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TITLE: Bone Regeneration Device for Compromised Wounds

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CONTRACTING ORGANIZATION: University of Pittsburgh, Pittsburgh, PA

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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
None listed.

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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 RNA-seq/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). Added in FY19: Submit manuscript on functional healing after 1-month post-surgery (pilot animal surgeries).

The statement of work follows and progress to date follows.

	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 80%
Subtask 2: Hydrogel Sponges (fabricate for implantable devices to be used in the grant year) 3 times each year	3-32	In progress 100%
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 80%
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 80%
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 80%
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 80%
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 100%

Subtask 2: Collection of blood fluid samples Pre-op and day 3 and 30 post-op for each animal	5-33	In progress 100%
Subtask 3: Cytokine profiling in blood serum Twice per year (0.5 month for samples from 3 swine)	7-35	In progress 90%
Subtask 4: Immune cells characterization from lymph node biopsy Twice per year (1 month for samples from 3 swine)	7-35	In progress 90%
Subtask 5: Transcriptome analysis of tissue via RNA-seq analysis Twice per year (2 months for samples from 3 swine)	8-28	In progress 80%
Subtask 6: Immunohistochemistry to identify cellular compliment Once per year (3 months for samples from 5 swine)	8-28	In progress 40%
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 80%
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 80%
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 / eliminated
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 60%
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
Milestone #4: NEW Co-author manuscript on bone healing after 1 months post-surgery using pilot animal data	41-48	100%

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.
5. CY19 Goal (Submit manuscript on functional healing after 1-month post-surgery): Completed

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.

Regarding Subtask 2, we no longer utilize the sponges because they were compressed onto the tibia by the plating system causing the hydrogel to extrude from the defect site, ultimately impairing regeneration. This was reported in the Y4Q1 report.

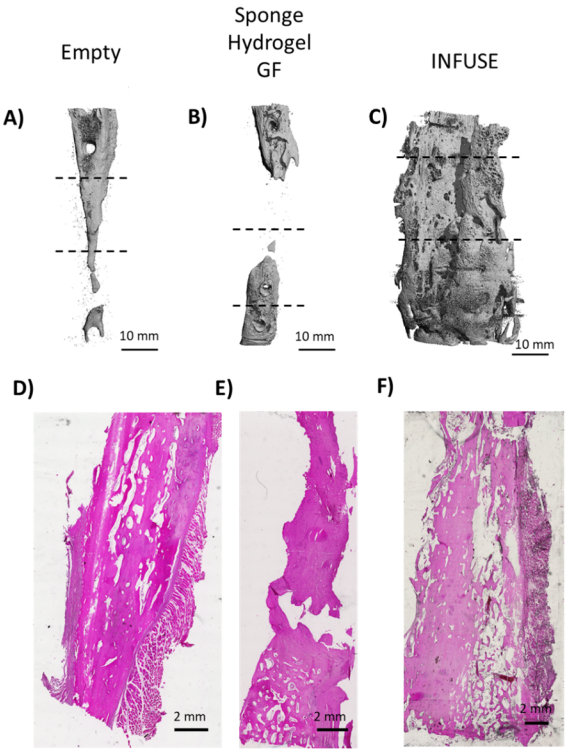


Figure 1. MicroCT and histology of 5 month samples with biphasic scaffold (sponge outer core and hydrogel filler). (B,E) The biphasic scaffold (Sponge with PGH Hydrogel and TGFβ-1) (showed less bone regeneration in the segmental defect (3 cm fibular excision near the distal end) (n=6) compared to (A,D) untreated (Empty) defects (n=2). The plating system used (screw holes evident in image) was found to compress the sponge, which in turn extruded out the hydrogel. Thus, the sponge was eliminated from the scaffold design. (C,F) The INFUSE controls showed excessive ectopic bone 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. We determined that a batch of our drug delivery

studies in vitro were faulty because the TGF-β1 came from the vendor dissolved in an organic solvent instead of PBS. We further studied the drug release profiles from the hydrogel in vitro including analysis of content retained in the hydrogel over time (Figure 1).

