoperative time and healing, animals became more comfortable walking on their fractured limb. When they tended to want to ride the treadmill again at the slower “postop” speed, additional trials were collected at “healthy” speed. At the end of the 12 week survival, all animals were capable of walking at the faster speed.

Greater than 500 data trials were captured for the Series 1 animals (12 animals x 7 time points x replicate trials/multiple speeds). We are in the process of evaluating all the data to determine limb usage and limb kinematics for these animals. Additionally, the instrumented treadmill comes with software that is specific for veterinary clinical applications of the tool in which relative foot pressures are sufficient for diagnosis and evaluating outcomes. We will need to calibrate the device in order to obtain actual foot loading data for evaluation of limb loading characteristics. This calibration and data analysis is ongoing.

Figure 4. Yucatan minipig undergoing postoperative gait analysis in the new gait lab. The harness and leash are primarily for guidance on and off the treadmill. The treadmill senses pressure under the feet and reports a pressure map (upper right), and the black patches with reflective markers facilitate motion capture of the limb movement by the Vicon cameras mounted around the room. The marker templates for the animal were developed specifically for this study, and the analysis routines are being developed (lower right).

Series 1 Animal Surgeries – Task 1.2
There were a total of 24 live animals surgeries planned for investigation of Aim 1, with these animals broken down into three different study group: fixation at time of fracture, fixation 3 days post-fracture, and fixation 3 days post-fracture with distraction. For logistical reasons, primarily
gait analysis and on-campus animal housing, these 24 animals were divided into two identical series of 12 animals each (n=4 from each group). We were able to begin Series 1 as scheduled in Q3 of the project. All animals in the immediate fracture fixation group tolerated the 12 week study period well. This was expected given our substantial experience with the model.

Animals in the delayed fixation group appeared more lame for a slightly longer postoperative period, however there were no complications with wound or fracture healing, and animals recovered to qualitatively similar overall health as the immediate fixation group. In order to ensure that the animals did not bear weight through their fractured and unfixed joints, we developed a flexed casting protocol to flex the hock joint out of a convenient weightbearing position. A separate fiberglass “ski” was applied extending over the toes on the affected side (Figure 5). The animals found placing their foot on the ground with this “ski” over their toes to be disturbing, and therefore they did not attempt to load the flexed joint. Radiographs were obtained immediately after fracture, and prior to fixation 3 days later to ensure that the fracture fragment was not grossly displaced, which would have been an indication of weightbearing. There was no radiographic evidence of fragment displacement as a result of weightbearing in any of the animals that had a delay in fracture fixation.

There were several complications with the animals wearing distractors. One of the four animals jumped off of the treadmill during the 2 week postoperative gait analysis session. This resulted in a broken calcaneal pin and early removal of the external fixator. The animal continued in the study for the full 12 week survival time. All pin tracts healed well, and the animal appeared to suffer no further complications from the broken pin or the distractor. The remaining 3 animals in the distraction group all has gross loosening of the calcaneal pins 4 weeks postoperatively. While the joints appeared distracted radiographically (while the animal was sedated and non-weightbearing), it was unlikely that the fixators with loose pins were maintaining distraction. All distractors were therefore removed at 4 weeks. There were only minimal superficial pin tract infections, and all pin tracts had healed fully in all animals at the 12 week euthanasia time.

Figure 5. (Top) Flexed casting technique to prevent weightbearing on an unfixed fracture. The hock is flexed under the body, and a “ski” made from fiberglass casting tape encourages animals to remain off the limb. (Bottom) The location of the fracture fragment (arrow) was monitored radiographically immediately postoperatively (left) and 3 days later when the flexed cast was removed for fixation surgery (right). Flexing the joint did not displace the fragment, and the animal appear to not have displaced the fragment through weightbearing.
Figure 6. Photographs of an external fixator installed during fracture fixation surgery 3 days after IAF. The immediate postoperative picture shows the frame before the transfixion pins and half pins were cut off, which prevented the pins from further irritating the animals. One week postoperatively, the incision, which was sutured closed is healing nicely and the pin tracts are infection-free.

Figure 7. Intraoperative fluoroscopy images for the animal shown in Figure 6. The native configuration of the joint (left, green arrow) is closed. With the external fixator applied, a symmetric joint distraction was applied (right, green arrow).

