AWARD NUMBER: W81XWH-16-1-0793

TITLE: “Bone Regeneration Device for Compromised Wounds”

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Pittsburgh, Pa 15213-3320

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Fort Detrick, MD 21702-5012

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Juan Taboas, PhD

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University of Pittsburgh
3520 Fifth Avenue
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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 65%
Subtask 2: Hydrogel Sponges (fabricate for implantable devices to be used in the grant year) 3 times each year	3-32	In progress 65%
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 100%
Milestone #1: Fabricate sufficient hydrogel (200 ml) and coacervates (2 ml) for device fabrication per year		In progress 65%
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 90%
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 60%
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 40%
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 60%
Major Task 3: Terminal assays at 2 weeks 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 90%
Subtask 2: Collection of blood fluid samples Pre-op and day 3 and 30 post-op for each animal	5-33	In progress 90%
Subtask 3: Cytokine profiling in blood serum Twice per year (0.5 month for samples from 3 swine)	7-35	In progress 50%

Subtask 4: Immune cells characterization from blood Twice per year (1 month for samples from 3 swine)	7-35	In progress 90%
Subtask 5: Transcriptome analysis of tissue via RT-qPCR analysis Twice per year (2 months for samples from 3 swine)	8-28	In progress 30%
Subtask 6: Immunohistochemistry to identify cellular compliment Once per year (3 months for samples from 5 swine)	8-28	In progress 20%
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	In Progress 20%
Subtask 2: Computed tomography (µCT) imaging to quantify bone volume and ultrastructure 4 times per year (0.5 months for 2-3 swine)	18-34	In Progress 20%
Subtask 3: 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 4: Histological assays for bone, cartilage, fibrous tissue, revascularization and reinnervation 4 times per year (3 months for 3 swine)	18-35	In Progress 20%
Subtask 5: Histological assays for immunological response in the mature engrafted tissue 4 times per year (3 months for 3 swine)	18-35	In Progress 5%
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?

Overview, grouped by Goal:

1. CY16 Goal (Manufacture bone regeneration devices): Met 100% of goal for CY18 (65% completion over life of grant).
2. CY17-CY19 Goal (Implant both device types and monitor animals): Met 100% of goal for CY18 (total of 60% completion over life of grant). We have operated a total of 26 animals under the experimental protocol. We changed the short term immuno-assay time-point from one month to two weeks based on the results of our pilot animals (discussed in prior quarterly reports).
3. CY18 Goal (Report initial results of terminal assays at 2-weeks): In progress. We are performing immunohistochemical analysis of the 2 week animals, which were operated June-Sept 2019. However, we are finalizing a manuscript describing results of the pilot one-month animals (discussed in prior quarterly report).
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 continue using the 8% (w/v) hydrogel formulation throughout all experimental treatments. We continue using the LAP initiator for irradiation activated crosslinking of the segmental defect. However we have pivoted to employ the same crosslinking mechanism for the comminuted defects; we did so to increase repeatability of treatments and ability to compare results across defect models. Namely, the injected hydrogel was found to flow from defect area before crosslinking. We now place the three bone fragments of the comminuted defect in a mold into which we pour the hydrogel precursor, and then crosslink (Figure 1). This encases the bone fragments into a construct we then implant into the boney defect, mimicking hydrogel formation around the bone fragments in situ.

Regarding Subtask 2, we continue manufacturing as described in the Y2 annual report. We utilize molds fabricated in house to cast the hydrogel into the half-cylinder sponges (Figure 1).

