AWARD NUMBER: W81XWH-16-1-0793

TITLE: Bone Regeneration Device for Compromised Wounds

PRINCIPAL INVESTIGATOR: Juan Taboas, PhD

CONTRACTING ORGANIZATION: University of Pittsburgh

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Fort Detrick, Maryland 21702-5012

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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Juan Taboas, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pittsburgh
3520 Fifth Avenue
Pittsburgh, Pa 15213-3320

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
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12. DISTRIBUTION / AVAILABILITY STATEMENT

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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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	16	

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Prescribed by ANSI Std. Z39.18

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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	Site 1 (Dr. Taboas)	Site 2 (Col Weitzel)	Fraction (%) Completed
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	1-51	Dr. Taboas		85%
Subtask 2: Hydrogel Sponges (fabricate for implantable devices to be used in the grant year) 3 times each year	3-32	Dr. Taboas		100%
Subtask 3: Coarcervate (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	Dr. Taboas		100%
Milestone #1: Fabricate sufficient hydrogel (200 ml) and coacervates (2 ml) for device fabrication per year				85%
Specific Aim 2: Assess the immunomodulatory effect and potential for endochondral ossification at 2 weeks 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	Dr. Taboas	Dr. Weitzel	100%

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	5-52	Dr. Taboas	Dr. Weitzel	95%
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	6-52	Drs. Taboas and Almarza	Dr. Weitzel	85%
Subtask 4: Sample Harvest (excise midshaft tibia/fibula of swine limb) Total of 0.25 months over max 5 times a year	7-57		Dr. Weitzel	85%
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				85%
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) Immediately post-op and at euthanasia	5-33		Dr. Weitzel	100%
Subtask 2: Collection of blood fluid samples Pre-op and day 3, 7 and 14 post-op for each animal	5-33		Dr. Weitzel	100%
Subtask 3: Cytokine profiling in blood serum Twice per year (0.5 month for samples from 3 swine)	7-35	Dr. Taboas	Dr. Weitzel	66%
Subtask 4: Immune cells characterization from blood and lymph nodes Twice per year (1 month for samples from 3 swine)	7-58	Dr. Taboas	Dr. Weitzel	80%

Subtask 5: Transcriptome analysis of tissue via RNA-seq analysis Twice per year (2 months for samples from 3 swine)	8-58	Dr. Taboas	Dr. Weitzel	90%
Subtask 6: Immunohistochemistry to identify cellular compliment Once per year (3 months for samples from 5 swine)	8-54	Dr. Taboas		70%
Milestone #1: Co-author manuscript on the immunomodulatory effect and potential for endochondral ossification at 1 month post-surgery.	18-58	Drs. Taboas, Almarza	Dr. Weitzel	
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	5-57		Dr. Weitzel	85%
Subtask 1: Computed tomography (μCT) imaging to quantify bone volume and ultrastructure 4 times per year (0.5 months for 2-3 swine)	18-58	Dr. Taboas		85%
Subtask 2: Mechanical 4-point bending (non-destructive) for bone strength 4 times per year (0.5 months for 2-3 swine)	18-34	Dr. Almarza		NC
Subtask 3: Histological assays for bone, cartilage, fibrous tissue, revascularization and reinnervation 4 times per year (3 months for 3 swine)	18-60	Dr. Almarza		60%
Subtask 4: Histological assays for immunological response in the mature engrafted tissue 4 times per year (3 months for 3 swine)	18-60	Dr. Taboas		20%

Milestone #1: NEW Co-author manuscript on bone healing after 1 months post-surgery using pilot animal data	41-48	Dr. Taboas	Dr. Weitzel	90%
Milestone #2: Co-author manuscript on functional bone healing response after 5 months post-surgery	33-60	Drs. Taboas, Almarza	Dr. Weitzel	
Milestone #3: Application to Coulter Foundation to perform GMP large animal pilot study	54	Drs. Taboas and Almarza		

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 CY19 (total of 100% completion over life of grant). All experimental animals were operated and samples harvested.
3. CY18 Goal (Report initial results of terminal assays at 2-weeks): In progress.
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 continued using the 8% (w/v) hydrogel formulation throughout all experimental treatments. We continued 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.

Regarding Subtask 3, we previously eliminated the coacervates, and we continue to evaluate the spatiotemporal profile of drug release (IL-10 and TGFβ-1) from the hydrogel. We instead delivered the cytokines from the hydrogel. This was reported in the Y4Q4 (annual) report. We determined that the simulated body conditions used in the assay preclude detection of TGFβ-1 bioactivity.

