AWARD NUMBER: W81XWH-18-1-0438

TITLE: Implantable Nanochannel System for the Controlled Delivery of Osteogenic Growth Peptide

PRINCIPAL INVESTIGATOR: Carly Filgueira

CONTRACTING ORGANIZATION: Methodist Hospital Research Institute, Houston, TX
Houston, TX 77030

REPORT DATE: August 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
AUG 2019	Annual	1 AUG 2018 – 31 Jul 2019

4. TITLE AND SUBTITLE

Implantable Nanochannel System for the Controlled Delivery of Osteogenic Growth Peptide

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0438

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Carly Filgueira

E-Mail:csfilgueira@houstonmethodist.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

METHODIST HOSPITAL RESEARCH INSTITUTE
6670 BERTNER AVE
HOUSTON TX 77030-2602

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During the research period, we have fabricated an implantable, medical grade polyether ether ketone (PEEK) device housing two silicon nanofluidic membranes for the sustained and constant release of osteogenic growth peptide (OGP). The device, which can be used to treat osteopenia, can be implanted without external fixation. Use of PEEK ensures some flexibility and radiolucency. In vitro release studies were performed with microfabricated silicon nanochannel membranes to determine the optimal nanochannel size (3.5 nm) to achieve sustained, constant release for two months. Release studies were performed through the use of custom cuvettes loaded with 200 uL of 3.12 mg/ml OGP into a top reservoir separated by a nanochannel membrane, such that the peptide was released into a sink reservoir below. The absorbance of the sink solution was measured at 275 nm (the wavelength where OGP displays a maximum absorbance) to obtain a dose release profile and quantitatively monitor diffusion of OGP through the nanofluidic membrane into the surrounding environment. OGP release is relatively linear over the two-month period, and at day 60, cumulative release profiles targeted ~30 ug of peptide.

15. SUBJECT TERMS
osteogenic growth peptide (OGP), nanofluidic membrane, nanochannel, osteopenia, sustained release, implant, polyether ether ketone (PEEK), spinal fusion, device

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

Table of Contents

1. INTRODUCTION 1
2. KEYWORDS 1
3. ACCOMPLISHMENTS 1
4. IMPACT 4
5. CHANGES/PROBLEMS 4
6. PRODUCTS 5
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS 5
8. SPECIAL REPORTING REQUIREMENTS 6
9. APPENDICES 6

1. INTRODUCTION

The scope of the research is to present a novel, nanotechnology-based spinal implant capable of sustained and constant release of osteogenic growth peptide (OGP). When administered to animals, systemic treatment with OGP (a small, 14 amino acid peptide) has been shown to promote new bone formation. Unfortunately, however, this requires extensive, frequent dosing and can result in off-target effects commonly associated with signaling pathway crosstalk as a result of poorly controlled drug delivery. Our delivery approach minimizes unwanted systemic side-effects by providing local, sustained release near the defect site (spine). We intend to evaluate our nanofluidic device in skeletally mature New Zealand White rabbits through use of imaging modalities (x-ray, DynaCT) and histology.

2. KEYWORDS

Osteogenic growth peptide (OGP), new bone formation, spinal fusion, Rabbit model, nanochannel membrane, nanofluidics, osteopenia, sustained release, implant, device

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major Task 1 Fabricate medical grade PEEK device in replicates. (Months 1-6)
Major Task 2 Prepare IACUC protocol for *in vivo* studies. (Months 1-3)
Major Task 3 Perform Rabbit study. (Months 6-18)

What was accomplished under these goals?

Specific Aim 1. Design and evaluate *in vitro* a spinal fusion implant that allows for sustained release of OGP.

Major Task 1 Fabricate medical grade PEEK device in replicates. (Months 1-6)

Major task 1 was completed 95% (See Figs. 1 & 2).

The subtasks achieved were: Drawing an autoCAD rendering (Figure 1c), Fabricating PEEK components (Figure 1a,b), Preparing the nanochannel membranes through a series of solvent washes including a basic piranha wash, Perform membrane gas testing to ensure the channels are not clogged and there is no leakage and visually inspect with microscopy, Clean the PEEK components with a series of detergents and solvents designed to remove any residue or contaminants and sterilize with Ethylene Oxide, Assemble the implants and membranes with UV and thermally cured epoxy and load with either OGP or vehicle under sterile conditions for *in vitro* testing, Perform in vitro release studies of OGP from three different nanochannel sized membranes (3.5 nm, 5 nm, and 20 nm), and Assemble the implants and membranes with UV and thermally cured epoxy and load with either OGP or vehicle under sterile conditions immediately prior to implantation.

The subtasks pending achievement: Assess biological activity of the released OGP by alkaline phosphatase assay in murine fibroblastic NIH 3T3 cells (ATCC).