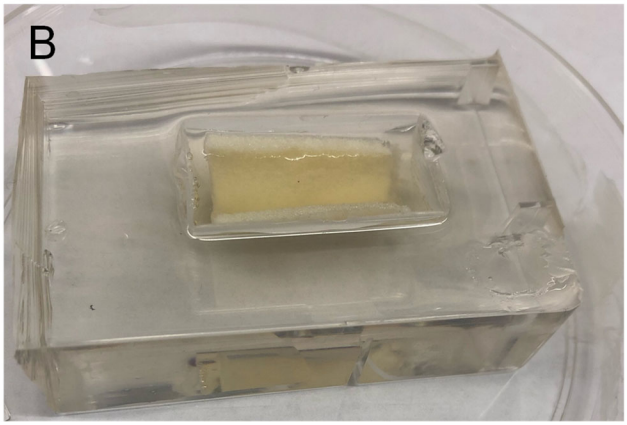

Figure 1: Final device for segmental and comminuted defects. For segmental defects, the external sponge is placed in a clear silicone mold, and then they hydrogel precursor cast into the interior (loaded +/- cells, TGFβ-1, and IL-10). The hydrogel is then irradiated yielding the final implant as depicted. For comminuted defects, the bone fragments are placed in the silicone mold, the hydrogel precursor cast, and the precursor irradiated to yield fragments embedded in the hydrogel. The mold serves to contain the liquid hydrogel precursor prior to gelation and hydrogel formation.

Regarding Subtask 3, we previously eliminated the coacervates, and we continue to evaluate the spatiotemporal profile of drug release (IL-10 and TGF-β1) from the hydrogel. The coacervates released the drugs too rapidly to offer any benefit over simple complexation with the hydrogel itself. We identified ELISA plates with antibodies that do not produce the background found with the R&D Systems plates. We further studied the drug release profiles from the hydrogel in vitro including analysis of content retained in the hydrogel over time (Figure 2).

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 have performed surgeries this year on 26 animals, a significant ramp-up compared to last year (when we performed the pilot surgeries on 9 animals). All surgeries this year are under the study protocol.

Regarding Subtask 1, task is complete.

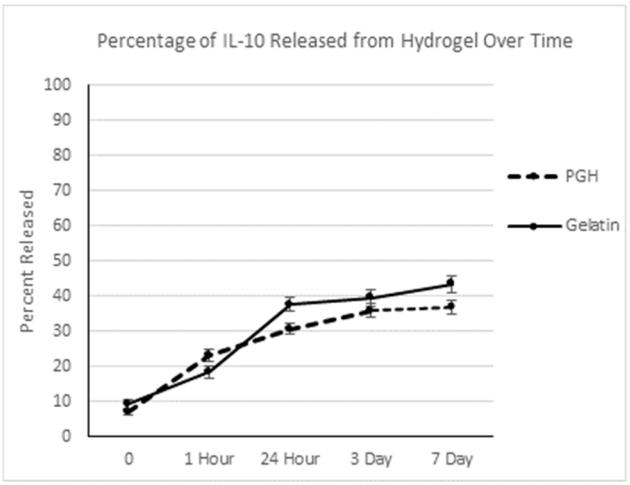

Figure 2: The hydrogel scaffold (PGH) delivers IL-10 more slowly than the gelatin control, with approximately 37% delivered after 7 days. Hydrogels were maintained in PBS at 37C and the PBS exchanges at the listed times. IL-10 was loaded at 1.0 µg/ml into the hydrogels and the ratio of hydrogel:PBS was 3:10. Higher loading is expected to lead to a greater percentage release because drug mobility increases as binding sites (e.g. Heparin) become saturated.

Regarding Subtask 2, cell expansion rates are rapid, such that about half the animals have had sufficient cell numbers at passage 3 for bilateral transplants. We have operated 2 of the 2-week animals with cells, and 3 of the 5-month animals with cells.

Regarding Subtask 3, surgeries were performed on 12 animals for the 2-week time-point and 14 animals for the 5-month time-point. All tissues for the 2-week animals have been harvested. We have completed almost all (40%) of the experimental treatments with autologous stem cells.

Regarding Subtask 4, all tissues for the 2-week animals have been harvested and are in process for immunohistochemical analysis. We are also processing the tissue biopsies for RNA sequencing. We have harvested about half of the 5-month animal tissues (from those animals operated this year). All of 5-month samples have been micro-CT scanned, and are now being sectioned for histochemical analysis.

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 at the 2-week necropsy. No mineralized tissue is observed at 2 weeks in the defect sites.

Regarding Subtask 2, we modified the protocol (as described in last year's report) to collect both blood and lymph node biopsies at pre-op, days 1, 3, 7 and 14 post-op. A portion of the blood was allocated for Subtask 3 (cytokine profiling assays), while second portion to Subtask 4 (flow cytometry assays). The node biopsies were all allocated to Subtask 4.

