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TITLE: “Bone Regeneration Device for Compromised Wounds”

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Juan Taboas, PhD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: This device will promote bone regeneration in compromised wounds. It addresses the critical limiting factors in repair: low osteo/chondro-progenitors, low vascular supply, and a fibrotic immune response. Our hypothesis that controlled prolonged delivery of the immunomodulatory and chondrogenic cytokines will promote bone regeneration in both comminuted fractures and critically sized bone void defects compared to no cytokine delivery. We also hypothesize that the hydrogel component will promote bone regeneration in both models via formation of a larger cartilaginous callus-like tissue. The device is designed to be applied via two different modalities depending on the nature of the bone injury: an Injectable Hydrogel device and an Implantable Hydrogel Infused Scaffold device. The injectable hydrogel is used to treat comminuted fractures and small bone deficits while the implantable hydrogel infused scaffold is used to treat large bone deficits. We will test the injectable device in a bi-lateral simulated comminuted fractures of the fibulas while the implantable device in bi-lateral fibular segmental defects in swine. The Specific Aims are: 1. Manufacture the bone regeneration devices; 2. Assess the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery; 3. Assess the functional bone healing response after 5 months post-surgery (bone formation and strength, revascularization and reinnervation).

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **Introduction**

Military personnel are substantially burdened with traumatic bone injury to the extremities, but no ideal therapy is available to regenerate large bone volumes in compromised wounds. These wounds are sub-optimal for regeneration because the vascular damage and immune response provoke oxygen deficiency and inflammation, which impair bone growth and drive formation of fibrous tissue. This project evaluates our technology to address these critical limiting factors in repair and to accelerate bone healing. It is an off-the-shelf biologic device that can be loaded with minimally manipulated autologous mesenchymal stem cells (MSCs) at the point-of-care. We evaluate its efficacy in two relevant models of bone injury, 1) a simulated comminuted fracture and 2) a critically sized bone void defect. We create these injuries in the distal fibula (bilateral) of minipigs and implant/inject the device with and without addition of autologous stem cells. We compare the device efficacy to an Infuse control group. We assess the immunomodulatory effect and potential for endochondral ossification over one month using x-ray imaging, cytokine and leukocyte profiling from blood samples, and RNAseq/gene array analysis of gene expression in regenerate tissue. We assess the functional bone healing response after 5 months post-surgery via mechanical, histological and micro-computed tomography analysis of bone formation and strength, revascularization and reinnervation.

2. Key words

Bone, cartilage, comminuted, endochondral ossification, fibrosis, fracture, gelatin, heparin, hydrogel, immunomodulation, IL-10, nanoparticles, minipig, non-union poly(ethylene glycol), scaffold, stem cell, TGF-β,

3 Summary/Specific Aims and Accomplishments

What were the major goals of the project?

The Aims of the project are:

1. Manufacture the bone regeneration devices
2. Assess the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery
3. Assess the functional bone healing response after 5 months post-surgery

The Major Goals to accomplish these are:

1. CY16 Goal – Manufacture bone regeneration devices: Fabricate sufficient hydrogel (200ml) and coacervates (2ml) for device fabrication per year (in 2-3 batches per year).
2. CY17-CY19 Goal – Implant both device types and monitor animals: Perform surgeries on 9 swine in year 1, 19 in year 2, and 17 in year 3.
3. CY18 Goal –Report initial results of terminal assays at 1 month. Co-author manuscript on the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery (months 18-28).
4. CY19 Goal – Report all assay results and advance device development. Co-author manuscript on functional bone healing response after 5 months post-surgery (months 33-36). Submit application to Coulter Foundation to perform GMP large animal pilot study (month 30)

	Timeline	**Status**
Specific Aim 1: Manufacture the bone regeneration devices.		
Major Task 1: Scaffold Manufacture	Months	

Subtask 1: Injectable Hydrogel (synthesize sufficient hydrogel for implantable and injectable devices to be used in the grant year and test the hydrogel quality with mechanical testing and NMR) 2 times each year	1-31	In progress 40%
Subtask 2: Hydrogel Sponges (fabricate for implantable devices to be used in the grant year) 3 times each year	3-32	In progress 40%
Subtask 3: Coacervate (synthesize sufficient nanoparticles and load with drugs for implantable and injectable devices to be used in the grant year. Evaluate drug delivery profile in year 1 with ELISA, e.g. release profile of IL-10) 3 times each year	3-32	In progress 40%
Milestone #1: Fabricate sufficient hydrogel (200 ml) and coacervates (2 ml) for device fabrication per year		In progress 40%
Specific Aim 2: Assess the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery		
Specific Aim 3: Assess the functional bone healing response after 5 months post-surgery		
Major Task 2: Animal Surgeries	Months	
Subtask 1: Animal Approval At least 3 to 4 months will be required for regulatory review and approval by the USAMRMC Animal Care and Use Review Office (ACURO)	1-4	Completed
Subtask 2: MSCs Preparation (isolation of autologous swine MSCs from marrow biopsy and expansion) 1.5-2 months per animal at 5 times a year	5-32	In progress
Subtask 3: Surgeries (marrow biopsy of swine receiving implants with MSCs and the implant surgeries) 0.25 months at a maximum of 5 times a year	6-32	In progress 20%
Subtask 4: Sample Harvest (excise midshaft tibia/fibula of swine limb) Total of 0.25 months over max 5 times a year	7-33	In progress 20%
Milestone #1: Perform surgeries on 9 swine in year 1, 19 in year 2 and 17 in year 3 for a total of 45 swine		In progress 20%
Major Task 3: Terminal assays at 1 month post implant surgery on 13 animals over 3 years (Year / animals: Y1 = 4, Y2 = 5, Y3 = 4)	Months	
Subtask 1: X-rays (hind limb tibia/fibula midshaft to determine orthopaedic hardware stability and qualitatively evaluate healing and bone formation) Every 2 weeks for each animal	5-33	In progress 20%
Subtask 2: Collection of blood fluid samples Pre-op and day 3 and 30 post-op for each animal	5-33	In progress 20%
Subtask 3: Cytokine profiling in blood serum Twice per year (0.5 month for samples from 3 swine)	7-35	Not initiated