Over twenty-four medical grade PEEK devices housing silicon nanochannel membranes were fabricated. Our design was slightly modified to include a silicone epoxy between the two membrane and drug reservoirs to allow for more flexibility in the implant since it will be placed near the spine (Fig 1B). This was achieved by joining two PEEK drug reservoirs using a silicone primer (MED-163, Nu-Sil) followed by application of a two-part fast-cure silicone adhesive (MED3-4213, Nu-Sil). This adhesive is compatible with PEEK and may be considered for use in human implantation for a period of greater than 29 days. The PEEK components were cleaned with a series of detergent washes (Tergazyme, Fisher Sci.) followed by rinses with water and isopropyl alcohol and sterilized using an autoclave. Membranes were cleaned using a piranha etch (mixture of sulfuric acid and hydrogen peroxide) to remove any organic residue, gas tested and optically inspected with a microscope to ensure the channels were not clogged and no holes were present in the membrane, and attached to the PEEK drug reservoir using UV Cure Optical Epoxy (EPO-TEK® OG116-31). Membranes with gas test values of ~0.032 sccm at a nitrogen flow rate of 15 psi were determined ideal for release.

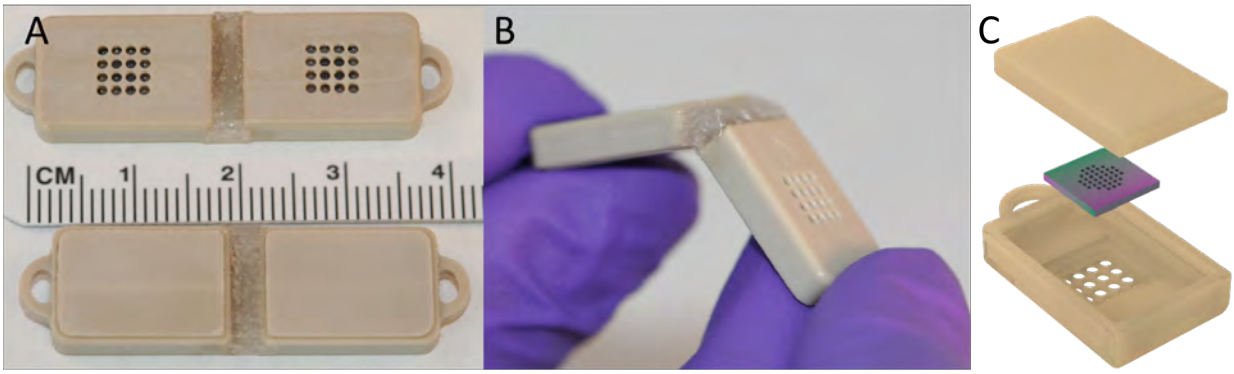

Figure 1. (A) Assembled PEEK device consisting of two nanochannel membranes with side loops for suturing capability to muscle tissue, (B) photo demonstrating implant flexibility, and (C) rendering of the osteoinducive implant.

To determine the appropriate nanochannel size for sustained release, in vitro release studies of OGP from three different nanochannel sized membranes (3.5 nm, 5 nm, and 20 nm) were conducted. Custom release cuvettes were loaded with 200 uL of 3.12 mg/ml OGP into a top reservoir separated by a nanochannel membrane, such that the peptide was released into a sink reservoir below. The absorbance of the sink solution was measured daily at 275 nm (the wavelength where OGP displays a maximum absorbance, Fig. 2A) for over two months. Using the standard curve shown in Fig. 2B, we were able to transform absorbance to concentration and show an achieved sustained, constant release of OGP from 3.5 nm membranes (Fig. 2C) for over 2 months. Release from the 5 and 20 nm nanochannel membranes occurred relatively quickly (weeks). Significantly, for the 3.5 nm nanochannel membranes, the cumulative release profile was relatively linear (n=4) over two months, and at day 60, targets ~30 ug of peptide. Therefore, we conclude that membranes housing 3.5 nm nanochannels are the most appropriate for *in vivo* studies.

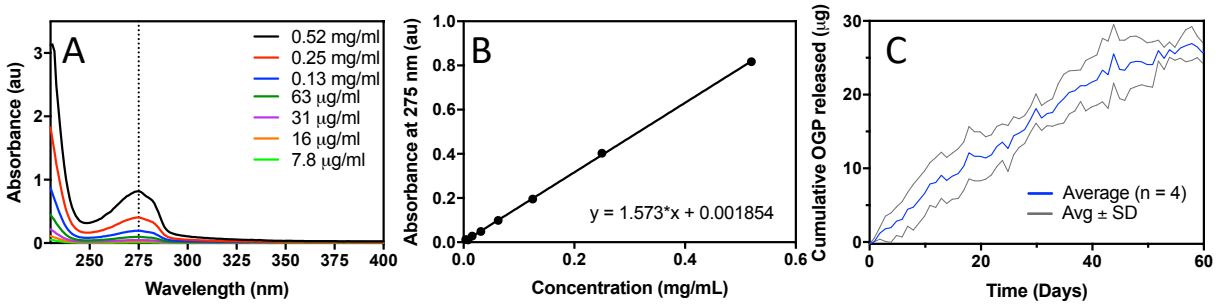

Figure 2. (A) Absorbance spectra of OGP at various concentrations demonstrating absorption maxima at 275 nm, (B) OGP concentration versus absorbance at 275 nm, and (C) cumulative OGP release profiles (n =4) from 3.5 nm nanochannel membranes over 60 days.