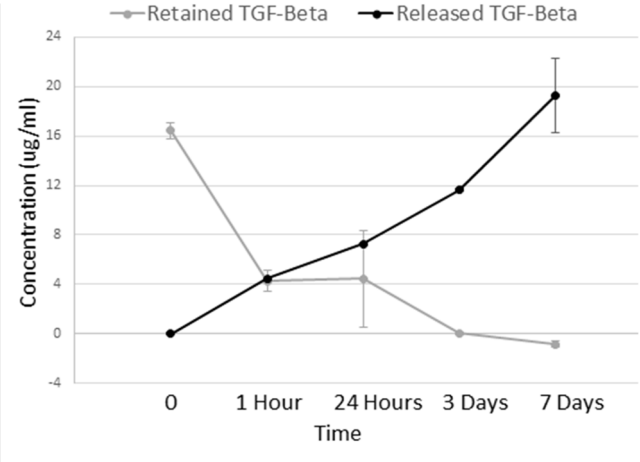


Figure 2. The PGH hydrogel releases TGFβ-1 over 1-week. PGH hydrogels 8% (w/v) containing 100 µg/ml TGFβ-1 were placed in PBS at a ratio of 10:1 PBS:hydrogel, and incubated at 37 °C. At the indicated time-points, PBS was collected and the hydrogels digested overnight in a collagenase solution. These were assayed via ELISA to quantify the TGFβ-1 released into the PBS and retained within the hydrogel (n=3 per time-point). Error bars = standard deviation of the mean.

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 and necropsies on 36 out of the 45 animals. Only animals in the 5 month group remain.

Regarding Subtask 1, task is complete.

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 completed all replicates for both 2-week and 5-month animals but have added 2 more replicates at 5-months for the two MSC groups (= MSC +/- (TGF-β1+IL-10)) to increase statistical power. These last two animals remain to be operated.

Regarding Subtask 3, we have completed surgeries for the 2-week time-point animals (3 replicates per treatment per experiment design) but have 9 animals remaining for the 5-month time-point animals (6 replicates per treatment). Three experimental replicates were allocated to each of the 8 treatment groups. However, we had to eliminate the remaining replicates to increase our sample number for the 5-month assay after determining that the biphasic sponge implant extruded hydrogel from the defect site. Below is a table of the completed groups and replicates.

			Replicates	
	Group Name	**Group Description**	**2-week**	**5-month**
1	Empty	Empty defect / untreated control	3	4
2	High dose drugs	Hydrogel + 100 ug/ml TGF-β1 + 10 ug/ml IL-10	3	4
3	Low dose drugs	Hydrogel + 10 ug/ml TGF-β1 + 10 ug/ml	2	2
4	MSCs + drugs	Hydrogel + MSCs + 100 ug/ml TGF-β1 + 10 ug/ml IL-10	3	7
5	MSCs	Hydrogel + MSCs	1	7
6	Infuse	Infuse	2	3

7	TGF only	Hydrogel + 100 ug/ml TGF-β1	3	2
8	Control	Hydrogel only control	3	4

Regarding Subtask 4, all tissues for the 2-week animals have been harvested and are in process for immunohistochemical analysis. All tissues for 5-month animals operated before the Covid-19 facilities shutdown have been harvested. Surgeries are estimated to resume in January 2021 for the remaining animals.

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, blood and lymph node biopsies were collected 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 (TNFα, IFNγ, Il-1a, Il-1b, Il-6, IL-8, IL-10) on the Illumina platform. Statistical power was low due to high animal variability such that we cannot determine if no differences exist. We are increasing our statistical power by sampling blood from the 5-month animals.

Regarding Subtask 4, flow analysis was performed in real-time when the samples were collected (blood and lymph node biopsies). Analysis is pending software purchase (FlowJo).