Subtask 4: Immune cells characterization from blood Twice per year (1 month for samples from 3 swine)	7-35	In progress 20%
Subtask 5: Transcriptome analysis of tissue via RT-qPCR analysis Twice per year (2 months for samples from 3 swine)	8-28	In progress 10%
Subtask 6: Immunohistochemistry to identify cellular compliment Once per year (3 months for samples from 5 swine)	8-28	In progress 10%
Milestone #1: Co-author manuscript on the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery.	18-28	Not initiated
Major Task 4: Terminal assays at 5 months post implant surgery on 32 animals over 3 years (Year / animals: Y1 = 5, Y2 = 14, Y3 = 13)	Months	
Subtask 1: X-rays Every 2 weeks for first month for each animal, then monthly	5-33	Not initiated
Subtask 1: Computed tomography (µCT) imaging to quantify bone volume and ultrastructure 4 times per year (0.5 months for 2-3 swine)	18-34	Not initiated
Subtask 2: Mechanical 4-point bending (non-destructive) for bone strength 4 times per year (0.5 months for 2-3 swine)	18-34	Not initiated
Subtask 3: Histological assays for bone, cartilage, fibrous tissue, revascularization and reinnervation 4 times per year (3 months for 3 swine)	18-35	Not initiated
Subtask 4: Histological assays for immunological response in the mature engrafted tissue 4 times per year (3 months for 3 swine)	18-35	Not initiated
Milestone #2: Co-author manuscript on functional bone healing response after 5 months post-surgery	33-36	Not initiated
Milestone #3: Application to Coulter Foundation to perform GMP large animal pilot study	30	Not initiated

What was accomplished under these goals?

In general grouped by Goal:

1. CY16 Goal: Met 100% for CY16 (40% completion over life of grant). Major developments include:
    a. Further characterized the kinetics of peroxide crosslinking method for hydrogels
    b. Determined that the TGFβ-1 ELISA plates manufactured by R&D Systems are unreliable.
    c. Optimized the sponge scaffold manufacturing procedure to enhance pore interconnectivity. We have now modified the sponge design so that it is a hollow cylinder (wall approximately 1-2mm thick.)
2. CY17-CY19 Goal: Met 20% for CY17. 9 animals were treated with both the segmental and comminuted defects, and sacrificed after one month of healing.
3. CY18 Goal – Report initial results: In progress. Our TGFβ-1 loaded devices (no IL-10 or stem cells) promoted significant bone regeneration, evidenced by woven bone and some small pockets of cartilage.
4. CY19 Goal – Report final results and advance device development: Not initiated

Major Task 1: Scaffold manufacture

This task is in support of Aim 1, to manufacture the bone regeneration devices (sans cells) which are composed of hydrogel, sponge scaffolds, and a drug delivery system.

Regarding Subtask 1, we are using 8% (w/v) hydrogel formulations instead of the proposed 10% (w/v). We are using the LAP initiator for irradiation activated crosslinking of the segmental defect devices while the persulfate based initiator for the comminuted defect devices; we abandoned the Tetrakis crosslinking because it was labile (the hydrogels dissolved after several days in vitro in PBS). We have further characterized the kinetics of persulfate crosslinking, and have tested sodium versus potassium persulfates. A 10 mM concentration for gelation (both persulfate and ascorbate) has been selected. Pilot mechanical testing of the scaffold strength under LAP and persulfate initiators has been performed; we found that the persulfate crosslinker does not yield a hydrogel with gelatin but does with PEG and PGH. We will perform an in-depth study comparing LAP and persulfate on the properties of the PGH scaffold.