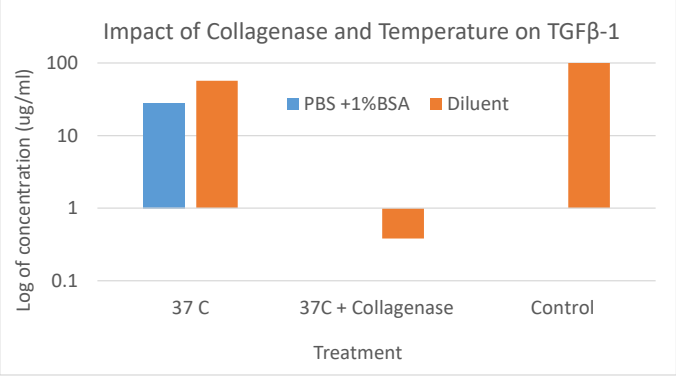


Figure 1: The bioactivity of TGFβ-1 decreases dramatically at simulated body conditions (37 C and PBS buffer) compared to controls. However, TGFβ-1 still exerts bioactive effects in our animal model as evidenced by increased bone formation in MSC groups with TGF. The diluent used in ELISA assays stabilizes the TGFβ to some degree. Collagenase digestion of the hydrogels cannot be used with available ELISA plates to detect TGF remaining in the hydrogel (not eluted). PBS = phosphate buffered saline.

Regarding Milestone #1: completed.

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 completed surgeries on the 5-month animals. Thus, all surgical work is complete (all animals operated and samples harvested).

Regarding Subtask 1, task is complete.

Regarding Subtask 2, we prepared cells from 3 animals for implantation into 5-month animals.

Regarding Subtask 3, we completed surgeries on the remaining 5-month animals, 9 in total. We were unable to acquire additional animals to repeat treatments with the modified implant design (removed the sponge component in Y4). We therefore focused on the following groups in the treatment table below: 1, 2, 4, 5, 6, and 7. We completed surgeries for the 2-week time-point animals in prior years, with decreased replicates to increase the number of replicate treatments for the 5-month animals.

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

Regarding Subtask 4, all tissues for all 9 of the 5-month animals have been harvested, micro-CT imaged, and in processing for histological analysis.

Regarding Milestone #1: completed.

Major Task 3: Terminal assays at 2-weeks post implantation

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

Regarding Subtask 1, completed in prior years.

Regarding Subtask 2, completed in prior years.

Regarding Subtask 3, all plasma samples gathered duplexing with the 5-month animals operated this year. We increased our statistical power by sampling blood from the remaining 5-month animals at same time-point as the 2-week animals. Assays are incomplete because the ELISA manufacturer reported fabrication delay due to plastic ware scarcity due to SARS-CoV-2. We were unable to purchase the kits this grant year.

Regarding Subtask 4, additional biopsies were performed and analyzed using the 5-month animals operated this year. FlowJo was purchased for analysis of results.

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 processed additional samples for histochemical and immunohistochemical staining.

Regarding Milestone #1: pending completion of subtasks above.

Major Task 4: Terminal assays at 5 months

This task focuses on the bone regeneration efficacy of the devices. We have sacrificed and harvested the tissues of the remaining 9 5-month animals operated January through March of 2021.

Regarding Subtask 1, we followed the surgeries with x-rays post-op and at 5 months.

Regarding Subtask 2, all samples from the remaining 9 animals were µCT scanned. The following summarizes µCT results for the study to date sans recent 9 animals. These CT results support our conclusion for Y4 that the compression plating used in this study (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.