Regarding Subtask 5, we completed the RNA-seq and are running the analysis, comparing across treatments and to control tissues (bone and cartilage).

Regarding Subtask 6, we are processing samples for histochemical and immunohistochemical staining. To date, we have found that the PGH hydrogel with 100 ug/ml TGF-β1 promotes a greater number of pro-regenerative T helper cells compared to PGH hydrogel without TGF-β1.

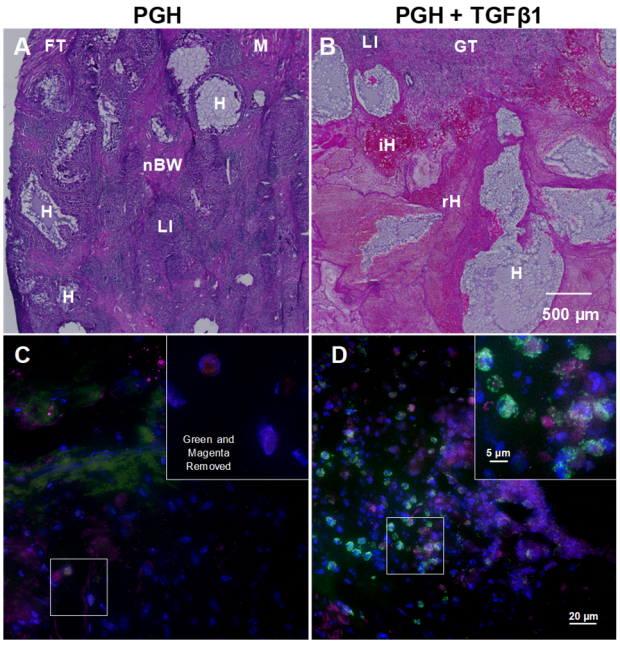


Figure 3. PGH alone (Left) versus PGH with TGFβ-1 (100 µg/ml, Right) at two weeks post-op. Top Row (A,B): H&E stain: The PGH hydrogel underwent resorption over two weeks in vivo through several stages, including cell infiltration (iH) and cell mediated resorption (rH). In that time, fibrous tissue (FT), leukocyte infiltrate (LI), and neo-woven bone (nBW) were formed. Delivery of TGFβ-1 (B) lead to faster resolution of the acute inflammation, as evidenced by less LI and more GT and matrix than defects treated with PGH only (A). Bottom Row (C,D): immunostaining for Type 1 and 2 helper T cells (Th1 and Th2): Defects treated with PGH without TGFβ-1 (C) showed a lower number of Th (green) than defects treated with PGH with TGFβ-1 (D). The few Th in the PGH only defect (C) express are Th1 expressing T-bet (red, inset). The majority of Th in the PGH with TGFβ-1 (D) are Th2 expressing Gata3. A large grouping of these is evident near the hydrogel. Remnants of hydrogel are evident as voids without cells. Top Row: 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. Bottom Row: Blue = DAPI, Green = CD4 (Th), Red = T-bet (Th1), Magenta = Gata3 (Th2).

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. The bone fragments of comminuted defects were reported in last year's annual report (Y3) to appear resorbing and eliminated from the study. This is likely arising for the use of compression plating in our model. This type of rigid fixation inhibits interfragmentary motion (micromotion) that is anabolic for boney healing (increases callus size). Future work should employ far locking screws or a more flexible plate which permit micromotion, consistent with the trend in orthopaedic practice. However, this model does provide a worst case scenario test of regenerative potential of therapeutics.

Regarding Subtask 2, all samples to date have been µCT scanned and boney bringing of the defect and volume or regenerate bone quantified. The following summarizes µCT results for the MSC treatments compared to controls.