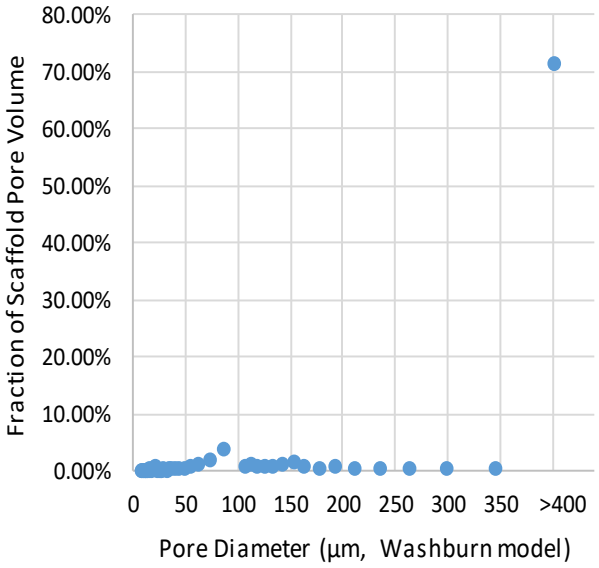

Figure 1: Mercury porosimetry results show over 70% (v/v) of the pore volume consists of cylindrical pores over 410µm in diameter.

Regarding Subtask 2, we have finished optimizing the sponge scaffold manufacturing procedure to enhance pore interconnectivity. We have now modified the sponge design so that it is a hollow cylinder (wall approximately 1-2 mm thick). The purpose is to facilitate infiltration with the stem cell laden PGH hydrogel without impairing cell viability. Mercury porosimetry results show over 70% (v/v) of the pore volume within the sponge scaffolds consists of cylindrical pores over 410 µm in effective diameter. The next largest volume of pores was in the 80-90 µm size range. Pores less than 50 µm compose only a small fraction of the scaffold (**Figure 1**.) The porosity and pore size of the sponge scaffold are in an optimal range for bone and cartilage formation. We determined that our de-waxing and thermal crosslinking method of sponge fabrication does not significantly change the scaffold internal architecture (via micro-CT imaging). . The cytotoxicity of our scaffolds has been evaluated using an in vitro transwell culture system over ten days. We found that the heat annealing, deparaffinization, rinsing and desiccation left no trace of toxic organic solvent in our sponge scaffolds, and that washout with isopropanol is unnecessary.

Gamma irradiation slightly decreases the hydrogel mechanical properties, but within an acceptable range; for example, irradiated pre-polymers yield a hydrogel (10% w/v) with 8.0-9.0 kPa relaxation modulus as opposed to un-irradiated polymers which yield a relaxation modulus between 10-15 kPa. However, gamma irradiation is unsuitable when the prepolymers are stored because they slowly crosslink over time. Therefore we use filtration sterilization for the hydrogels, and irradiation for the sponges

Regarding Subtask 3, we found that the coacervates release the drugs rapidly from the hydrogels as opposed to from distilled water. We have used ELISA assays to evaluate drug release from our devices in vitro (e.g. **Figure 2B**). However, ELISA does not indicate bioactivity of a drug (functional signaling,) merely the presence of the drug. We piloted a reporter cell line to evaluate functional signaling of TGF-βs via the receptor. It was found that the Preprotech TGF-β1 was 70% as potent as the R&D brand (**Figure 2A**).We improved the reliability of our ELISA assays by changing the plastic ware from polystyrene to polypropylene, and by including 0.1% w/v BSA in the elution solution (both serve to inhibit fouling of the plastic ware with the drug.) It was determined that the TGFβ-1 ELISA plates manufactured by R&D Systems are unreliable; they yield background signal when the analyte has any

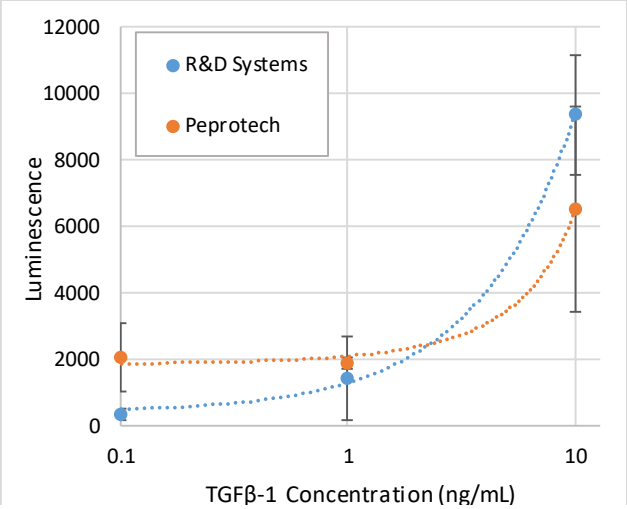

Figure 2A. The Preprotech brand TGF-β1 is not as potent as the R&D Systems brand. We used a reporter cell line to evaluate functional signaling of TGF-βs via the receptor. At 10 ng/ml, the Preprotech TGF-β1 is approximately 70% as potent as the R&D brand. We therefore switched supplier to R&D.