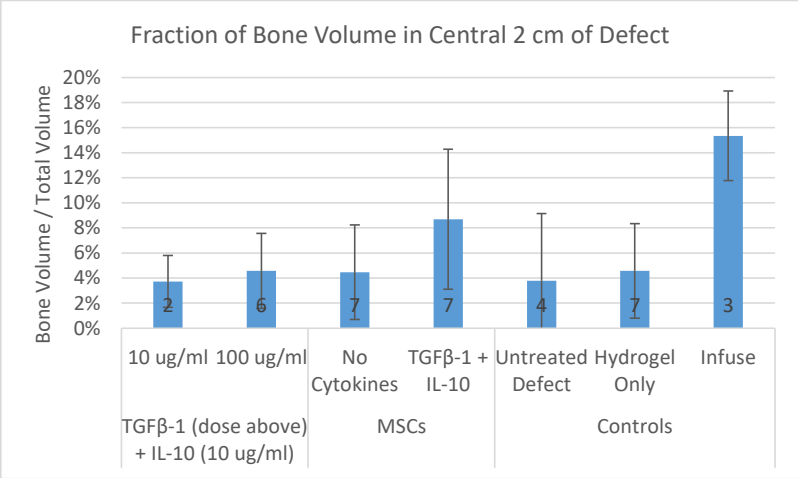


Figure 2: .The MSC treatment with cytokines (TGFβ-1 @ 100 ug/ml + IL-10 at 10 ug/ml) appears to yield the greatest bone regeneration at 5 months compared to all other experimental treatments. All experimental treatments employ the hydrogel (8% w/v) as a carrier for the cells and cytokines. Infuse (BMP-2 @ 1.5 mg/ml in a collagenous sponge) shows greater regenerate bone, but suffers from extensive heterotopic ossification in the surrounding musculature. The bone volume is quantified in a 2 cm long rectangular volume placed at the center of the defect. Numbers in bars indicate replicates to date. Error bars indicate standard deviation of the mean.

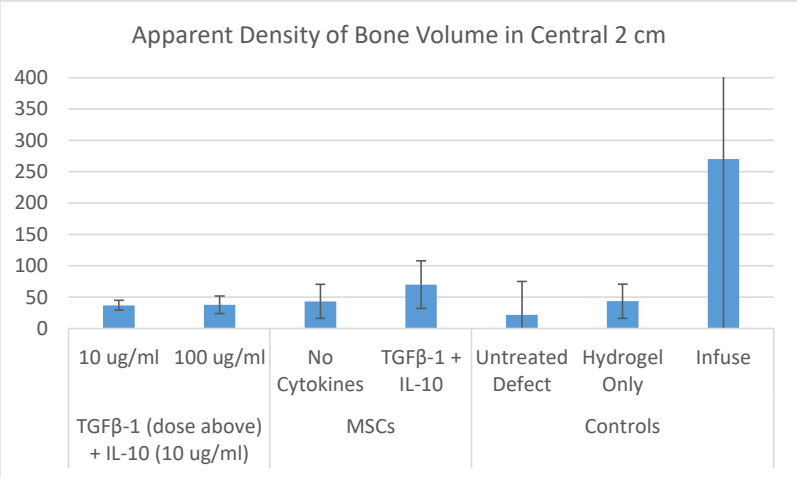


Figure 3: .The bone produced by Infuse treatment appears denser than all other experimental treatments. This suggests that Infuse has the most mature (oldest) bone, consistent 2-week x-rays showing that the Infuse group has significant amount of woven bone in the defect and surrounding musculature while other treatments do not have much woven bone in the defect. However, this has not been validated. Numbers in bars indicate replicates to date. Error bars indicate standard deviation of the mean.

Regarding Subtask 3, the remaining 18 samples from the 5-month animals operated this year are in histological processing (decalcification and embedding). Sectioning and staining will be performed after processing is complete.

Regarding Subtask 4, pending histological processing in Subtask 3

Regarding Milestone #1: Manuscript rejected due to insufficient replicates, which we understood would be an issue.

Regarding Milestone #2: Pending completion of Subtasks above

Regarding Milestone #3: We continued prosecution of our patent application through the University of Pittsburgh Innovation Institute, but require completion of the Histology for a Coulter grant.

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)

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

1. Two surgical residents participated in the non-survival surgery this year
2. One new research 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. "Nothing to Report."

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

1. Rerun our analysis of the temporal-spatial profile of TGFβ-1 delivery from the PGH hydrogels at 4C.
2. Complete histological processing and morphometric analysis of the 5-month animals.
3. Complete cytokine profiling (run samples from 5-month animals) when ELISA kits are available from manufacturer
4. Complete analysis of RNA-seq data
5. Complete flow cytometry analysis

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) was published on July 15, 2021, as publication number US-2021-0213170-A1
2.

What was the impact on society beyond science and technology?

1. "Nothing to Report."

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

"Nothing to Report."

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

We encountered significant delays in the project this year for the following reasons:

1. Personnel changes at the University of Pittsburgh delayed histological processing of the final eight animals in the study and analysis of the transcriptome. These animals were re-scheduled for surgery (due to SARS-CoV-2 shut-down of animal facilities at the USAISR) on January through March of 2021. All biopsies were

received at Pittsburgh by August, 2021. However, the histological processing was delayed due to change of staff at Pittsburgh and training of the new hire. The research technician resigned on May 28th, 2021.