At the time of euthanasia (early September), a detailed dissection of the joints was performed. Macroscopically, the joints fixed immediately after fracture appeared similar to those in our previous work with the model. Qualitatively, the joints that had a delay in fixation appeared to be slightly more degenerated than those fixed immediately. This could potentially have implications about the decision to delay surgical fixation of IAFs in human patients. Unfortunately the joints from animals with 4 weeks of joint distraction were qualitatively worse in appearance than those without distraction. There were more focal defects, areas of cartilage approaching lesions, overall thinner looking cartilage, discolored subchondral bone, and roughened, pitted (non-lustrous) appearance (Figures 8 & 9).
Figure 8. Composite of Series 1 tibias. Anterior is up and medial is on the left. A three-day delay appeared to prolong the visibility of the (healed) fracture line, and distraction appeared to result in larger areas of erosion around the fracture line.

Figure 9. Composite of Series 1 taluses. Anterior is down and medial is on the left. A three-day delay appeared to be associated with thinner talar cartilage, and distraction appeared to result in larger erosions and discoloration of the underlying bone.
Unfortunately, we have not been able to begin Series 2 of these animals on our original timeline as a result of challenges procuring animals from our preferred vendor, Exemplar Genetics. Their herd was infected with PRRS (porcine respiratory and reproduction syndrome). The mortality rate associated with this virus is very high, especially in young animals, but it can pass to older animals as well. It appears that the vendor elected to cull the herd and repopulate with clean animals. The timing of this event left them with insufficient numbers of 24 month animals for our study. We have had to explore other options for animal vendors, which has and will continue to significantly delay our progress and is discussed in greater detail below.

**Post-Euthanasia Analysis – Tasks 2.1, 2.2, 2.4**

The tissue and cellular level analysis of the Series 1 animals is ongoing. This includes contrast enhanced micro CT imaging and image analysis. Imaging is being performed on formalin fixed tissues as a result of insufficient time for imaging on euthanasia days. Samples were stored for Western blot analysis and these will be analyzed in batches in the upcoming project year. We replaced the originally proposed measurements of mitochondrial respiration using a Seahorse Extracellular Flux Analyzer with a confocal microscopy based approach that could be more readily integrated into the workflow of our live/dead confocal analysis that occurred on the euthanasia and harvest days. While this sacrifices per/cell measures of oxygen consumption, this approach maintains spatial registration of mitochondrial oxidant production at sites relative to the fracture, erosions, and cartilage zones. These images were acquired at the time of euthanasia and their quantitative analysis is ongoing.

### 3.3 Opportunities for Training & Professional Development

This project was not specifically intended to provide training or professional development. However, we do have a graduate research assistant working on the project who is receiving training in the form of one-on-one mentorship with the Project PI and several other research team members. The training has included learning the necessary skills for analyzing animal gait data and analyzing the mechanical effects of joint distraction. That training will contribute to the graduate student’s thesis.

### 3.4 Dissemination of Results

Nothing to Report

### 3.5 Plans for Next Reporting Period

Adhering to the tasks and a slightly delayed timeline from the approved SOW, post-euthanasia analysis of the tissues harvested from the Series 1 animals will be ongoing. This will include quantitation of the confocal images acquired on euthanasia day, which now consist of both chondrocyte viability images, as well as mitochondrial content and oxidative stress data. Finalizing that data set will be a goal for the next reporting period. Western blot analysis will also be completed on specimens harvested at euthanasia of the Series 1 animals. Micro CT imaging will continue, and the specimens will be processed into the variety of histological and immunohistochemical sections outlined in the proposal. The quantitation techniques needed for those sections will continue to be developed and then implemented.

Similarly, gait analysis will be completed for the Series 1 animals and the techniques required for all animal gait data will be established for more rapid implementation in all future animal series. In order to complete development and actual analysis of the gait data, the instrumented treadmill will need to be calibrated during the next reporting period.

An important developmental activity for the next reporting period will be the design, construction and testing of the articulated joint distraction device for animal surgeries to address specific Aim 2. Those design and testing activities will be iteratively ongoing throughout the next reporting period.
The delays and challenges associated with obtaining sufficient numbers of appropriately aged animals will delay the surgical schedule that was originally planned for the second project year. In the first quarter of the second project year, the PI will work with the Science Officer on the project to determine the best course of action regarding contracting study groups or requesting an extension on the project timeline. We will continue to attempt to identify alternative animal vendors that can provide the necessary animals in a timely and economically feasible manner.