The sink solution from these studies has been saved and murine fibroblastic NIH 3T3 cells (ATCC) are currently being cultured and expanded so that the the biological activity of the released OGP can be assessed by alkaline phosphatase assay.

Major Task 2 Prepare IACUC protocol for *in vivo* studies. (Months 1-3)

The IACUC protocol for this study entitled “Development of Nanochannel Delivery System for Large Animal Models” has been approved.

Major task 2 has been completed, 100% complete.

Specific Aim 2. Release of OGP in an established large animal (rabbit model).

Major Task 3 Perform Rabbit study. (Months 6-18)

We are waiting for ACURO approval of our IACUC protocol to initate animal work.

Major task 3 has not been completed, 0% complete.

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

During the next reporting period we intend to 1) perform the alkaline phosphatase assay on murine fibroblastic NIH 3T3 cells treated with released OGP (from the sink solution) to assess if the biological activity of released OGP is maintained and 2) initiate the *in vivo* analysis of our devices in skeletally mature New Zealand white rabbits. To examine the devices *in vivo*, the transverse process of the rabbits will be decorticated and devices implanted on both sides of the spine for release over 8 weeks. During this 8-week period, animal weight will be monitored weekly and serum and plasma collected. Non-invasive imaging modalities (X-ray and DynaCT) will be used to characterize devices and assess surrounding tissue and bone *in vivo*.

Euthanasia will be performed at day 60 post-implantation and organs and tissue harvested and processed, embedded in paraffin, sectioned and stained with H&E for histological analysis.

4. IMPACT

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

Our major accomplishments thus far were: 1) sustained release in vitro of OGP for over two months using 3.5 nanochannel membranes and 2) the ability to custom fabricate our device in PEEK. This will impact the principle discipline of restorative bone healing as similar customizable devices can be made for any skeletal deformity which allow for constant, sustained and biologically appropriate concentrations of OGP directly at a target site for bone regeneration.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

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

Our IACUC protocol went up for a three year renewal in April. A new protocol was resubmitted and IACUC approval granted but there was a lengthy delay on ACURO approval for this renewal. Our study will be on track after we receive approval to continue.

Changes that had a significant impact on expenditures

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Carly Filgueira**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-3246-303X
Nearest person month worked:	5
Contribution to Project:	Dr. Filgueira drew the device elements in AutoCAD, fabricated and cleaned the implants and membranes, assembled and loaded the implants for the in vitro work and performed the in vitro release testing by monitoring OGP release daily. She is also maintaining the NIH 3T3 cells and assembling the implants for the in vivo rabbit studies. During these studies Dr. Filgueira will monitor the implant in the rabbits (migration/reaction)

	as well as analyze the images (DynaCT & X-ray) and histology to assess ossification and formation of new trabecular & cortical bone.

Name:	**Bradley Weiner**
Project Role:	Co-I
Nearest person month worked:	1
Contribution to Project:	Dr. Weiner will perform the implant surgery in rabbits.

Name:	**Dennis Wang**
Project Role:	Research Assistant
Nearest person month worked:	7
Contribution to Project:	Mr. Wang assisted Dr. Filgueira in cleaning the devices and membranes, performing gas testing and optical inspection of the membranes, and measuring the OGP in vitro release profiles.

Name:	**Alessandro Grattoni**
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0001-7888-422X
Nearest person month worked:	1
Contribution to Project:	Dr. Grattoni assisted in the design and fabrication of the spine fusion implant.

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

Yes, attached are the other support documents for Dr. Filgueira, Dr. Weiner and Dr. Grattoni.

What other organizations were involved as partners?

Not applicable

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

None

FILGUEIRA, C. (PI)

Previous Support

Grattoni/**Filgueira**/Bruckner/Igo 02/01/2017-08/31/2018 50% effort, 6.0 calendar
Kostas Cardiovascular Nanomedicine Grant Award $99,299 total
Controlled Intrapericardial Delivery of PGE-1 PLGA Nanoformulations for Heart Failure
Our goal is to fabricate a drug eluting device in the peri-coronary epicardial fat for implantation in healthy pigs as a novel means for treating vulnerable plaque.
Our Specific Aims include: 1) Pharmacodynamics of intrapericardial PGE-1 via HeartPAS™ infusion, 2) Release of intrapericardial nanoformulations via HeartPAS™ infusion in an established large animal (pig) model, and 3) Repeated delivery of agents in the established pig model.
Role: Co-Principal Investigator
Point of Contact: Tiffany Polk
This project relates to the use of fluoroscopy to evaluate device placement in a healthy pig model. There is **no scientific or budgetary overlap**.