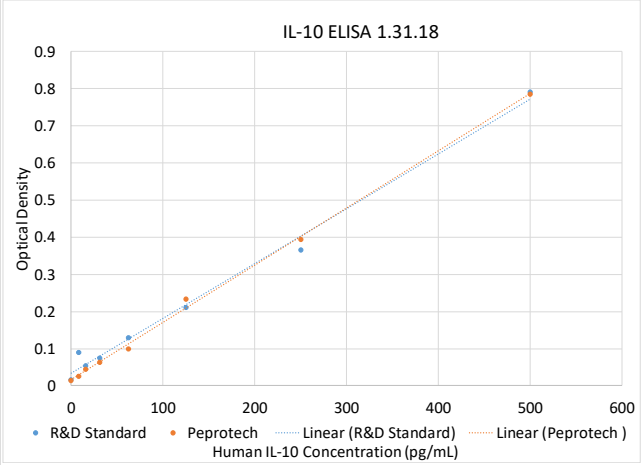

Figure 2B. The concentration of IL-10 sourced Peprotech and R&D is similar, via ELISA.

of the polymer precursors that compose our PGH hydrogel. We have ordered ELISA plates that use antibodies which recognize a different epitope of the TGFβ. It has been verified that the IL-10 ELISA plates do not suffer the same issue. We have not analyzed the effect of the hydrogel crosslinkers on the bioactivity of the drugs because of the aforementioned background problem.

Major Task 2: Animal Surgeries

The animal surgeries task is a major component of Specific Aims 2 and 3. These aims asses the immunomodulatory and regenerative potential of the devices in the pig model. We performed surgery on animals in May and June. The main purpose of this run of surgeries was to gather data on the short-term disposition of the implants (1 month), and to perform quality control testing of our outcome assays.

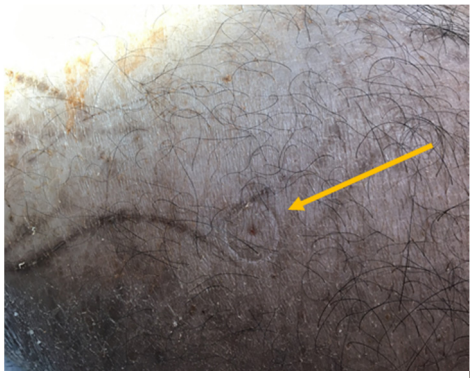

Figure 3. Bone marrow aspirates were taken from iliac crest of the pilot animal post-sacrifice to evaluate the cell isolation and expansion protocol.

Regarding Subtask 1, we performed surgery on one pilot animal in December to satisfy concerns voiced by the IACUC veterinarian. To demonstrate that the bilateral defect would be well tolerated, one comminuted and one segmental defect were made, both were left unplated. The pilot animal was taken down on 1/18/18 (operated to satisfy IACUC review). We received ACURO approval on 01/31/2018.

Regarding Subtask 2, we tested our bone marrow aspiration procedure using the cadavers of our pilot animal but the total aspirate volume was low (**Figure 3**). The bone marrow aspiration procedure was optimized using fresh pig cadavers from another study. We found that a multi-perforated needle is best for extracting the necessary marrow volume. We verified that the cells grow well with our culture protocol and found Ficol works well to purify the aspirate before plating mononuclear cells.

Regarding Subtask 3, surgeries were performed on nine animals in May and June. The main purpose of this run of surgeries was to gather data on the short-term disposition of the implants (1 month), and to perform quality control testing of our outcome assays. We created both defect models (critical size and comminuted) in bilateral defects, as proposed. We treated the defects with some of the proposed experimental groups. We used LAP

crosslinking for the devices implanted into the critical sized defect, and persulfate crosslinking for the injectable solutions into the comminuted defects.

Regarding Subtask 4, we have euthanized the animals and are processing the tissue samples for RNA sequencing and histological analysis. We also collected blood samples and subjected them to flow cytometry assays for some of the proposed cellular markers. We also biopsied lymph node tissue to test the potential for cytokine profiling/histological assay. These animals were off protocol in age and did not incur cost to acquire.

Major Task 3: Terminal assays at 1 month

This task focuses on analyzing the immunomodulatory effect and endochondral ossification potential of the devices.

Regarding Subtask 1, we followed surgeries with x-rays post-op and then every 2 weeks for each animal (**Figure 4**.)