Based on the uniformly poor results of acute joint distraction, there appears to be no reason to finish the remaining 4 animals in that group that would be in Series 2. Series 2 surgeries will move forward in the upcoming project year with the immediate and the delayed fixation groups only. The decision to redesign the distraction device and attempt another surgical series or the decision to no longer include this group in the study will be made based on the quantitative outcomes of the tissue harvested at euthanasia. This decision will be made with input from the project's Science Officer.

Due to lack of animal availability, all activities listed as occurring in months 12-24 will be delayed to an as-yet unknown degree. One of the primary tasks to be accomplished early in the next reporting period is an acceptable modification of the timeline for the remainder of the project.

4. IMPACT

As this was the first year of the project, the focus has been on methodology development and data generation. There is presently insufficient information available to yet have an impact on clinical treatment of IAFs.

4.1 Impact on the Principal Discipline

The initial, macroscopic findings of this work would indicate that delays between an IAF and the time that fracture is surgically reconstructed increase the subsequent cartilage damage. Presently, this finding has only been present in 4 animals (the first in the project). Additional animals are needed to confirm this finding and lend support to the conclusion that delays in fixation are not positive events in the context of PTOA development. Similarly, immediate distraction of a joint, while necessary for clinical treatment, appears to have a negative effect on joint health as it relates to future cartilage degeneration. Again, these findings are simply general observation of a small number of replicate animals, and additional data and detailed analysis is needed before this information will impact clinical care of IAFs.

4.2 Impact on Other Disciplines

Nothing to Report

4.3 Impact on Technology Transfer

Nothing to Report

4.4 Impact on Society

Nothing to Report

5. CHANGES/PROBLEMS

This project has and will continue to be impacted by the lack of animals of an appropriate age from our preferred vendor. For this work, we require Yucatan minipigs that are skeletally mature, dictating that animals >24 months of age are needed for the study. This age animal is necessary because prior to skeletal maturity, the physis in the distal tibia is variably open (dependent on animal age and individual), presenting a confounding factor in the IAF creation. The IAF methodology uses a stress rising saw cut to guide the intra-articular fragment created
by the impact. The presence of an open physis creates another stress riser in the distal tibia that can confound the resultant fracture fragment or result in additional fracture of the distal tibia beyond the intended IAF.

At the time of submission, Exemplar Genetics was our Yucatan minipig vendor. We had an established relationship with them, and they had been the source for our two previous studies using the minipig. They are a relatively local source (for cheaper shipping) with economically priced animals. Unfortunately, as described above, this vendor was struck by the PRRS virus, and decided the best course of treatment was to depopulate the herd and repopulate with non-infected animals. This resulted in the euthanasia of the animals that would have been appropriately aged for our study. After discussions with this vendor, it was learned that they were able to start their breeding program again in early 2016, so they would not have any animals of an appropriate age until early 2018, the final year of this project.

This lack of animals from our preferred source has severely disrupted the budget and the timeline of the project. It will likely result in changes to the approach, although attempts will be made to minimize those changes. There have been a few minor problems and changes associated with particular study aspects, but animal availability has and will continue to be the greatest driver of change in this work.

5.1 Changes in Approach

Presently, no significant changes in approach have been made. However, the seemingly negative effects of immediate joint distraction, which was intended to be a treatment, would indicate this is no longer a group worth pursuing. If, upon quantification of the microscopic and biological outcome measures, cartilage health is confirmed to be degraded by distraction, we will work with the Science Officer and obtain approval to modify our study design to eliminate this group. It is possible that the animals originally intended to fill out the n=8 in this group would serve better as a pilot group for the articulated distractor. A pilot would potentially be beneficial given the challenges associated with calcaneal pin loosening in this group. The Aim 2 animals are even more valuable as they will be 12 weeks after fracture before distraction is applied. Complications at that stage would be even more unfortunate as replacing animals would be extremely expensive both in terms of dollars and effort by research personnel.