Ongoing Research Support

Grattoni/**Filgueira**/Bruckner/Igo 11/15/2017-01/18/2020 12.5% effort, 1.5 calendar
Kostas Cardiovascular Nanomedicine Grant Award $100,000 total
Controlled Intrapericardial Delivery of PGI2 PLGA Nanoformulations for Heart Failure
Our goal is to proceed in the development and implantation through thoracotomy, in a healthy pig model, of a device that sits on the pericardium with minimal epicardial contact to provide drug access after myocardial infarction.
Our Specific Aims include: 1) Deployment of the HeartPAS™ in vivo (pig) with concurrent fluoroscopy imaging (mo. 1 to 6), 2) In vitro assessment of prostacyclin analogues in a diseased cell line, and 3) Delivery of agents in the established porcine model.
Role: Co-Principal Investigator
Point of Contact: Tiffany Polk
This project relates to deployment and assessment of a device that sits on the pericardium in a healthy pig model. There is **no scientific or budgetary overlap**.

Grattoni/**Filgueira**/Bruckner/Igo 03/01/2019-02/29/2020 12.5% effort, 1.5 calendar
Kostas Cardiovascular Nanomedicine Grant Award $50,000 total
Pharmacokinetic Study of Intrapericardial Delivery of PGI-2 Polymeric Nanoformulations for Heart Failure
Our goal is to demonstrate that PGI-2 administered intrapericardial as either unformulated drug or nanoformulated will result in higher drug concentrations than those obtained when the drug is administered systemically.
Our Specific Aims include: 1) To generate a direct comparison of the pharmacokinetics of PGI-2 and PGI-2 loaded polymeric-NPs administered by intravenous injection versus intrapericardial administration.
Role: Co-Principal Investigator
Point of Contact: Tiffany Polk
This project relates to deployment in a healthy pig model. There is **no scientific or budgetary overlap**.

Grattoni/Butler/**Filgueira** 09/01/2016-12/31/2019 2% effort, 0.24 calendar
Golfer's Against Cancer $80,000 total
From Local Delivery to Systemic Immune Activation: One-Two Punch to Cancer
Our goal is to intratumorally deliver gold nanoparticles through an innovative device and use a one-two punch of photothermal and radiation therapies to eradicate solid tumors and trigger an anti-tumor immune response to eliminate metastases around the body.
Our Aims are to 1) accurately quantitate the amount of gold nanoparticles released from our device into the

tumor and demonstrate a higher yield when compared with intravenously injected nanoparticles, 2) excite the particles through both the photothermal effect and radiotherapy and show cancer cell death by measuring tumor size, and 3) monitor the immune response induced by both photothermal and radiation therapy destruction of the tumor and assess the abscopal effect of distal metastasis.
Role: Co-Principal Investigator
Point of Contact: Tiffany Polk
This project relates to lung cancer and gold nanoparticle radiotherapy. There is **no scientific or budgetary overlap**.

Butler/**Filgueira** 09/01/2018-12/31/2019 2% effort, 0.24 calendar
Golfer's Against Cancer $50,000 total
Nanoparticle Enhanced Radioimmunotherapy for Lung Cancer
Our goal is to intratumorally deliver gold nanoparticles and immunoadjuvants to significantly enhance radiotherapy and produce synergistic effects.
Our aims are to: 1) determine the dose dependent effects of irradiation coupled with gold nanoparticle treatment on lung cell tumor regression (measure tumor size, change in luminescence), 2) quantify the amount of gold nanoparticles required to achieve tumor regression, and 3) perform radiotherapy of the primary tumor in combination with immunoadjuvants (CD40 monoclonal antibody) to test for increased survival and immune-mediated regression of metastasis outside the radiation field, based on an abscopal effect.
Role: Co- Principal Investigator
Point of Contact: Tiffany Polk
This project relates to lung cancer and gold nanoparticle radiotherapy. There is **no scientific or budgetary overlap**.

Butler/**Filgueira** 03/22/2019-12/31/2019 2% effort, 0.24 calendar
Golfer's Against Cancer $50,000 total
Nanoparticle Induced Anti-tumor Immunity for Lung Cancer
Our goal is to improve cancer treatment and promote cancer immunity by inducing the abscopal effect in a more robust manner to generate a tumor-specific immune response using an antibody-gold nanoparticle construct.
Our aims are to: 1) develop an antibody-gold nanoparticle construct, 2) demonstrate with computed tomography (CT) imaging that our chemically modified nanoparticles distribute differently in the tumor environment than unmodified nanoparticles and monitor length of particle entrapment and clearance in a solid tumor, 3) determine the effects of treatment with irradiation and chemically modified nanoparticles (changes in tumor growth, immune activation, and prevalence of lung metatasis).
Role: Co- Principal Investigator
Point of Contact: Tiffany Polk
This project relates to lung cancer and gold nanoparticle radiotherapy. There is **no scientific or budgetary overlap**.