2. Personnel leave at the USAISR impacted reporting of project delays at the site. The laboratory manager at the USAISR requested family medical leave twice during the summer and fall of 2021. During this time, reporting of delays from the subsidiary labs performing assays lapsed.
3. The cytokine assays at the USAISR were delayed due to unavailability of assay kits. The ELISA manufacturer reported fabrication delay due to plastic ware scarcity due to SARS-CoV-2. The backordered kits became available this year and have been received by the CIRS laboratory. These assays are two thirds complete. Note that the SOW reported cytokine assays as completed, but we added further assays for the final eight animals since the submission of the SOW.

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
    d. USPCT published July 15, 2021, as publication number US-2021-0213170-A1

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		4	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		12	Preparation and analysis of all implantable device materials, cell culture.	
Ingrid McNamara	Research Technician		2	Histological processing of samples	

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

Funding Agency	CDMRP, DoD
Grant Number:	CDMRP # OR200187, Grants.gov # GRANT13202780
Title of Grant:	Injectable Antimicrobial Hydrogel for Extremity Wound Management
Principal Investigator	Juan Taboas
Taboas Role on Grant	PI (3 Co-Is)
Years Inclusive	9/01/2021-8/31/2023
Percent Effort	5.0 %
Total Direct Costs	

Funding Agency	NIDCR, NIH
Grant Number:	1R01DE030296
Title of Grant:	Polymer Scaffolds for Mandibular Condyle Cartilage Regeneration
Principal Investigator	Juan Taboas and Alejandro Almarza

Taboas Role on Grant	Co-PI (2 Co-Pis)
Years Inclusive	12/1/2020-11/30/2024
Percent Effort	20 %
Total Direct Costs	

Funding Agency	NIDCR, NIH
Grant Number:	AR074981
Title of Grant:	Phosphate Signaling in Biomineralization
Principal Investigator	Dobrawa Napierala
Taboas Role on Grant	Co-Investigator
Years Inclusive	9/1/2019-8/30/2024
Percent Effort	5 %
Total Direct Costs	

Funding Agency	NIDCR, NIH
Grant Number:	U24DE026915/ Interdisciplinary Translational Project, Cycle 3
Title of Grant:	Vital Dent, A Revitalizing Root Canal Implant
Principal Investigator	Juan Taboas
Taboas Role on Grant	PI (multi co-investigators)
Years Inclusive	3/1/2019-12/31/2021 (NCE)
Percent Effort	15 % (no salary requested)
Total Direct Costs	

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

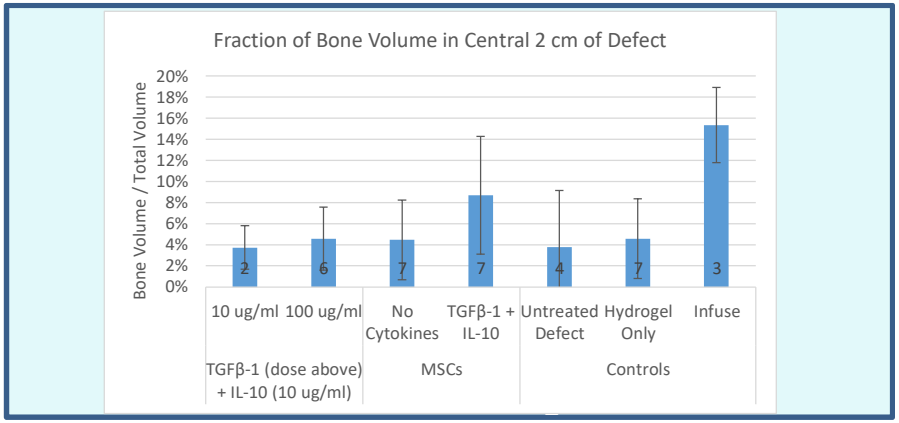
Study/Product Aim(s)

- 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.



The MSC treatment with TGFβ-1 @ 100 ug/ml and IL-10 at 10 ug/ml appears to yield the greatest bone regeneration at 5 months compared to all other experimental treatments (all employ the hydrogel, 8% w/v, as a carrier). Infuse (BMP-2 @ 1.5 mg/ml in a collagen sponge) yields greater volume of bone, but suffers extensive heterotopic ossification in the surrounding musculature. Numbers in bars = replicates to date, error bars = standard deviation of the mean.

Timeline and Cost

Activities CY	16	17	18	19	20	21
Scaffold Manufacture						
Animal Surgeries						
Terminal assays 1 month post-op						
Terminal assays 5 months post-op						
Budget in $K, (estimated)	**$35**	**$336**	**$400**	**$615**	**$370**	**$343**

Updated: 6/15/2022

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: $ 2,099,411 (direct + indirect)