Figure 10. Direct comparison of talus appearance associated with distraction. Distraction was intended to prevent cartilage degeneration; however, it appears to have exacerbated degeneration.
There were two minor changes to the approach specified in the proposal. The first was that the duration of distraction was shortened from the proposed 6 weeks to 4 weeks. This was as a result of radiographic and gross evidence of calcaneal pin loosing 4 weeks postoperatively. Rather than introduce additional trauma or confounding factors by trying to maintain stability of the distraction for an additional 2 weeks, the period of the distraction was simply shortened. Secondly, a decision was made to omit the per/cell measures of oxygen consumption by the mitochondria as specified in the proposal. While this is an extremely detailed measure of chondrocyte respiratory function, the technique is necessarily destructive, requiring the extraction of live chondrocytes from cartilage, and a period of several days of in culture before the measurements can be made. It also makes subsequent histological analysis challenging on the remaining tissue. The decision was made to switch from the more destructive technique to a confocal imaging based assessment of equivalent information. The confocal technique uses MitoTracker Deep Red and Cell ROX Orange stains (both commercially available) to stain for mitochondrial content and oxidative stress. The advantage of this information is that the chondrocytes can be evaluated in situ, preserving their phenotype, minimizing confound factors from time in culture, and maintains spatial information through the depth of the cartilage. This provides much more useful information for interpretation against the immunohistological and histological data being collected from the same joints. As both of these changes were very minor variations on the original approach, and the aims, resultant data, and conclusions to be drawn all remain as approved, no formal approval was solicited for these slight modifications in approach.

5.2 Problems & Delays

As described above, the problem encountered thus far is animal availability. This has led to delays and financial problems. When informed that we would not be able to obtain animals from our original vendor, we identified alternative sources. The first source was Sinclair Bioresources, and that vendor did have animals of the necessary age available. However purchase price was approximately ~$2,300/animal, and the project budget was based upon purchasing from Exemplar at ~$950/animal. We were able to make arrangements to house our longer-term survival Aim 2 animals at Iowa State University, our nearby sister institution, which has a Veterinary School and less expensive per diems, which allowed us to make up some of the greatly increased costs. This change in housing was cleared with the grants officer through email discussion, however no formal changes have yet been made to the institutional or ACURO animal use protocols to reflect this change in housing due to the as-yet un-finalized timeline. Those approvals will be obtained prior to being relevant.

A further challenge with the animals obtained from Sinclair was that in addition to being significantly more expensive, they were significantly larger. The animals from Exemplar had tended to be in the approximately 60-70 kg range for bodyweight. The animals we were able to obtain from Sinclair were approximately 100 kg. This required a delay while working with the University Animal Resources to modify their regulations, which did not allow them to accept animals that were that large/heavy. Furthermore, the dramatically increased size and weight of these animals required significantly larger doses of drugs and anesthesia. Unfortunately, the budgeted amounts for those were based on a 60 kg animal.

We have identified another approved vendor of Yucatan minipigs who offers animals at an intermediate price. We have delayed the start of Aim 1 – Series 2 animals until we can determine animal availability through that vendor in 2017. An updated timeline guided by budgetary considerations and outcomes from the Series 1 animals will be created early in Project Year 2.
5.3 Changes in Expenditures

The significantly increased purchase price and higher drug costs of the available Yucatan minipigs has caused the need for changing the allocations of expenditures. As all of these costs are considered supply costs in some form or another, there are not major shifts in expenditures from one category to another. However, it has been necessary to find alternative options to permit greatly increased animal purchase costs.

Current expenditures at the end of Project Year 1, are $333,310. This figure is lower than the original Project Year 1 budget of $496,184. The reason for the discrepancy is two-fold. First, the Animal Surgicenter has not yet posted the surgical charges for the Series 1 animal surgeries. This will occur at the beginning of Project Year 2. Secondly, and the dominant factor, is that we have been unable to begin Series 2 of Aim 1 animals, which were originally planned for this first year. Therefore, animal purchase costs, surgical costs, and per diem costs have not yet been expended. As soon as a vendor with animals that meet our needs is located, these funds will be expended.

5.4 Changes in Use/Care of Human Subjects

Not Applicable

5.5 Changes in Use/Care of Vertebrate Animals

There have been no major changes in the use or care of Vertebrate Animals since our original proposal submission and IACUC and ACURO approvals of our protocols. The minor amendments to the IACUC approved protocol were detailed above and amounted to additional analgesia and anti-anxiety medication to improve the comfort of the animals after the prescribed surgical procedures. IACUC and ACURO approval will be obtained prior to a change in housing location for the Aim 2 animals that will be used in Years 2 and 3 of this project.

5.6 Changes in Use of Biohazards/Select Agents

Not Applicable

6. PRODUCTS

There are no publications or presentations to report.

The primary product developed in this reporting period is the external fixator for applying distraction to the minipig hock. There are no intentions to pursue a patent for this device as it is simply specific hardware to this project and has very limited other applications.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.1 Individuals Working on the Project

Jessica Goetz
Nearest person month Worked: 3
Contribution to Project: Project PI
Dr. Goetz has been actively involved in designing and testing the external fixator, assisting with the gait analysis, live animal surgery, and followup gait analysis and care. She has also been performing administrative and organizational duties, writing reports, managing budgets and coordinating the research team.