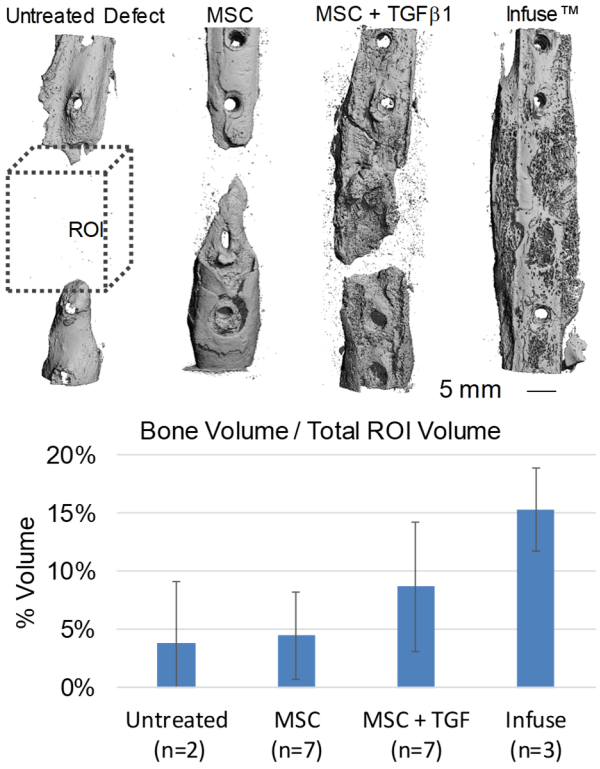


Figure 4. The PGH hydrogel (1.5 ml) loaded with MSCs (30x106/ml), TGFβ-1 (100 µg/ml) and IL-10 (10 µg/ml) led to significant woven bone formation in the 3.0 cm segmental fibular defects after five-months growth in minipigs. Infuse™ yielded more bone in the ROI but produced copious heterotopic ossification in the surrounding musculature (cut away to view defect). ROI = region of interest = 20 x 15 x 10 mm3

Regarding Subtask 3, the mechanical testing was deemed incompatible with the study (reported in last year's annual report, Y3) and eliminated from the study. . Mechanical testing was removed in favor of the µCT and immunohistochemical analysis.

Regarding Subtask 4, all of 5-month samples to date have been sectioned and samples stained with Hematoxylin and Eosin.

Regarding Subtask 5, staining is pending Subtask 4. However, test samples show that the inflammatory response is well resolved by 5-months.

Regarding Milestone #4 (new, reporting results of 1-month pilot animals), we submitted the manuscript but have to revise for resubmission. The following is the summary data for these animals.