Filgueira 08/01/2018-1/31/2020
Department of Defense PRMRP Discovery Award $200,000 direct costs 65% effort, 7.8 calendar
Implantable Nanochannel System for the Controlled Delivery of Osteogenic Growth Peptide
Our objective is to design a spinal implant permitting sustained release of Osteogenic Growth Peptide (OGP) and to perform *in vivo* efficacy testing in a large animal (rabbit) model.
Specific Aim 1: Design a spinal fusion implant that allows for sustained release of OGP and Specific Aim 2: Release of OGP in an established large animal (rabbit) model.
Role: Principal Investigator
Point of Contact: Allison Milutinovich, Ph.D. Program Manager
This project involves use of an implantable nanofluidic membrane for the controlled administration of OGP and there is **no scientific or budgetary overlap**.

Pending Research Support

Filgueira 09/30/2020-3/29/2022
Department of Defense PRMRP Discovery Award $200,000 direct costs 50% effort, 6.0 calendar
Sustained Release of Platelet Rich Plasma Growth Factors for Improved Graft Remodeling in Post-Traumatic Osteoarthritis
Our objective is to develop phosphorylated alginate nanocarriers encapsulating platelet-rich plasma (PRP) for the sustained release of autologous growth factors to provide symptomatic relief in osteoarthritis of the knee.
Specific Aim 1: Design blended phosphorylated alginate-based carriers that allow for sustained release of PRP and assess them *in vitro* and Specific Aim 2: Release of PRP in an established large animal (porcine) anterior cruciate ligament transection and reconstruction model.
Role: Principal Investigator
Point of Contact: TBD
This project involves use of alginate nanocarriers encapsulating platelet-rich plasma and there is **no scientific or budgetary overlap**.

Filgueira 09/30/2020-3/29/2022
Department of Defense PRMRP Discovery Award $200,000 direct costs 50% effort, 6.0 calendar
Heart-Specific Therapy for Cardiomyopathy
Our objective is to engineer poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles loaded with a prostaglandin (PGE1, PGI2) and deliver them intrapericardially via our pericardial access system to provide a targeted heart-specific therapy to promote cardiac tissue regeneration.
Specific Aim 1: To develop prostaglandin loaded poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles and assess them *in vitro* in comparison to unmodified drug and Specific Aim 2: To assess pharmacokinetics and efficacy in vivo (sheep) in a model of ischemic cardiomyopathy.
Role: Principal Investigator
Point of Contact: TBD
This project involves use of polymeric nanoparticles in a sheep model and there is **no scientific or budgetary overlap**.

Filgueira 09/30/2020-09/29/2021
Department of Defense LCRP Concept Award $100,000 direct costs 50% effort, 6.0 calendar
A radioimmunotherapeutic approach to lung cancer
Our objective is to investigate the tumor microenvironment and explore how radiotherapy (RT) establishes an *in situ* vaccination that promotes and enhances the local and abscopal anti-tumor effect of immune checkpoint inhibition (ICI) treatment.
Specific Aim 1: To investigate the primary tumor (local) response to combined RT and mAb PD-L1 ICI treatment in a lung cancer mouse model and Specific Aim 2. To assess contralateral and metastatic tumor (abscopal) response to combined RT and mAb PD-L1 ICI treatment in a lung cancer mouse model.
Role: Principal Investigator
Point of Contact: TBD
This project involves use of radio- and immuno-therapy in a murine lung cancer model and there is **no scientific or budgetary overlap**.

WEINER, B. (Co-I)

Ongoing Research Support

W81XWH-15-1-0718 09/30/2015 - 09/29/2019 0.24 Calendar Months
Department of the Army, The Combat Casualty Care Research Program (CCCRP)
A GMG/GLP investigation of degradable polymeric shells for traumatic osteogeneration
Project Goal: Further development of the PEU and Collagen Shells will help to solve the devastating problem of bridging critically sized lower extremity defects. Clinical applications will include the ability to quickly and safely form bone, avoid repeated surgeries or extensive rehabilitation, eliminate permanent infection-prone hardware, and quickly return to duty for military personnel and normal routine for civilians. Specific Aims/Study Design: We will complete the preliminary assessment of PEU and Collagen Shells effective in critical-sized osteoregeneration. GLP studies in sheep will be performed using GMP-grade PEU and Collagen Shells, and the data will be rigorously analyzed for a pre-IDE regulatory consultation with the Food and Drug Administration. We have worked with industry partners and regulatory experts and policy makers to identify an accelerated path to clinical application.
Role: Co-Investigator
Point of Contact: McKean, Joshua D. (Grants Specialist) Joshua.d.mckean3.civ@mail.mil