Thomas Baer
Nearest Person Month Worked: 2
Engineer
Contribution to Project: Mr. Baer was responsible for layout and building of the multiple iterations of distraction devices described in this report. This includes the incremental version modifications and large scale production to build multiple full versions for the live animal work.

**Mitchell Coleman**  
Nearest Person Month Worked: 1  
Contribution to Project: Dr. Coleman was responsible for guiding the biological and histological analysis techniques initiated during euthanasia. He identified the new mitochondrial confocal microscopy stains and developed the protocols for their implementation.

**Douglas Fredericks**  
Nearest Person Month Worked: 1  
Contribution to Project: Mr. Fredericks assisted with modifications to animal care documents, assisted with the performance of the all surgeries, and assisted with general project planning.

**Christopher Heck**  
Nearest Person Month Worked: 6  
Contribution to Project: Mr. Heck has been learning/implementing quadruped gait analysis techniques and assisted with all surgeries and follow up care, dissection, and confocal imaging.

**Emily Petersen**  
Nearest Person Month Worked: 1  
Contribution to Project: Dr. Petersen organized all animal ordering, animal surgeries and performed all postoperative care. She assisted in modifications to animal care documents and with gait analysis.

**M. James Rudert**  
Nearest Person Month Worked: 1  
Contribution to Project: Dr. Rudert developed and built the new MTS testing device and assisted with the cadaveric testing and the development of the new pin design.

**Michael Willey**  
Nearest Person Month Worked: 1  
Contribution to Project: Dr. Willey provided clinical expertise used to design the different fixator hardware systems, and performed all the live animal surgeries.

**Other Personnel**  
Various other personnel contributed to this project in the first year, several were paid through this grant, and the remainder were paid by the University of Iowa Department of Orthopedics & Rehabilitation. Their efforts were less than 1 person month, and they are therefore not listed above.
James Martin – biological analysis input  Keli McLaughlin – animal monitoring
Gail Kurriger – dissection, histology  Amie Pluskowski – gait analysis
Barb Laughlin – dissection  Amanda Weibold – animal monitoring
Abagail Lehman – dissection  Nicole Watson – gait analysis
Marc Brouillette – confocal microscopy

7.2 Changes in Active Other Support

The last update in Other Support for key investigators on this project was made in the pre-award documents submitted in June 2015. All updates in completed support and new support reported here are relative to that time.

Goetz – Dr. Goetz had two Institutional grants end and one new grant begin since the previous reporting period. The completion of the two grants provided 2.1 calendar months of time, only 1.2 of which is filled with the new grant, leaving sufficient time for the proposed effort on this work.

Completed:
1. Identification of Histologically Based Biomarkers Contributing to a Mathematical Model of Pathological Fracture
   Sponsor Agency: University of Iowa OVPRED Internal Funding Initiative
   06/01/2014 – 09/30/2015, $36,609 Direct Costs
   Jessica E. Goetz, PhD (PI), 1.8 Calendar months

2. Effects of Radiation Dosing Regimen on Mechanical Strength of Bone
   Sponsor Agency: University of Iowa Sarcoma Research Program
   05/01/2014 – 06/30/2015, $10,500 Annual Direct Costs
   Jessica E. Goetz, PhD (PI), 0.3 Calendar months

New:
1. Engineering Endogenous Cartilage Repair
   Sponsor Agency: Arthritis Foundation
   Arthritis Foundation National Office
   1330 W. Peachtree St., Suite 100
   Atlanta, GA 30309
   01/01/2016-01/01/2019, $892,057 Total Direct Costs
   James A. Martin, PhD (PI)
   Jessica E. Goetz, PhD (Co-Investigator), 1.2 Calendar months

The overall goal of these studies is to test the feasibility of a novel method for in situ progenitor cell-mediated cartilage regeneration in a large animal (goat) model. We propose to package the chondrogenic factor TGFbeta3 in biodegradable poly(lactic co-glycolic acid (PLGA) microparticles and suspend them in hydrogel before arthroscopic in vivo delivery into a goat femoral condyle chondral defect. This project will include device design for delivering the hydrogel into a chondral defect, mechanical testing of the integration and structural integrity of the repair tissue, and biological/histological analysis of the new repair tissue originating from the hydrogel material.
OVERLAP: There is no overlap with this work.