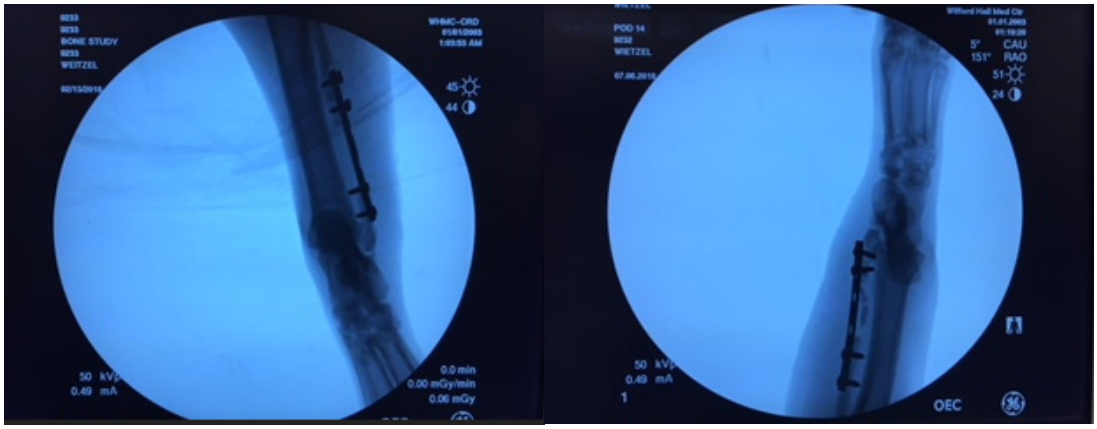

Figure 4. (Left) Plated segmental bone defect treated with a sponge scaffold loaded with TGFβ-1. The sponge is radiotranslucent **(Right)** Plated comminuted fracture defect left untreated.

Regarding Subtask 2, we collected blood samples at pre-op and 3 and 30 days post-op. A portion of each was subjected them to flow cytometry assays for several of the proposed cellular markers (Subtask 4), while another were stored for future cytokine profiling assays.

Regarding Subtask 3, cytokine profiling validation is pending. Total serum was gathered instead of plasma, which is not according to our protocol and not ideal for cytokine profiling; platelet factors are released into serum which masks systemic cytokine changes.

Regarding Subtask 4, we acquired the needed antibodies for flow cytometry and all our panels are complete. The circulating leukocyte populations are similar across treatments (**Figure 5**). They reflect an upregulation of

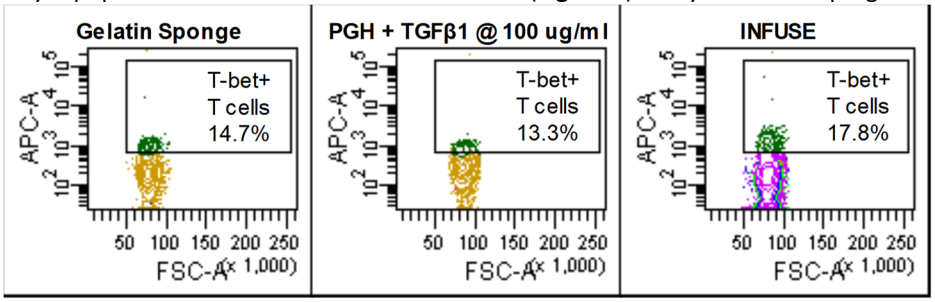

Figure 5. The circulating lymphocyte population maintains a Th1 phenotype after 1 month healing. Flow cytometry of blood draws shows Th1 cells (T-bet+) among devices, but no circulating Th2 cells (GATA-3+, not shown). The PGH+TGFβ-1 device appears to decrease Th1 levels, but this is not significant.

inflammatory cells post-op. We also attempted isolating lymphocytes from the draining lymph nodes (post-sacrifice), but the yield was low; we are currently optimizing our protocol.

Regarding Subtask 5, we changed the RNA isolation protocol to use a tissue homogenizer instead of mortar and pestle grinding of frozen tissues and continue to preserve tissues with RNAlater. RNA quality is good. Currently, the RNA-seq validation is pending. Next quarter we will harvest control tissues for RNA sequencing.

Regarding Subtask 6, histological sectioning has been validated, however, validation of the immunohistochemical staining is incomplete at this time. We have stained a set of samples from these 9 animals with 1) hematoxylin and eosin to identify bone, fibrous tissues and cells, 2) Safranin O and fast green counterstain to identify cartilaginous tissue and cells, 3) two antibodies to identify M1 type macrophages and total macrophages. It was found that the TGFβ-1 delivery from our devices enhances bone regeneration in segmental defects at one month compared to untreated controls (**Figure 6**). The INFUSE clinical standard also regenerates bone, but also yields ectopic bone. Furthermore, the INFUSE group shows inflammatory macrophages unlike defects treated with our devices, or left untreated (**Figure 7**). It was found that pan-macrophage antibody stains osteoblasts as well, which makes it unideal for our purposes. We will test alternates.

