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

PRINCIPAL INVESTIGATOR: Carly Filgueira

CONTRACTING ORGANIZATION:
Methodist Hospital Research Institute, Houston, TX

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Carly Filgueira

E-Mail:csfilgueira@houstonmethodist.org

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

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14. ABSTRACT

We have fabricated implantable, medical grade polyether ether ketone (PEEK) devices housing two silicon nanofluidic membranes for the sustained and constant release of osteogenic growth peptide (OGP) and tested their tolerability and efficacy to stimulate local bone growth in New Zealand White Rabbits. The devices, 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 the microfabricated silicon nanochannel membranes to determine the optimal nanochannel size (3.5 nm) to achieve sustained, constant release. OGP release was relatively linear over the two-month period, and at day 60, cumulative release profiles targeted ~30 ug of peptide. Vehicle releasing (PBS:PBS), mixed (PBS:OGP), or treatment releasing (OGP:OGP) devices were implanted on both sides of the spine in rabbits (n=12) for two months to assess the promotion new bone formation in vivo. Signs of osteogenesis were evaluated via X-ray, cone beam CT, and histology. Overall, we demonstrate that our nanofluidic platform for constant OGP delivery can result in local ossification near the device delivery site.

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

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Table of Contents

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. NIH 3T3 cells were obtained from

ATCC and Alkaline Phosphatase Assay Kits (Colorimetric) were obtained from Abcam (Cat. No. ab83369). We had difficulty in measuring alkaline phosphatase due to detachment of the 3T3 cells during media exchange prior to OGP treatment. Phenol red DMEM (from ATCC) was used to culture, expand, and passage the cells prior to running the assay. However, once the cells were seeded in 96-well plates at a seeding density of 3.2x10^3 cells/well, when the media was exchanged for serum free, phenol red free media, (required since this is a colorimetric assay) the cells detached from the plate.

Since higher concentrations of sodium bicarbonate are known to affect cell attachment, in order try to resolve the problem different types of media (ATCC and Gibco) with different sodium bicarbonate concentrations (1500 mg/L versus 3700 mg/L) were tested. This still resulted in cell detachment, so an in house media formulation using a phenol free DMEM base was prepared by dissolving DMEM powder (Sigma) in sterile water with a lower amount of sodium bicarbonate. Coating the wells with Poly-L-Lysine (Sigma) to promote attachment was also tried. In each of the scenarios, the cells detached. A solution has not been found but we are still trying to problem solve potential resolutions.

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.

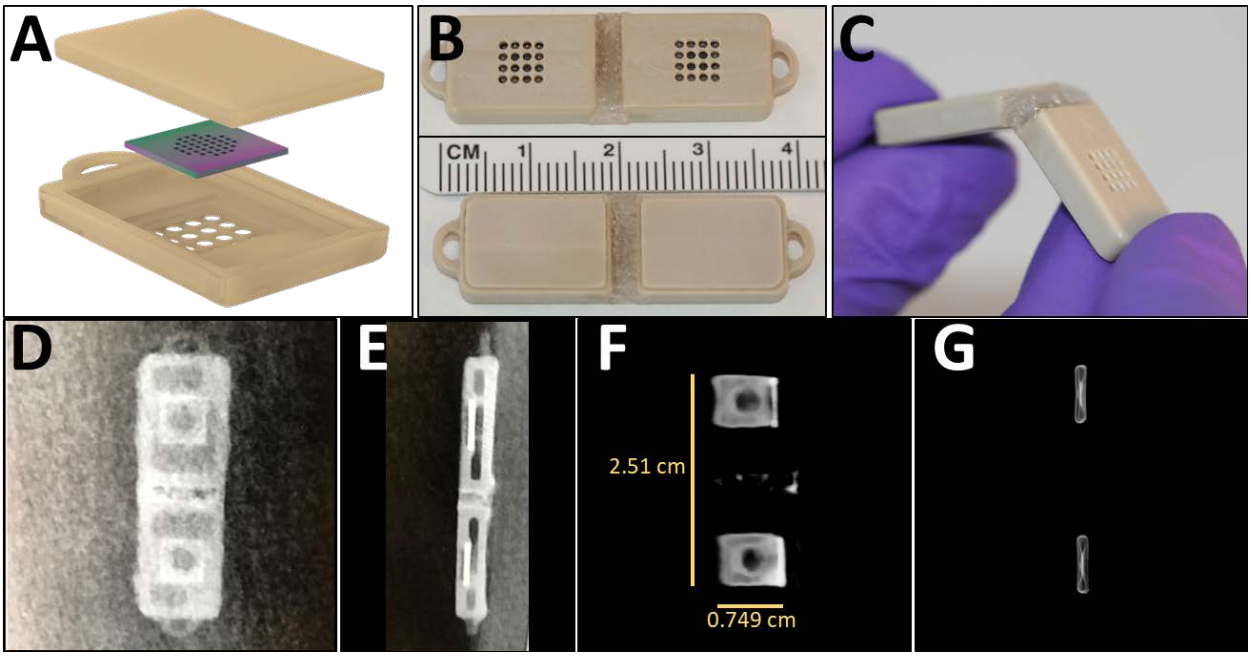

Figure 1. (A) 3D rendering of the osteoinductive implant with side loop for suturing capability, (B) assembled PEEK device consisting of two drug reservoirs and nanochannel membranes, (C) photo demonstrating implant flexibility, (D, E) X-ray and (F, G) DynaCT images of the implant.