Cullen Trust Foundation 05/01/2014-12/31/2018 0.60 Calendar Months
Houston Methodist Research Institute
Project Goal: To mimic the body's healing function and repair damaged tissues, utilizing combinations of tissue-engineered constructs, nanoscale smart delivery systems, and patient-derived stem cells. By leveraging on our multidisciplinary strengths we will bridge life sciences with physics, medicine with mathematics, device engineering with molecular imaging, and nanotechnology with systems biology. This project aims at the creation of the Center for Regenerative Medicine, which perfectly embody the spirit, vision and commitment of my laboratory to the development of unconventional technologies and solutions inspired by nature.
Role: Principal Investigator
Point of Contact: Sun, Tong (Managing Fund Custodian) tsun@houstonmethodist.org

W81XWH-14-1-0600 09/30/2014-09/29/2019 0.36 Calendar Months
Department of Defense, Spinal Cord Injury Research
Assessment of Spinal Injuries Using Novel Ultrasound Techniques
Project Goal: Develop new spinal 3D US imaging techniques to assess spinal injuries, fractures and abnormalities and monitor bone regeneration in vivo, Develop new spinal elastography techniques to assess the mechanical response of the soft tissue in proximity of the spine and at the soft tissue/bone interface in the presence of a SCI, spinal fracture or abnormality and during tissue healing and bone regeneration in vivo and Test and statistically analyze the performance of the developed spinal US imaging techniques (3D ultrasound and elastography) in the assessment of SCI in a small animal study in vivo and validate the results using MRI, CT and histopathology.
Role: Co-Investigator
Point of Contact: Dr. Tilghman

W81XWH-18-1-0438 08/01/2018-1/31/2020 0.24 Calendar Months
Department of Defense PRMRP Discovery Award $200,000 direct costs
Implantable Nanochannel System for the Controlled Delivery of Osteogenic Growth Peptide
Our objective is to design a spinal implant permitting sustained release of Osteogenic Growth Peptide (OGP) and to perform *in vivo* efficacy testing in a large animal (rabbit) model.
Specific Aim 1: Design a spinal fusion implant that allows for sustained release of OGP and Specific Aim 2: Release of OGP in an established large animal (rabbit) model.
Role: Co-Investigator

Point of Contact: Allison Milutinovich, Ph.D. Program Manager
This project involves use of an implantable nanofluidic membrane for the controlled administration of OGP and there is **no scientific or budgetary overlap**.

Pending Research Support (Approved. Await funds)

Integrated Solutions for Cranial and Maxillofacial Reconstruction $10,000,000
Department of Defense / Senate Appropriations

Overlap
No overlap

GRATTONI, A. (Co-I)

Previous Support

Grattoni 08/01/2014-07/31/2019 23% effort, calendar
CASIS GA-2014-145 $1,420,000
Remote controlled nanochannel implant for tunable drug delivery.
Our goal is to develop a technology for the tunable drug dosing of animals on the International Space Station.
Our specific aim includes: 1) To design a tunable nanochannel delivery implant based on ionic concentration polarization. 2) To characterize the implant in vitro. 3) To test the implant for the tunable delivery of ibandronic acid in healthy Sprague-Dawley rats in microgravity.
Role: Principal Investigator
Point of Contact: Kenneth Shields
This project relates to expanding in vivo study capabilities on the ISS by developing a tunable drug delivery system. There is no scientific or budgetary overlap.

Ongoing Research Support

Grattoni 03/01/2018 – 02/28/2020 1% effort, 0.12 calendar
Wilfred Masterson Burke Medical Research Institute $29,025
Controlled delivery of butyrate from a nanofluidic implant
Our goal is to develop a sustained delivery system for the administration of butyrate.
Our specific aim includes: 1) to develop HPLC methods for the quantification of butyrate in vitro. 2) To test the release of butyrate from nanofluidic membranes and determine release rates adequate for in vivo testing.
Role: Principal Investigator
Point of Contact: Rajiv Ratan
This project relates to assessing the sustained release of butyrate in vitro. There is no scientific or budgetary overlap.

Grattoni/Chen 08/01/2018-07/31/2019 1% effort, calendar
Golfers Against Cancer $90,000
Leveraging synergistic effects of local radio-immunotherapy to eradicate breast cancer.
Our goal is: To combine intratumoral immunotherapy delivery with radiation to induce a potent systemic anti-tumor immune response to eliminate primary and metastatic tumors. If successful, the potential to revolutionize treatment extends beyond breast cancer.
Our specific aim includes: 1) Evaluate effects of intratumoral release of monoclonal antibody, 4-1BB, alone or in combination with radiation on tumor growth and immune response. 2) Compare conventional systemic 4-1BB delivery with sustained intratumoral delivery to examine efficacy and effects on toxicity. 3) Assess efficacy of 4-1BB antibody alone or in combination with radiation to prevent tumor recurrence and metastasis.
Role: Principal Investigator
Point of Contact: Tiffany Polk
There is no scientific or budgetary overlap.