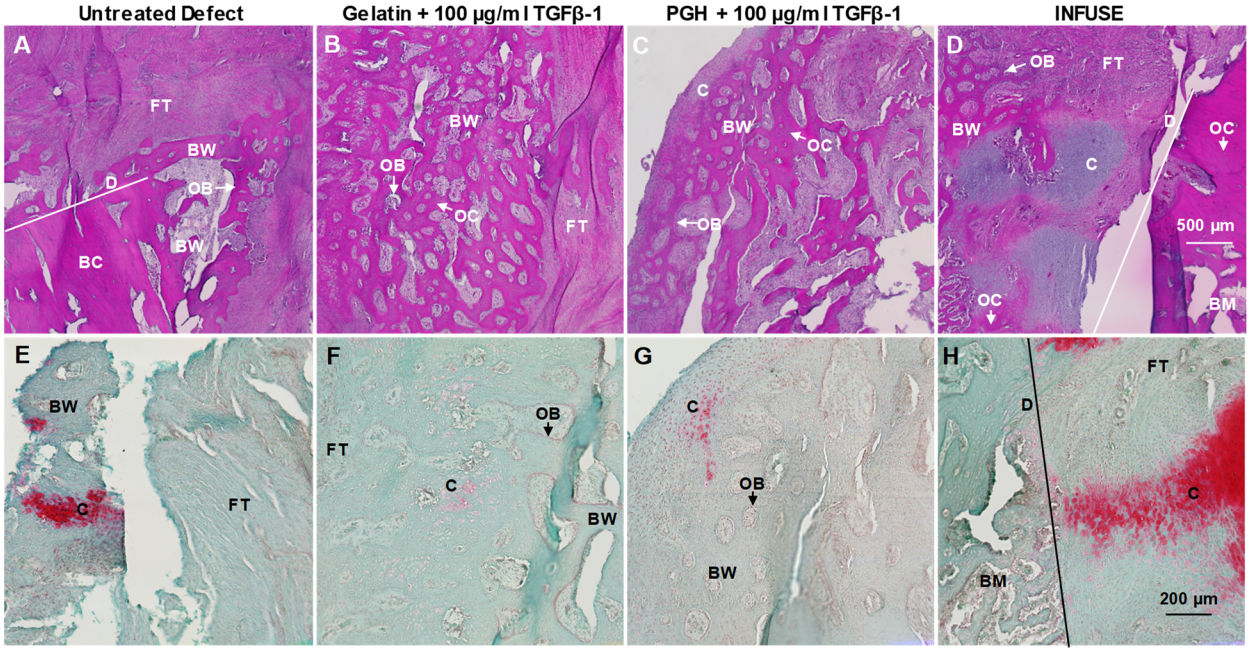

Figure 6. TGFβ-1 devices (with PGH hydrogels or gelatin sponges) lead to significant woven bone formation in the 3cm segmental fibular defects after one month healing (woven bone as evidenced by lack of lamellar structure and high osteocyte content compared to the cortical bone). This effect occurred with both PGH hydrogels alone and gelatin sponges alone (the two material components of our device). (A,E) Untreated defects showed woven bone near the osteotomy site (A) but no bone in the defect proper. (B,F) Gelatin scaffolds + 100 µg/ml TGFβ-1 produced significant woven bone in the defect proper (B) with some evidence of cartilage (F). (C,G) PGH scaffolds + 100 µg/ml TGFβ-1 also yielded woven bone (C) and some cartilage (G). Woven bone contained high osteocyte density (OCs) and active osteoid surfaces lined with osteoblasts (OBs). (D,H) INFUSE also yielded regeneration but with more cartilage (H) evident at one month than treatments with TGFβ-1. INFUSE also yielded ectopic bone (not shown). BC = Cortical Bone, BW = Woven Bone, BM = Bone Marrow, FT = Fibrous Tissue, C = Cartilage, M = Muscle, D = Osteotomy Margin, OB = Osteoblast, OC = Osteocyte. (A-D stains: pink = bone and fibrous tissue via eosin, violet = cartilage and dark purple = cell nucleus via hematoxylin. E-F stains: red = cartilage via Safranin O, Green = bone and fibrous tissue via fast green)

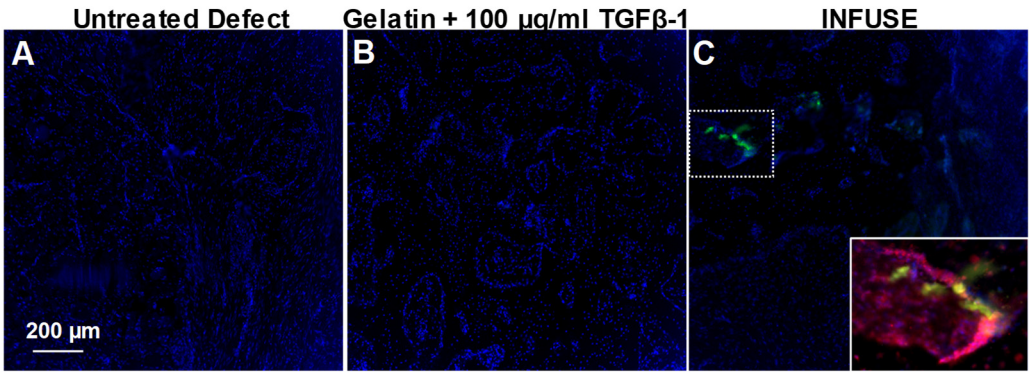

Figure 7. Untreated (A) and TGFβ-1 (100 ug/ml, B) defects show no M1 inflammatory macrophages after 1 month healing. However, INFUSE defects (C) still show M1 cells (Green). Inset of dashed box shows the M1 marker co-localizes (yellow) with a pan-macrophage marker (Red = CD86, also stains osteoblasts which line the woven bone). Only TGFβ-1 and INFUSE treated defects showed woven bone in the defect proper. (Green = MAC387, Blue=DAPI)

Major Task 4: Terminal assays at 5 months

This task focuses on the bone regeneration efficacy of the devices. It was not initiated because the animal surgeries have not begun.