Regarding Subtask 3, we gathered the plasma and subjected samples to cytokine profiling on the Illumina platform. Results to date using the pilot animals show no consistent expression pattern of cytokines (TNFα, IFNγ, Il-1a, Il-1b, Il-6, IL-8, IL-10) among treatment groups (Figure 3). Patient (animal) variability is the major contributor to variance of the data.

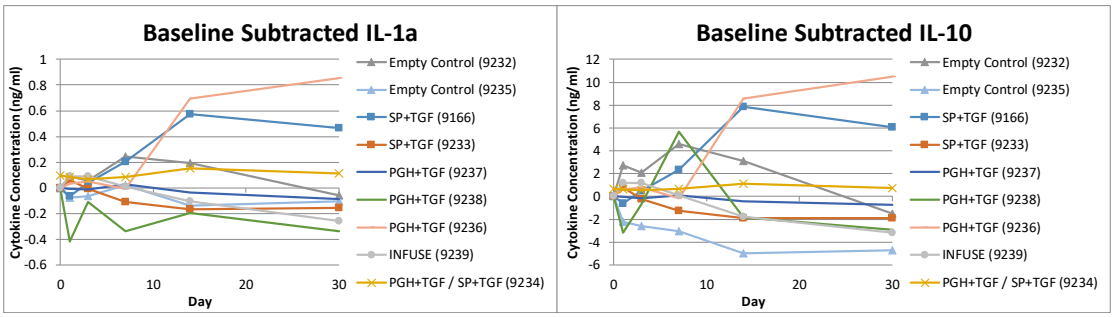

Figure 3: IL-1a and IL-10 plasma cytokine levels at pre-op and post-op days 1, 3, 7, 14 and 30 in the pilot animals. SP=sponge, PGH=hydrogel, TGF=TGFβ-1 at 100 µg/ml, INFUSE=commercial kit with collagen sponge and 1.5 mg/ml BMP-2. Baseline levels pre-op subtracted from all readings. The number in parenthesis is the animal code.

Regarding Subtask 4, flow analysis was performed in real-time when the samples were collected (blood and lymph node biopsies). We perfected our isolation of cells from the lymph nodes. We harvest biopsies from some of the 5 month animals to account for poor cellular yield from some of the biopsies. Results to date using the pilot animals show that we have perfected our assay (Figure 4). Analysis of subsequent assays is pending.

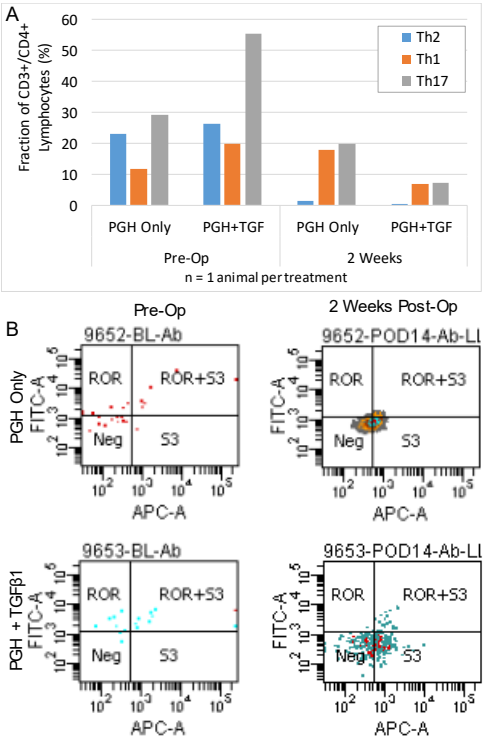

Figure 4. Flow cytometry analysis of T helper cell phenotypes in inguinal nodes at pre-op and 2 weeks post-op in the pilot animals. This subset of animals were treated with either the PGH+TGFβ-1 (100 µg/ml) or PGH alone (n=1 each). (A) The fraction of T helper cells in CD3+/CD4+ lymphocytes. Delivery of TGFβ-1 appears to reduce pro-inflammatory Th1 and Th17. (B) Representative gating for Th17. The 2 week samples reflect improved isolation of cells from the lymph node samples.

Regarding Subtask 5, we are currently completing the RNA-seq and analysis is pending, comparing across treatments and to control tissues (bone and cartilage).