Grattoni/Liu 04/15/2018 – 01/31/2022 20% effort, calendar
NIH/NIGMS R01GM127558 $1,572,802
A nanofluidic platform for tunable drug delivery
Our goal is to demonstrate in small and large animal models an implantable drug delivery systems based on electrostatic gating for the remotely controlled delivery of therapeutics.
Our specific aim includes: 1) To design and assemble remotely controlled delivery implants. 2) To investigate the tunable and remote controlled release of drugs *in vitro*. 3) To test the RF-controlled implant for the tunable delivery of drugs in small and large animals.
Role: Principal Investigator

Point of Contact: Richard Okita
This project relates to the development of a remotely controlled gated drug delivery system. There is no scientific or budgetary overlap.

Grattoni 03/01/2018-02/28/2020 1% effort, calendar
JDRF Innovative Grant $109,000
3D printed biomaterial encapsulation with localized immunomodulation for islet transplantation.
Our goal is to develop a 3D printed polymeric encapsulation system for the transplantation of pancreatic islets.
Our specific aim includes: 1) Development and in vitro characterization of a hydrogel based matrix for localized and sustained release of growth factors and immunosuppressants. 2) Demonstration of islet survival and function in the prevascularized encapsulation system in immunocompetent mice
Role: Principal Investigator
Point of Contact: Esther Latres
This project relates to a cell transplantation technology. There is no scientific or budgetary overlap.

Grattoni/Shen 08/01/2017-02/01/2020 1% effort, calendar
Golfers Against Cancer $80,000
Triggering the abscopal effect in triple negative breast cancer with nDSmini.
Our goal is: To reproducibly trigger a systemic immunological response that could eradicate both primary tumor and metastasis.
Our specific aim includes: 1) Demonstrate release of chemoimmunotherapeutic drugs (doxorobicin, CD40 and PD-1 antibodies) directly into the tumor via intratumoral drug delivery implant, towards achieving tumor regression. 2) Establish that prolonged tumor exposure to chemoimmunotherapeutic drugs will maximize drug uptake and induce systemic anti-tumor immune response, and thereby enhance treatment efficacy. 3) Treat primary tumor and prevent cancer recurrence and metastasis.
Role: Principal Investigator
Point of Contact: Tiffany Polk
There is no scientific or budgetary overlap.

Grattoni/Butler/Filgueira 09/01/2016-8/31/2017 2% effort, 0.24 calendar
Golfer's Against Cancer $80,000 total
From Local Delivery to Systemic Immune Activation: One-Two Punch to Cancer
Our goal is to intratumorally deliver gold nanoparticles through an innovative device and use a one-two punch of photothermal and radiation therapies to eradicate solid tumors and trigger an anti-tumor immune response to eliminate metastases around the body.
Our Aims are to 1) accurately quantitate the amount of gold nanoparticles released from our device into the tumor and demonstrate a higher yield when compared with intravenously injected nanoparticles, 2) excite the particles through both the photothermal effect and radiotherapy and show cancer cell death by measuring tumor size, and 3) monitor the immune response induced by both photothermal and radiation therapy destruction of the tumor and assess the abscopal effect of distal metastasis.
Role: Co-Principal Investigator
Point of Contact: Tiffany Polk
This project relates to lung cancer and gold nanoparticle radiotherapy. There is no scientific or budgetary overlap.

Grattoni 07/01/2017-06/30/2020 5% effort, calendar
Lamborghini Auto $750,000
Investigation of the biocompatibility of carbon fibers composites for implantable medical devices.
Our goal is to assess the biocompatibility of 16 carbon fiber materials for potential biomedical implantable applications.
Our specific aim includes: 1) To test in vitro the cytotoxicity and genotoxicity of CFRPs and assess their effects on osteoblast and macrophages. 2) To evaluate acute systemic toxicity and sub-chronic toxicity of CFRPs after

subcutaneous implantation. 3) To investigate chronic toxicity and foreign body response to CFRP implants during 6 month implantation in a domestic pig model.
Role: Principal Investigator
Point of Contact: Luciano De Oto
This project relates to the evaluation of the biocompatibility of new materials. There is no scientific or budgetary overlap.

Grattoni 09/01/2016-08/31/2021 23% effort, calendar
NIH/NIAID R01AI120749 $3,889,078
A novel nanochannel system for sustained delivery of Tenofovir Alafenamide Fumarate and Emtricitabine for HIV pre-exposure prophylaxis.
Our goal is to develop a transcutaneously refillable drug delivery implant of TAF and FTC and evaluate the PK and preventive efficacy in the context of HIV pre-exposure prophylaxis.
Our specific aim includes: 1) To develop nDS implants capable of sustained and constant release of TAF/FTC in rats and NHP. 2) To assess the pharmacokinetics of constant delivery of TAF/FTC from nDS implants at target release rates for 60 days in NHP. 3) To evaluate prevention of SHIV infection through rectal challenge by release of TAF/FTC from nDS implants in NHP.
Role: Principal Investigator
Point of Contact: Jim Turpin
This project relates to the demonstration of an implant for HIV PrEP. There is no scientific or budgetary overlap.