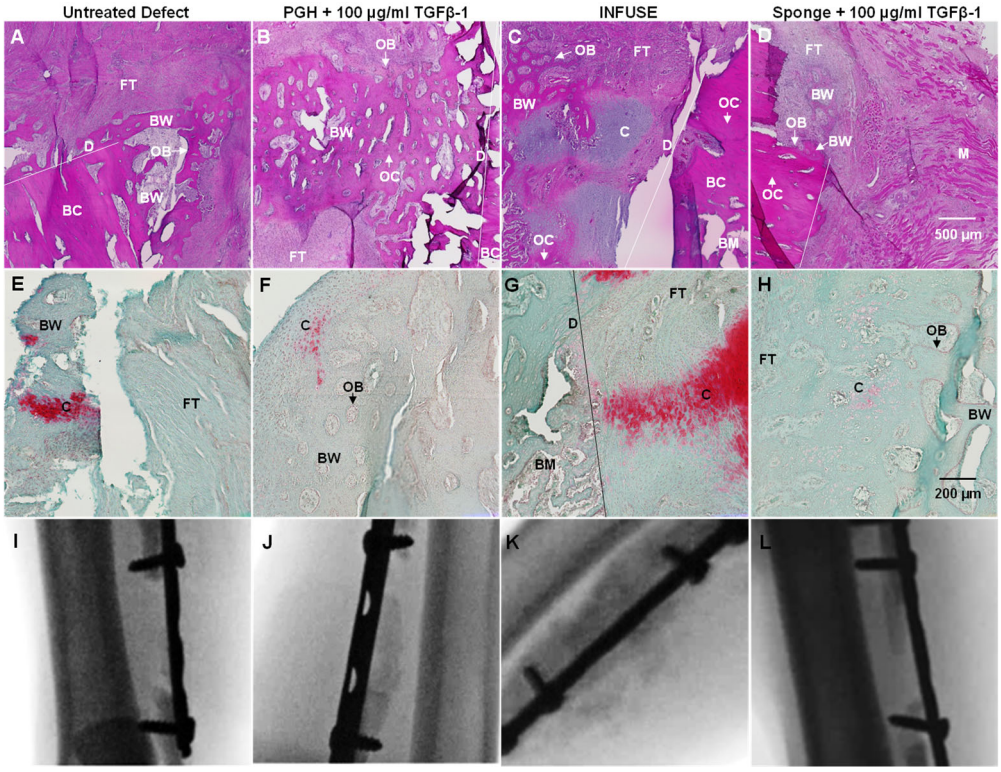


Figure 5. PGH with TGFβ-1 lead to significant woven bone formation the 3cm segmental fibular defects after one-month growth as evidenced by lack of lamellar structure and high osteocyte content compared to the cortical bone. (A,E,I; n=2): Untreated defects showed woven bone near the osteotomy site (A) but no bone in the defect proper (I). (B,F,J; n=4) PGH scaffolds + 100 µg/ml TGFβ-1 yielded woven bone (B), some cartilage (F), and bone in the defect proper (J). Woven bone contained high osteocyte density (OCs) and active osteoid surfaces lined with osteoblasts (OBs). (C,G,K; n=2): Infuse™ also yielded significant bone regeneration (C), but with more cartilage (G) and ectopic bone (K) than treatments with TGFβ-1. (D,H,L; n=5): Gelatin sponge scaffolds + 100 µg/ml TGFβ-1 did not produced some woven bone in the defect proper (D,L). 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)

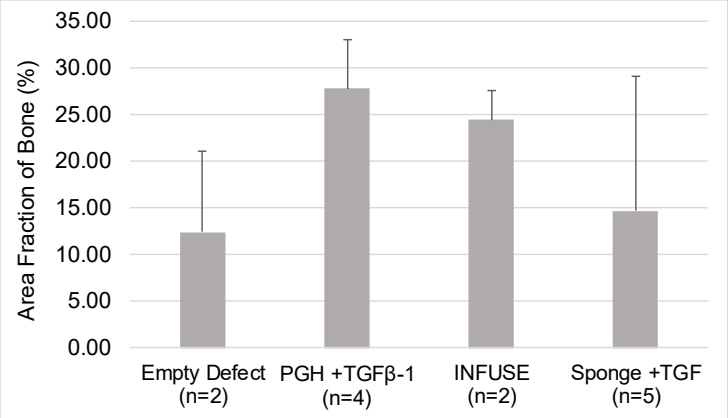


Figure 6. The Infuse™ (1.5 mg/ml BMP-2 in sponge) and PGH hydrogel with TGFβ-1 (100 µg/ml) appeared to yield similar volumes of bone within the 3.0 cm segmental defects after one month growth. However, the PGH with TGFβ-1 did not form ectopic bone like Infuse™. The treatment groups were not significantly different at an alpha ≤ 0.05. PGH+TGF-1 was non-inferior to Infuse™ within an equivalence margin of 25%. Error bars = standard deviation of the mean.

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 a manuscript on the results of the pilot 1-month animals. We will revise the manuscript based on reviewer feedback.
2. We did not present at the MHSRS Conference (Military Health System Research Symposium) due to Covid-19

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

1. Continue fabricating scaffolds implants as needed.
2. After replacing the TGFβ-1 with lyophilized powder that we dissolve in PBS, we will rerun our analysis of 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 9 pigs with segmental defects. As noted in Y3 annual report, we allocated the MSC treatments as priority and most of the remaining samples are for the other treatments.
4. Complete cytokine profiling (run samples from 5-month animals)
5. Complete analysis of RNA-seq data
6. Complete flow cytometry analysis
7. Continue µCT scanning of samples at necropsy
8. 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. Our US patent application (PCT/US2019/037081) will be nationalized in October.