Coleman – Dr. Coleman had 2 Institutional grants close, a no-cost extension on a previously reported project, and one new grant begin since the last report.
**Completed:**

1. **Radiosensitization of Leiomyosarcoma and Liposarcoma via Metabolic Oxidative Stress Induced by Inhibition of Glucose Metabolism**  
   Sponsor Agency: University of Iowa Sarcoma Research Group  
   5/01/2014 – 5/01/2016, $19,435 Total Direct Costs  
   Mitchell C. Coleman, PhD (PI), 1 Calendar month

2. **Sarcoma Cells of Different Subtypes Produce Excess Superoxide Relative to Normal Tissue Counterparts**  
   Sponsor Agency: University of Iowa Free Radical and Radiation Biology, Research Program of Excellence  
   5/01/2014 – 5/01/2016, $4,985 Total Direct Costs  
   Mitchell C. Coleman, PhD (PI), 0.5 Calendar months

**Extended:**

1. **Mitochondrial Based Treatments that Prevent Posttraumatic Osteoarthritis in a Translational Large Animal Intraarticular Fracture Survival Model (W81XWH-11-1-0583)**  
   Sponsor Agency: United States Department of Defense, Army Medical Research Acquisition Activity  
   09/01/11 – 08/31/2017, $2,559,912 Total Costs  
   James A. Martin, PhD (PI)  
   Mitchell C. Coleman, PhD (Co-Investigator), 6.00 Calendar months (effort reduced to 3.00 cal months, no salary support after 9/30/2015)

**New**

1. **The Effect of Sirtuin-1 on Chondrocyte Progenitor Cell Activity in Acute Cartilage Injury**  
   Sponsor Agency: Orthopaedic Trauma Association  
   9400 W. Higgins Road, Suite 305  
   Rosemont IL 60018-4226  
   01/01/2016 – 12/31/2016, $20,000 total direct costs  
   Jocelyn Compton, MD (PI)  
   Mitchell C. Coleman, PhD (Co-Investigator), 0.12 Calendar months (no salary support)

Stem cell-based treatments have been under investigation for adult human articular repair, and harnessing the regenerative potential of CPCs has attracted significant attention as a method to alleviate the disease burden associated with PTOA. If successful, this study will become the basis for further experiments studying the mechanism of PTOA progression as mediated by the SIRT1 pathway and aid in understanding of mitochondrial function as driven by SIRT1 expression as a critical mediator of CPC activity.

**OVERLAP:** There is no overlap.

**Fredericks** – Mr. Fredericks has had 4 industry contracts unrelated to this project that were granted and completed since the last report. He had a no-cost extension on a previously reported project. He also has 5 new grants and contracts since the previous reporting period that are ongoing at the time of this report. His total effort on these new projects is only 2.28 calendar months, leaving sufficient time for the proposed effort on this work.
Extended:
1. Mitochondrial Based Treatments that Prevent Posttraumatic Osteoarthritis in a Translational Large Animal Intraarticular Fracture Survival Model (W81XWH-11-1-0583)
   Sponsor Agency: United States Department of Defense, Army Medical Research Acquisition Activity
   09/01/11 – 08/31/2017, $2,559,912 Total Costs
   James A. Martin, PhD (PI)
   Douglas C. Fredericks, BS (Co-Investigator), 1.2 Calendar months (no salary support after 9/30/2015)

New:
1. ReBOSSIS in a Lapine Posterolateral Fusion Model and ReBOSSIS Bone Void Filler in a Rabbit Metaphyseal Defect Model
   Sponsor Agency: OrthoRebirth Co, Ltd.
   Sponsor Contact: Yasutoshi Nishikawa, President & CEO
   Phone: +81-90-7175-0001; Fax: +82-45-532-3650; E-mail: nishikawa@orthorebirth.com
   04/25/2016 – 04/24/2017, $201,914.17 Total Direct Costs
   Douglas C. Fredericks, BS (PI), 0.12 Calendar months

   The objective of the rabbit PLF model is to evaluate the performance of the test article, a novel synthetic bone graft substitute, by determining the effect of the test article on posterolateral spine fusions, relative to Actifuse ABX on the fusion rate, quantity and quality of new bone formed, quantity and quality of graft resorption, and host response.

   The Metaphyseal Defect Model is to evaluate the performance of ReBOSSIS 85, a resorbable bone void filler, to the repair of bone defects in the rabbit metaphyseal defect model, by determining the effect of the test article relative to a Actifuse ABX on quantity and quality of new bone formed, and host response.