Regarding Subtask 6, we have validated our immunohistochemical stains and are processing samples for histochemical and immunohistochemical staining. To date, we observe that the hydrogel and sponge scaffold are almost fully resorbed at 2 weeks (approximately 5%-35% by area of histological sections, Figure 5). We have analyze the effect of the of TGFβ-1 delivery from PGH on the leukocyte milieu within the segmental defect at two weeks post-op in two animals. Compared to PGH alone (without of TGFβ-1), delivery of TGFβ-1 decreased the Th (T helper cell) infiltrate at 2 weeks (Figure 6).

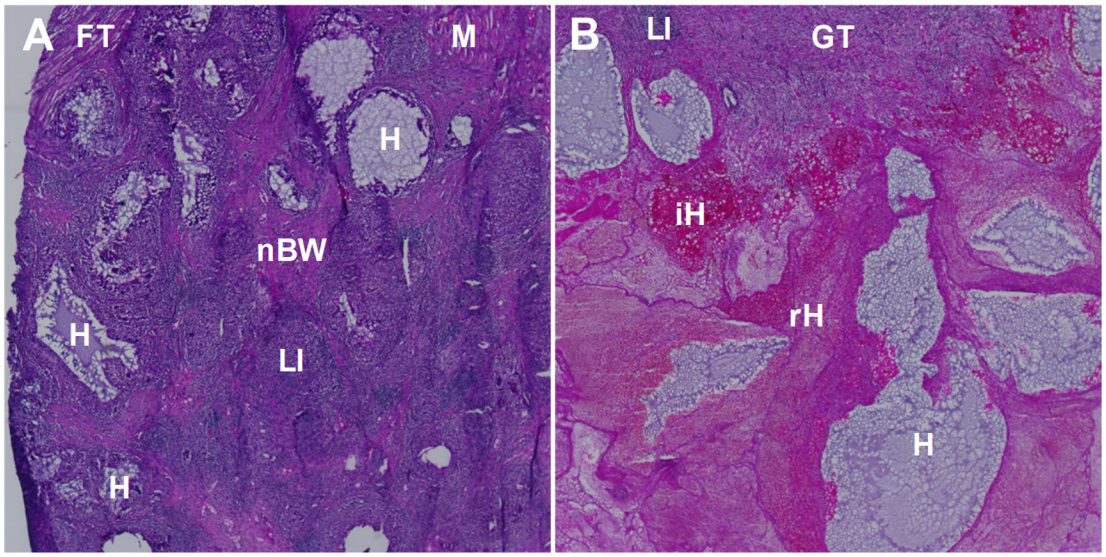

Figure 5: PGH alone (Left) versus PGH + TGFβ-1 (100 µg/ml, Right). The PGH hydrogel undergoes resorption over two weeks in vivo through several stages, including cell infiltration (iH) and cell mediated resorption (rH). In that time, fibrous tissue (FT), leukocyte infiltrate (LI), and neo-woven bone (nBW) are formed. Delivery of TGFβ-1 leads to faster resolution of the acute inflammation, as evidenced by less LI and more GT and matrix. H = PGH hydrogel, iH = cell infiltrated PGH, rH = degrading PGH, GT = Granulation Tissue, M = Muscle (Eosin stain: pink = bone, fibrous tissue, & cell cytoplasm, dark pink = iH, light diffuse pink = rH; Hematoxylin stain: light violet = PGH hydrogel, violet = cartilage, and dark purple = cell nucleus)

Major Task 4: Terminal assays at 5 months

This task focuses on the bone regeneration efficacy of the devices. We have sacrificed 7 out of the 14 5-month animals at the end of August and in September.

Regarding Subtask 1, we followed the surgeries with x-rays post-op and at 5 months, but the animal protocol did not permit additional monthly x-rays. No evidence of regenerate mineralized tissue was observed at 5 months in both the segmental and comminuted defects, irrespective of the treatment group (at this time, no INFUSE groups have been sacrificed at this time). In addition, the bone fragments of comminuted defects appear to be resorbing.

Regarding Subtask 2, all 7 samples harvested to date have been µCT scanned. Results support interpretation of x-rays at 5 months.