What opportunities for training and professional development has the project provided?

1. Two surgical residents participated in the non-survival surgery
2. The technician was trained in numerous techniques needed in the project (scaffold fabrication, material modification, immunohistochemistry, cell culture etc.)

How were the results disseminated to communities of interest?

1. We submitted a US patent application on our devices, application No. 62/684,401, filed June 13, 2018.
2. We also filed the final patent application on our hydrogel formulations: USA # 62/385,363 and international application # PCT/US2017/020765 on March 3, 2017
3. Presentation at the University of Texas Health Science Center Symposium, June 15, 2018, San Antonio, TX
4. No other presentations specific to this work at this time due to delay in animal protocol approval by Air Force lab.

What do you plan to do during the next reporting period to accomplish the goals?

1. Evaluate the TGFβ-1 and IL-10 delivery profiles from the PGH hydrogels.
    a. Test the effect of persulfate crosslinkers on the bioactivity of the drugs.
    b. Test BSA as a carrier for TGF on the scaffolds to improve solubility on implantation
2. Perform mechanical tests on our hydrogel formulation under both crosslinkers (LAP and persulfate system).
3. Modify IACUC protocol for isolation of lymphatic tissue
4. Perform experiments on 3 pigs (on-protocol surgeries) evaluating the first of stem cell devices.
    a. Perform survival bone marrow biopsies
    b. Run responder experiments with the pig cells (osteogenic and chondrogenic assays), including the effect of the combined drugs (planned in vitro).
    c. Perform lymph tissue biopsies
5. Harvest control tissues for validating RNA-seq

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

1. "Nothing to Report."

What was the impact on other disciplines?

1. "Nothing to Report."

What was the impact on technology transfer?

1. "Nothing to Report."

What was the impact on society beyond science and technology?

1. "Nothing to Report."

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

1. "Nothing to Report".

Actual or anticipated problems or delays and actions or plans to resolve them

1. The animal surgeries were delayed by one year because we changed animal facilities from the TSRL (Tri-Service Research Laboratory) to the Air Force Laboratory (AF lab) in San Antonio. Though the IACUC had already approved our animal protocol at the TSRL, the chief veterinarian at the AF lab wanted verification that the bilateral fibular defect model did not affect the animal ambulation and well-being (though it is a published model). The protocol was finally approved Q2 after were performed a surgical procedure on a pilot animal. We were subsequently able to perform the first surgeries in Q3 and have scheduled our next surgeries for Q1 2018.
2. The flow cytometer required maintenance which delayed testing the antibody panel. It has been subsequently repaired and the antibody panels verified
3. The TGFβ ELISA plates from the vendor R&D Systems gave high background with our polymers. We have purchased ELISA plates from an alternate vendor that employs antibodies which recognize different antigens than the R&D plates. These will be tested for accuracy. Should these prove unreliable, we wil use mass spectroscopy to quantify TGF concentration and the luciferase reporter system to quantify bioactivity (see Figure 2A).

Changes that had a significant impact on expenditures

1. The above delay in IACUC approval has effectively delayed the project by one year. The animal procedures incur a large cost. This decreased expenditures
2. PI effort was decreased to compensate for the above, seeking no effort in Q1 while funds were not released, and decreasing effort by 50% last quarter.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

1. Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. Examples of products include:

Publications, conference papers, and presentations

1. Presentation at the University of Texas Health Science Center Symposium, June 15, 2018, San Antonio, TX

Books or other non-periodical, one-time publications.

1. "Nothing to Report."

Other publications, conference papers, and presentations.

1. "Nothing to Report."

Website(s) or other Internet site(s)

1. "Nothing to Report."

Technologies or techniques

1. "Nothing to Report."

Inventions, patent applications, and/or licenses

1. US patent application on our devices, application No. 62/684,401, filed June 13, 2018.
2. Final patent application filed on the hydrogel technology: USA # 62/385,363 and international application # PCT/US2017/020765 on March 3, 2017

Other Products

1. "Nothing to Report."

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Project Role	Research Identifier	Person Months Worked	Contribution to Project	Funding Support
Alejandro Almarza	Co-I		2	Mechanical testing for biomaterials and tissues. Animal surgeries, data acquisition and interpretation.	
Jingming Chen	Graduate Student		12	Methacrylation, NMR, and mechanical testing of hydrogels. Biocompatibility assays.	
Jennifer Cox	Laboratory Administrator		1	Management of sub-award laboratory, supplies ordering, schedule coordination.	ISR

Tyler Swenson	Research Technician		12	Preparation and analysis of all implantable device materials, cell culture.	
Michael Sippel	Research Resident		4	IACUC approval, surgical work.	USISR
Juan Taboas	PI		3	Preparation of animal protocol. Development of biomaterials and devices. Animal surgeries, data acquisition and interpretation. Overall management of project	
Yadong Wang	Co-I	0000-0003-2067-382X	1	Development of PEAD polymer and coacervate drug delivery system. Zeta testing of coacervates. Now sub-award PI with Cornell university (left Pittsburgh)	
Erik Weitzel	Co-I		1	Sub-award PI. Animal surgeries, data acquisition, and interpretation. Foster collaboration with sub-award	USISR
Bijaya Parida	Research Scientist		2	Flow cytometry	USISR

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

1. "Nothing to Report."