   OVERLAP: There is no overlap.

2. Statement of Work #1: HA-TCP-45S5 Bioactive Glass-collagen Composite Lapine Posterolateral Fusion Model
   Statement of Work #2: HA-TCP-45S5 Bioactive Glass-collagen composite Bone Void Filler in a Rabbit Metaphyseal Defect Model
   Statement of Work #3: DBM-Glycerol Lapine Posterolateral Fusion Model
   Statement of Work #4: DBM-Glycerol Bone Void Filler in a Rabbit Metaphyseal Defect Model
   Sponsor Agency: Berkeley Advanced Biomaterials
   Sponsor Contact: François Génin, CEO and Tissue Bank Director
   901 Grayson, Suite 101
   Berkeley, CA 94710
   Phone: 510-883-0500 x12
   03/01/2016 – 07/31/2016, $159,459.57 Total Direct Costs,
   (01/27/2016 – 01/26/2021 Master Agreement), $132,867.93 Total Direct Costs
   Douglas C. Fredericks, BS (PI), 0.12 Calendar months each

   The objective of the study is to evaluate the performance of HA-TCP-45S5 Bioactive Glass-collagen Composite, a novel bone graft substitute, in a rabbit posterolateral spine fusion model, by determining the effect of the test article on posterolateral spine fusions, relative to Formagraft by assessing: fusion rate, quantity and quality of new bone formed, quantity and quality of graft resorption, and host response.

   OVERLAP: There is no overlap.
3. Engineering Endogenous Cartilage Repair (details above)  
Sponsor Agency: Arthritis Foundation  
01/01/2016-01/01/2019, $892,057 Total Direct Costs  
James A. Martin, PhD (PI)  
Douglas C. Fredericks, BS (Co-Investigator), 0.6 Calendar months

4. Silhouette Lapine Posterolateral Fusion Model  
Sponsor Agency: Biostructures, LLC  
1201 Dove Street, Suite 470  
Newport Beach, CA 92660  
09/01/2015 – 12/01/2016, $235,019 Total Direct Costs  
Douglas C. Fredericks, BS (PI), 1.2 Calendar months

Silhouette (Strip and Putty) is a bone void filler device intended for the treatment of surgical and trauma related osseous defects of the skeletal system (i.e., extremities, pelvis & spine) that are not intrinsic to the stability of the bony structure. The device comprises biphasic (HA/βTCP) granules suspended in a porous type I collagen matrix that is chemically crosslinked and lyophilized to impart porosity, hydration capability and handling properties. The goals of the study are to determine the effect of the test article (Silhouette) in posterolateral fusion and determine volume of new bone formation relative to Vitoss on the following outcomes: fusion rate (measured by palpation, motion analysis, radiography, CT, and histopathology), quantity and quality of new bone formed (measured by radiography, CT and histomorphometry), quantity and quality of graft resorption (measured by radiography, CT and histomorphometry), and host response (measured by histopathology).  
OVERLAP: There is no overlap.

5. Direct Delivery of Bone Morphogenetic Protein-2 and Fibroblast Growth Factor-2 Plasmid Genes for Diabetic Fracture Healing in a Rabbit Model  
Sponsor Agency: American Orthopaedic Foot & Ankle Society  
9400 West Higgins Road, Suite 220  
Rosemont IL 60018  
05/12/2015 – 10/31/2016, $20,000 Total Costs  
Nathan A. Nicholson, MD (PI)  
Douglas C. Fredericks (Co-Investigator), 0.12 Calendar months (no salary support)

This is an animal study investigating the effects of diabetes mellitus on bone healing in a diabetic rabbit femur fracture model. Specific aims include 1)Determine if long-standing type 1 diabetes mellitus results in alterations in gene expression, architecture, and amount of new bone at regenerate sites and 2)Determine if nonviral gene delivery with bone morphogenetic protein-2 (BMP-2) and Fibroblast Growth Factor-2 (FGF-2) will attenuate the deleterious effects of Type 1 diabetes mellitus on healing of a bone defect site.  
OVERLAP: There is no overlap.

Martin – Dr. Martin had a no-cost extension on a previously reported project and 2 new grants begin since the previous reporting period.