Regarding Subtask 3, the mechanical testing was deemed incompatible with the study because of the delay in sample fixation due to shipping and µCT. The shipment of samples from our vivarium takes 24 hours, and the µCT scan times are 7 hours per sample. Mechanical testing requires unfixed tissues (to test native properties), while the µCT fixed to eliminate tissue decay and loss of epitopes. Thus mechanical testing was removed in favor of the µCT and immunohistochemical analysis.

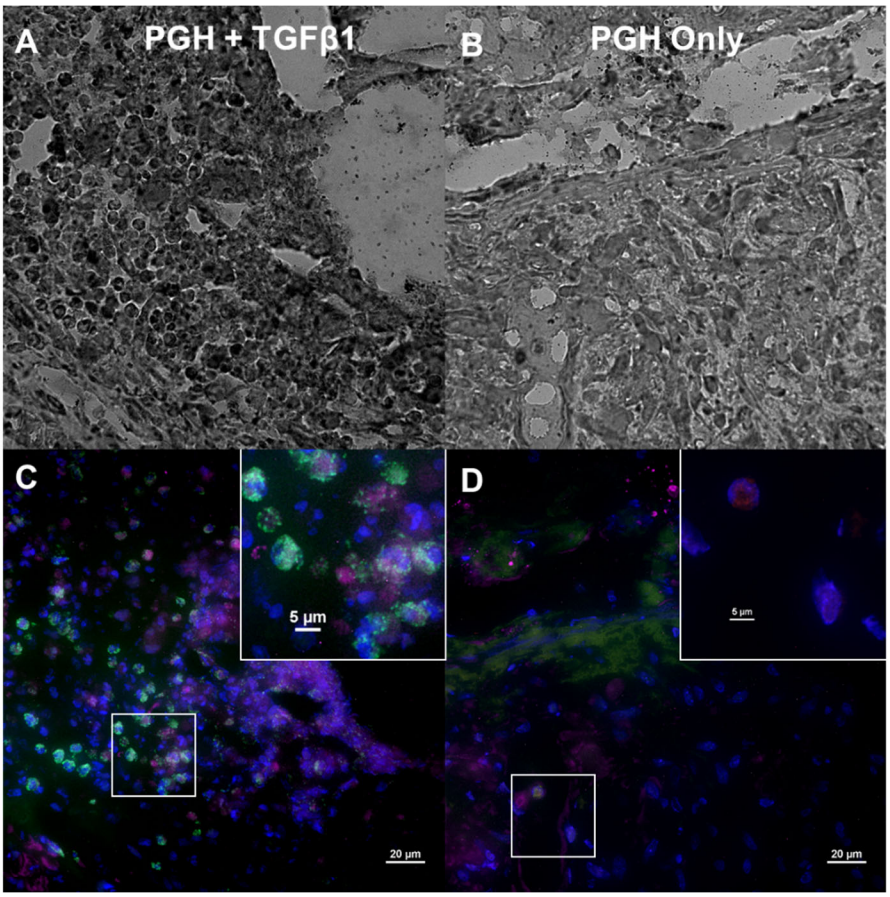

Figure 6: Delivery of TGFβ-1 (100 µg/ml) from PGH decreases the abundance of leukocyte infiltrate in the segmental defect at two weeks of growth (Right) compared to PGH without the cytokine (Left). Top row (A, B), Bright field image of regenerate tissue. Remnants of hydrogel are evident as voids without cells. Bottom Row (C, D), Immunostaining for Th1 and Th2 cells. The PGH control treated defects (C) show a greater number of T helper cells (green) than defects treated with PGH+TGFβ-1 (D). The majority of T cells in the PGH control (C) express Gata3. A large grouping of these is evident near the hydrogel. The few T cells in the PGH+TGFβ-1 defect (D) express T-bet (red, inset). Blue = DAPI, Green = CD4 (helper T cells), Red = T-bet (Th1), Magenta = Gata3 (Th2).

Regarding Subtask 4, we are currently processing the tissues from the 7 5-month animals for sectioning and subsequent biochemical and immunohistochemical analysis. However, we have completed the histological analysis of the pilot animals sacrificed at 1-month. The PGH hydrogel with TGFβ-1 (100 µg/ml) produced equivalent volume of bone after one month growth compared to INFUSE (1.5 mg/ml BMP-2 in sponge) in the 3.0 cm segmental defects, without forming ectopic bone like INFUSE (Figure 7).