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. We no longer utilize the biphasic scaffold design because they were compressed onto the tibia by the plating system causing the hydrogel to extrude from the defect site, ultimately impairing regeneration. This was reported in the Y4Q1 report.

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

1. Research activities were halted at the University of Pittsburgh and USAISR in mid-March due to the coronavirus (Covid-19) pandemic. During this time, no bench-work or animal surgeries were permitted. We were permitted to continue essential duties, which entailed 1) animal husbandry, 2) terminal necropsies of animals previously operated, and 3) preservation of necropsied tissues. The Pittsburgh site reopened research facilities on June 15 for essential personnel only. This allowed us to start processing the recent necropsies for histological analysis (decalcification). The USAISR site remains shut-down through the summer. At this time, the USAISR animal facilities have a backlog of projects and we are expecting surgeries to be rescheduled for January 2021.
2. The animals we had in house for surgeries to take place during the shutdown have grown too large for surgery. We will need to purchase 5 more animals than anticipated. We expect that the large sows can be used by other projects at the USAISR.

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. "Nothing to Report."

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.
    a. Nationalization on 10/15/2020
    b. World application under PCT = 2019/241577
    c. European application filed 10/23/2020 = 19819392.2-1109

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		1	Mechanical testing for biomaterials and tissues. Animal surgeries, data acquisition and interpretation.	
Jennifer Cox	Laboratory Administrator		6	Management of sub-award laboratory, supplies ordering, schedule coordination.	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		6	Surgical work. Left project Y3Q3	USISR
Quintin Letavic	Research Technician		8	Preparation and analysis of all implantable device materials, cell culture.	
Sindhu Gopalaswamy	Research Technician		8	Preparation and analysis of all implantable device materials, cell culture.	

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

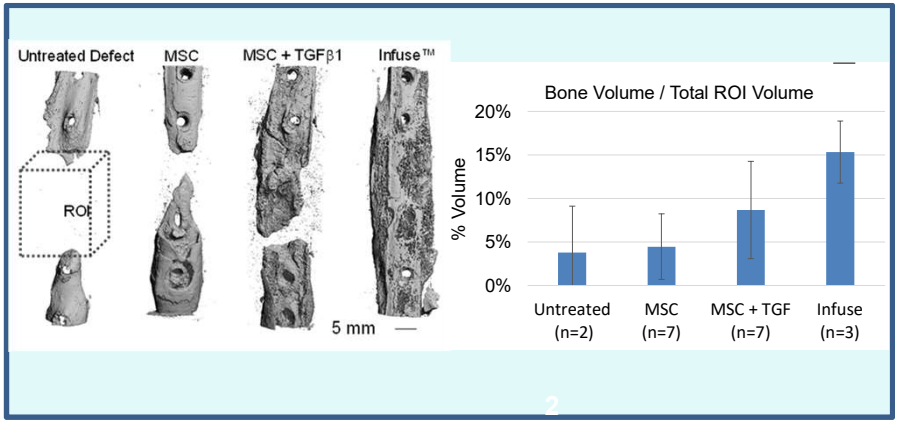
Study/Product Aim(s)

- 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.



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**	**$336**	**$400**	**$615**	**$316 ($395)**

Updated: 11/1/2020

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)

☑ Submit manuscript on functional healing after 1-month post-surgery.

Comments/Challenges/Issues/Concerns

- The timeline reflects no-cost extension due to surgery delay.
- Project delayed due to facilities shutdown for Covid-19 pandemic.

Budget Expenditure to Date: $1,688,302.29 (direct + indirect)