Extended:

1. Mitochondrial Based Treatments that Prevent Posttraumatic Osteoarthritis in a Translational Large Animal Intraarticular Fracture Survival Model (W81XWH-11-1-0583)
Sponsor Agency: United States Department of Defense, Army Medical Research Acquisition Activity  
09/01/11 – 08/31/2017, $2,559,912 Total Costs  
James A. Martin, PhD (PI), 1.73 Calendar months (no salary support after 9/30/2015)

New:
1. Engineering Endogenous Cartilage Repair (details above)  
   Sponsor Agency: Arthritis Foundation  
   01/01/2016-01/01/2019, $892,057 Total Direct Costs  
   James A. Martin, PhD (PI), 1.2 Calendar months

2. Direct Delivery of Bone Morphogenetic Protein-2 and Fibroblast Growth Factor-2 Plasmid Genes for Diabetic Fracture Healing in a Rabbit Model  
   Sponsor Agency: American Orthopaedic Foot & Ankle Society  
   05/12/2015 – 10/31/2016, $20,000 Total Costs  
   Nathan A. Nicholson, MD (PI)  
   James A. Martin (Co-Investigator), 0.12 Calendar months (no salary support)

Willey – Dr. Willey had one grant end and two new grants begin since the previous reporting period. His effort on the new projects is

Completed:
1. Reducing Surgical Site Infections by Identifying Modifiable Risk Factors in Orthopaedic Trauma Patients  
   Sponsor Agency: University of Iowa Injury Prevention Research Center  
   07/01/2014-06/30/2015, $20,250  
   Michael C. Willey, MD (PI), 0.6 Calendar months (no salary support)

New:
1. Engineering Endogenous Cartilage Repair (details above)  
   Sponsor Agency: Arthritis Foundation  
   01/01/2016-01/01/2019, $892,057 Total Direct Costs  
   James A. Martin, PhD (PI)  
   Michael C. Willey, MD (Co-Investigator), 0.6 Calendar months

2. The Effect of Sirtuin-1 on Chondrocyte Progenitor Cell Activity in Acute Cartilage Injury (details above)  
   01/01/2016 – 12/31/2016, $20,000 total direct costs  
   Jocelyn Compton, MD (PI)  
   Michael C. Willey, MD (Co-PI, Mentor), 0.12 Calendar months (no salary support)

7.3 Other Organization Involvement  
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS  
Updated Quad Chart attached

9. APPENDICES  
None
Joint Distraction Treatments of Intra-articular Fracture-Induced Post-traumatic Osteoarthritis in a Large Animal Model

OR140355
W81XWH-15-1-0642
PI: Jessica E. Goetz, PhD
Org: University of Iowa
Award Amount: $1,591,922

Study/Product Aim(s)
- Establish effect of delayed fracture fixation on PTOA development
- Determine efficacy of joint distraction for preventing PTOA when applied acutely after intra-articular fracture (IAF)
- Determine efficacy of mid-term joint distraction for reversing PTOA pathology that develops in the weeks after IAF
- Determine if articulated joint distraction results in superior tissue organization and tissue/joint function compared to static distraction

Approach
Building on previous work supported by W81XWH-10-1-0864 to create a large animal model of IAF-induced PTOA, this project will evaluate the ability of joint distraction to prevent or treat PTOA. In the first aim, joint distraction will be applied at the time of definitive fixation, and in the second aim, the joint will be distracted 12 weeks after fixation when the model is in early stages of PTOA pathology. Joint function, histological tissue structure, and cartilage biological function will provide outcome measures.

Goals/Milestones

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<tr>
<th>Goal 1 – Finalize distraction protocol</th>
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<td>✓ Mechanical validation testing of distractor configuration</td>
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Goal 2 – Determine efficacy of immediate postoperative distraction
- Develop data analysis routines (histology, µCT, confocal)
- Complete live animal work
- Analyze Specific Aim 1 data (mechanical/biological/histological)

Goal 3 – Determine efficacy of delayed joint distraction
- Finalize articulated distractor methodology
- Complete live animal work
- Analyze Specific Aim 2 data (mechanical/biological/histological)

Comments/Challenges/Issues/Concerns
- Purchasing appropriately aged animals has incurred significant costs over budget and caused major delays in surgery
- Calcaneal pin loosening required early removal of joint distraction

Budget Expenditure to Date
Projected Expenditure: $497,887
Actual Expenditure: $333,310

Timeline and Cost

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<td>(Distraction, Histology Analysis, µCT Analysis, etc.)</td>
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Updated: 11/4/2016

Accomplishment: We have completed 12 animals of Aim 1. Quantitative data analysis is ongoing, however qualitative observation suggests a delay in fixation has negative effects on cartilage health, and acute distraction has further negative effects on cartilage health.