Regarding Subtask 5, staining is pending Subtask 4.

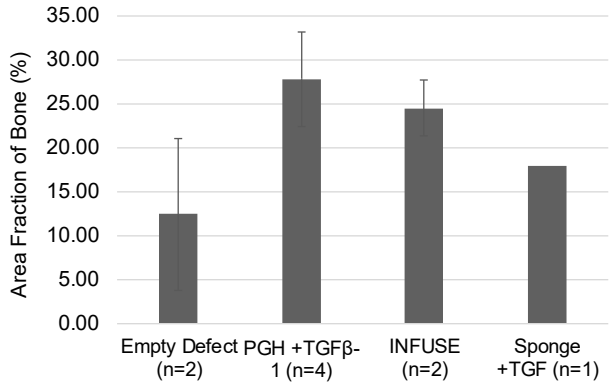

Figure 7: Area of regenerate bone tissue within the segmental defect at 1-month in pilot animals. The PGH (hydrogel component of our device) with TGFβ-1 at 100 μg/ml is superior to the commercial INFUSE (collagenous sponge with BMP-2) at 1/15th of the drug dose in INFUSE, yielding significant woven bone and pockets of cartilage at one month, with less inflammation and ectopic bone.

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

1. Four surgical residents participated in the non-survival surgery
2. Two new technicians were 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 the final US patent application on our devices, application # PCT/US2019/037081, filed June 13, 2019. We filed the provisional patent application the year prior.
2. We presented the work at the AMSUS annual meeting
    a. Sippel M, Yracheta J, Robbins N, Gerall C, Cox J, Parida BK, Salas MM, Antebi B, Gorantla VS, Weitzel EK, Chen J, Swenson T, Almarza AJ, Taboas JM. Novel Delivery System of Autologous Mesenchymal Stem Cells utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa). The 2018 AMSUS Annual Meeting. National Harbor, MD, November 26-30, 2018.
3. We presented the work at the McGowan Institute for Regenerative Medicine Annual Retreat.
    a. Yracheta J, Sippel M, Parida B, Salas MM, Antebi B, Gorantla VS, Weitzel EK, Chen J, Swenson T, Almarza AA, Taboas JM. Novel Delivery System of TGFβ-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa).McGowan Institute for Regenerative Medicine Annual Retreat, Pittsburgh, PA, March 2019.
4. We presented the work at the 2019 MHSRS Conference (Military Health System Research Symposium)
    a. Sippel M, Yracheta J, Weitzel EK, Chen J, Swenson T, Almarza AJ, Taboas JM. Novel Delivery System of TGFβ-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa). MHSRS, Orlando, FL, August 2019.

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

1. Continue fabricating scaffolds implants as needed.
2. Evaluate the temporal-spatial profile of dual TGFβ-1 and IL-10 delivery from the PGH hydrogels.
    a. Dual load the scaffolds with both cytokines and place them in PBS in vitro

    b. Analyze drug eluted into the PBS and remaining in the hydrogel.
3. Perform experiments on the remaining 20 pigs with segmental defects.
    a. Complete remaining MSC transplant groups as priority.
4. Analyze RNA-seq data
5. Complete cytokine profiling
6. Continue µCT scanning of samples at necropsy
7. Perform histological and immunohistochemical assays on all necropsy tissues.

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. We are processing the 5-month samples now to determine if the inclusion of the sponge as depicted in Figure 1 impaired regeneration. We may redesign the implant to be similar to that tested with the pilot animals, which showed significant bone regeneration in the segmental defects.

Changes that had a significant impact on expenditures

1. "Nothing to Report".