Grattoni 5/01/2016-12/31/2019 10% effort, calendar
CASIS GA-2016-234 $172,275
Implantable nanochannel system for the controlled delivery of therapeutics for muscle atrophy.
Our goal is to develop and test a small subcutaneous implantable system for the prevention of muscle atrophy in microgravity.
Our specific aim includes: 1) To optimize the implant for the constant and sustained delivery of FMT.
2) To assess the optimal release rate of FMT in vivo by PK analysis and dosing evaluation in mice on-ground, in comparison to conventional bolus administration. 3) To test the efficacy of sustained subcutaneous delivery of FMT released from nanochannel implants in the microgravity mouse model of muscle atrophy.
Role: Principal Investigator
Point of Contact: Kenneth Shields.
This project relates to muscle atrophy investigation in microgravity. There is no scientific or budgetary overlap.

Gaber/**Grattoni** 11/01/2011 – 12/31/2018 1% effort, 0.12calendar
Vivian Smith Foundation $650,000
Examining the potential of human Mesenchymal stem cells and osteocalcin in augmenting human islet mass and improving islet engraftment and long-term function.
Our goal is to develop a protocol for the differentiation of stem cells into islet like insulin producing cells and assess their ability to secrete insulin in vivo in a polymeric encapsulation system.
Our specific aim includes: 1) to develop and optimize MSC differentiation protocol to achieve islet like insulin producing aggregates (ILIPA) of cells. 2) To develop a 3D printed encapsulation for the delivery of cells and assess its degradation and biocompatibility in vitro. 3) To test the ILIPA in the encapsulation system in vivo in rodents.
Role: Co-Principal Investigator
Point of Contact: Jackie Callies
This project relates to cell transplantation for the treatment of diabetes. There is no scientific or budgetary overlap.

Filgueira 01/01/2019-6/30/2020
Department of Defense PRMRP Discovery Award $322,568 total 1.5% effort, 0.18 calendar

Implantable Nanochannel System for the Controlled Delivery of Osteogenic Growth Peptide
Our objective is to design a spinal implant permitting sustained release of Osteogenic Growth Peptide (OGP) and to perform in vivo efficacy testing in a large animal (rabbit) model.
Specific Aim 1: Design a spinal fusion implant that allows for sustained release of OGP and Specific Aim 2: Release of OGP in an established large animal (rabbit) model.
Role: Co-Investigator
Point of Contact: Allison Milutinovich, Ph.D. Program Manager
This project involves use of an implantable nanofluidic membrane for the controlled administration of OGP and there is no scientific or budgetary overlap with any of the previous, current, or pending funding support.

Grattoni 08/01/2014-07/31/2019 5% effort, 0.6 calendar
CASIS GA-2019-003 $49,674
Study of Lamborghini's carbon fiber composites for aerospace applications
Specific aim: To investigate the performances of 5 selected carbon fiber materials developed by Automobili Lamborghini for aerospace applications.
Role: Principal Investigator
Point of Contact: Kenneth Shields
There is no scientific or budgetary overlap.

Grattoni 1/01/2019-12/31/2019 1% effort, 0.12 calendar
Nancy Owens Memorial Foundation $35,000
Intratumoral Implant for Breast Cancer Immunotherapy.
Specific Aim: To evaluate efficacy of nanofluidic implant in murine and rodent models of breast cancer.
Role: Principal Investigator (2% effort)
Overlap: None

Grattoni 08/01/2019-07/31/2020 1% effort, calendar
Men of Distinction $100,000
Overcoming the epidemic of pediatric obesity and prediabetes via a nanofluidic technology
Specific Aim: To evaluate anti-obesity efficacy of sustained delivery of GC-1 in non-human primates
Role: Principal Investigator
Point of Contact: Tiffany Polk
There is no scientific or budgetary overlap.

Pending Research Support

Chua/**Grattoni** 11/16/2019-07/31/2019 2% effort, calendar
CASIS GA-2019-00953 $49,674
Sustained delivery of a bisphosphonate-prostaglandin analog complex (C3) for the prevention and treatment of osteopenia
Our goal is to study our nanofluidic implant for zero-order and sustained delivery of C3 for effective and safe prevention of osteoporosis in microgravity.
Our specific aim includes: 1) To optimize the nanofluidic implants for constant and sustained delivery of C3 in preparation for the microgravity flight experiment. 2) To test the efficacy of sustained subcutaneous delivery of C3 released from nanofluidic implants in microgravity-induced spontaneous mouse model of osteoporosis.
Role: Co-Principal Investigator
Point of Contact: Kenneth Shields
There is no scientific or budgetary overlap.