What other organizations were involved as partners?

We have two sub-awards in this grant. They do not provide financial or in-kind support, but naturally are collaborators on the project and provide facilities and personnel for the work:

1. Metis Foundation. 300 Convent St, San Antonio, TX 78205. The role of the metis is to manage the sub-award with the DOD co-investigators (Dr. Eric Weitzel).
2. Cornell University, Ithaca, NY 14854. This is the new institution of our Co-I Yadong Wang whom left the University of Pittsburgh.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

1. "Nothing to report"

QUAD CHARTS:

1. Attached

9. APPENDICES:

None

Bone Regeneration Device for Compromised Wounds

W81XWH-16-1-0793

PI: Juan M Taboas, PhD **Org:** University of Pittsburgh **Award Amount:** $2,099,557

Study/Product Aim(s)

- Manufacture the bone regeneration devices
- Assess the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery
- Assess the functional bone healing response after 5 months post-surgery

Approach

We will evaluate two devices that accelerate healing of large bone injuries using a bilateral porcine fibula injury model. We will test an injectable device to treat comminuted fractures using a 3 cm simulated comminuted fracture of the fibula, and an implantable device to treat large bone defects using a 3 cm fibular defects.

We will evaluate the host immune response (systemic and in neo-tissue) and functional bone healing using biochemical, mechanical, histological and immunohistochemical assays.

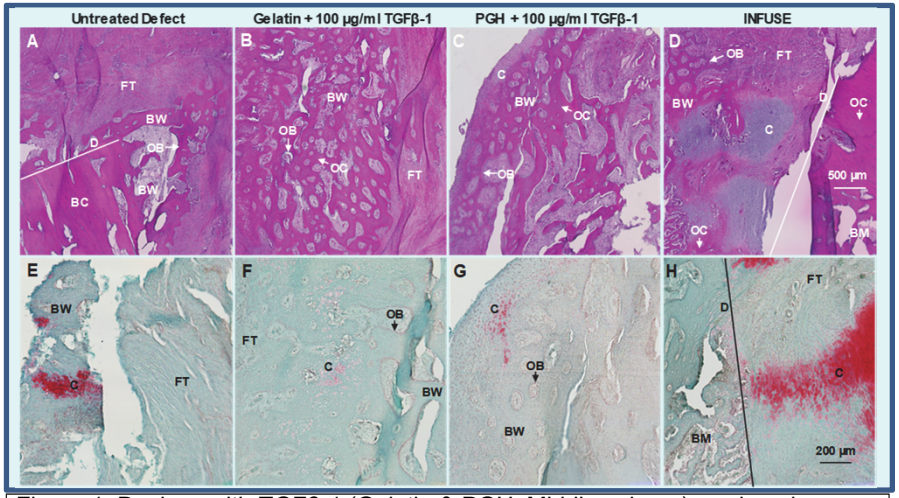

Figure 1. Devices with TGFβ-1 (Gelatin & PGH, Middle column) produced woven bone (BW) in 3 cm segmental fibular defects after one month healing. Untreated (Left) showed fibrous tissue (FT). INFUSE (Right) yielded bone and cartilage (C). (Top row = eosin, dark pink = bone; Bottom row = safranin O, red = cartilage)

Timeline and Cost

Activities CY	16	17	18	19	20
Scaffold Manufacture					
Animal Surgeries					
Terminal assays 1 month post-op					
Terminal assays 5 months post-op					
Budget in $K, (estimated)	**$35**	**$301**	**($405)**	**($686)**	**($673)**

Updated: 10/15/2018

Goals/Milestones (Example)

CY16 Goal – Manufacture bone regeneration devices

☑ Fabricate sufficient hydrogel (200ml) and coacervates (2ml) for device fabrication per year (in 2-3 batches per year).

CY17-CY19 Goal – Implant both device types and monitor animals

☑ Perform surgeries on 9 swine in year 1, 19 in year 2, and 17 in year 3

CY18 Goal –Report initial results of terminal assays at 1 month

☐ Co-author manuscript on the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery (months 18-28)

CY19 Goal – Report all assay results and advance device development

☐Co-author manuscript on functional bone healing response after 5 months post-surgery (months 33-36)

☐ Submit application to Coulter Foundation to perform GMP large animal pilot study (month 30)

Comments/Challenges/Issues/Concerns

- We evaluated the outcome of our surgeries to evaluate our assays and the short-term disposition of our implant devices.
- The timeline reflects no-cost extension due to surgery delay.

Budget Expenditure to Date: $ 615,216