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 2018 AMSUS Annual Meeting: Sippel M, Yracheta J, Robbins N, Gerall C, Cox J, Parida BK, Salas MM, Antebi B, Gorantla VS, Weitzel EK, Chen J, Swenson T, Almarza AJ, Taboas JM. Novel Delivery System of Autologous Mesenchymal Stem Cells utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa). National Harbor, MD, November 26-30, 2018.
2. Presentation at the McGowan Institute for Regenerative Medicine Annual Retreat: Yracheta J, Sippel M, Parida B, Salas MM, Antebi B, Gorantla VS, Weitzel EK, Chen J, Swenson T, Almarza AA, Taboas JM. Novel Delivery System of TGFβ-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa). Pittsburgh, PA, March 2019.
3. Presentation at the 2019 MHSRS Conference (Military Health System Research Symposium): Sippel M, Yracheta J, Weitzel EK, Chen J, Swenson T, Almarza AJ, Taboas JM. Novel Delivery System of TGFβ-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa). Orlando, FL, August 2019.

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. Final patent application PCT/US2019/037081, filed June 13, 2019.

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		10	Methacrylation, NMR, and mechanical testing of hydrogels. Biocompatibility assays. Left project Y3Q4,	
Jennifer Cox	Laboratory Administrator		1	Management of sub-award laboratory, supplies ordering, schedule coordination.	USISR
Tyler Swenson	Research Technician		8	Preparation and analysis of all implantable device materials, cell culture. Left Y3Q3.	
Michael Sippel	Research Resident		2	IACUC approval, surgical work. Left project Y3Q3	USISR
Juan Taboas	PI		3	Preparation of animal protocol. Development of biomaterials and devices. Animal surgeries, data acquisition and interpretation. Overall management of project	
Erik Weitzel	Co-I		1	Sub-award PI. Animal surgeries, data acquisition, and interpretation. Foster collaboration with sub-award	USISR
Jacklyn Yrachetta	Research Resident		2	Surgical work. Jackie replaced Dr. Sippel Y3Q3	USISR
Bijaya Parida	Research Scientist		1	Left project at end of Y3Q1	
Quintin Letavic	Research Technician		4.5	Preparation and analysis of all implantable device materials, cell culture. Replaced Tyler Swenson	
Sindhu Gopalaswamy	Research Technician		3.5	Preparation and analysis of all implantable device materials, cell culture. Replaced Jingming Chen	

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 one 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).

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.

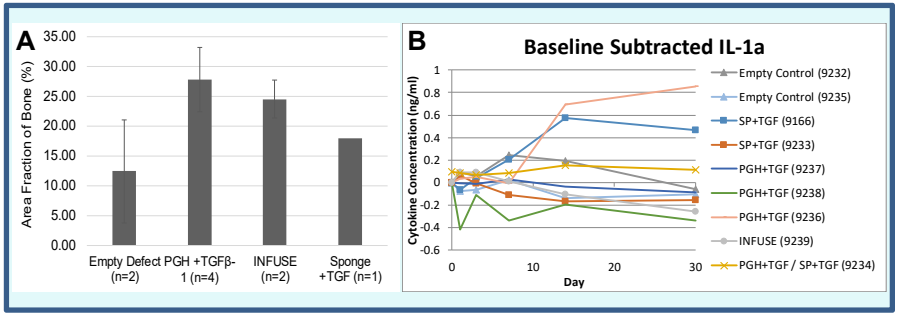

Figure 1. (**A**) Area of regenerate bone tissue within the segmental defect at 1-month in pilot animals. The PGH (hydrogel component of our device) with TGFβ-1 at 100 µg/ml is superior to INFUSE (commercial collagenous sponge with BMP-2) at 1/15th of the drug dose in INFUSE, yielding significant woven bone and pockets of cartilage at one month, with less inflammation and ectopic bone. (**B**) IL-1a and IL-10 plasma cytokine levels at pre-op and post-op days 1, 3, 7, 14 and 30 in the pilot animals. SP=sponge, PGH=hydrogel, TGF=TGFβ-1 loaded at 100 µg/ml, INFUSE=commercial kit with collagen sponge and 500 µg/ml BMP-2. Baseline levels at pre-op were subtracted from all readings. (# = animal code).

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**	**$324**	**$435**	**$526 ($700)**	**($606)**

Updated: 10/17/2019

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 2 weeks

☐ Co-author manuscript on the immunomodulatory effect and potential for endochondral ossification at 2 weeks 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

- The timeline reflects no-cost extension due to surgery delay.

Budget Expenditure to Date: $1,319,534.09 (direct + indirect)