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.

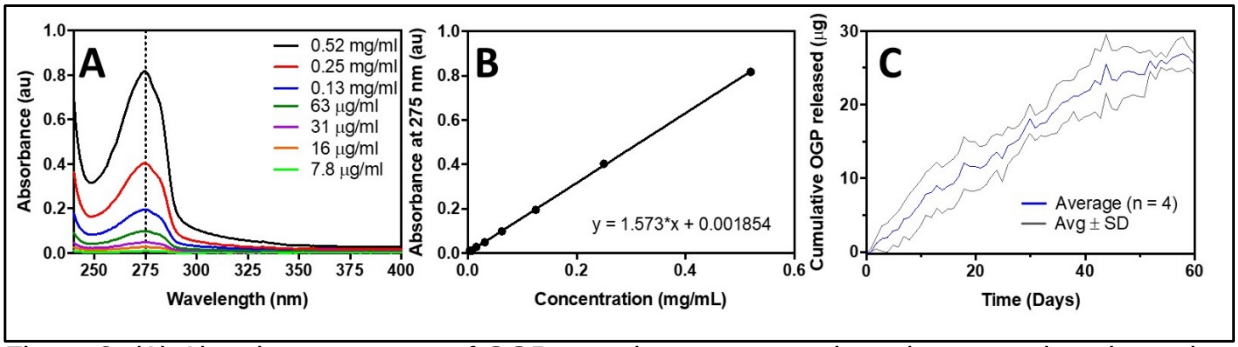

Figure 2. (A) Absorbance spectra of OGP at various concentrations demonstrating absorption maxima at 275 nm, (B) OGP concentration versus absorbance at 275 nm, and (C) cumulative OGP release profiles (n = 4) from 3.5 nm nanochannel membranes over 60 days.

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)

Major task 3 was completed 100% (See Figs. 3 – 7 and table 1).

The subtasks achieved were: 1) Ordering and acclimation of skeletally mature New Zealand White rabbits, acclimation, baseline weights and blood collection, 2) Practice device implantation, 3) Implantation across larger rabbit cohort (Fig. 3), 4) Monitoring healing and any reactions to implant (Table 1), 5) Performing non-invasive imaging with x-ray (Fig. 4) and DynaCT (Fig. 5), 6) Image analysis to determine bone volumes, 7) Euthanasia and histology of spine (Fig. 6) and fibrotic capsule (Fig. 7), 8) Device retrieval, and 9) Analyses, interpretations and report writing.

All experiments were approved by our local institutional animal care and use committee. Briefly, the study was conducted in twelve 14-week-old female New Zealand White laboratory rabbits (*Oryctolagus cuniculus*), New Zealand strain, weighing approximately 3 Kg. Rabbits arrived at the facility and acclimated for at least ten days. Body weight and blood collection were performed 14 days before surgery and at 7, 28, 42, and 56 days after device implantation was done. Blood analyses were performed by The University of Texas MD Anderson Cancer Center laboratory (MDACC). Previous to a bilateral posterolateral intratransverse process spinal arthrodesis at either L4-L5 or L5-L6, animals received a subcutaneous injection of buprenorphine SR (0.12 mg/kg body weight) and carprofen (5 mg/kg body weight) for postoperative analgesia. Intramuscular (IM) injections of ketamine hydrochloride (40 mg/kg body weight) and IM injections of midazolam (0.5–2 mg/kg) were administered as pre-anesthetic agents to help with anesthetic induction. General anesthesia was induced using a mask delivering a 2.5–3 % isoflurane/medical air, while anesthesia was maintained with a 2 %

isoflurane/medical air administered through an endotracheal tube, with the animal laying proneon the operating room table. Aseptic procedures, sterile gauze/sutures/drapes, draping of the surgical site, preparation of the surgeon (sterile gloves, cap, mask, gowns), and appropriate procedures during surgery to minimize the contamination of the surgery site were observed. A new sterile package of instruments was used on each animal. No preoperative antibiotics were given. Intraoperative antibiotics were administered (22mg/kg ceftriaxone (Rocephin)) IV every 2 hours. A dorsal midline incision extended through the skin, underlying subcutaneous (SC) fat, and the fascia. The musculature along the spine was removed with electrocautery to expose the lamina of the vertebrae, and an electric burr (Ideal Micro-Drill) was used to decorticate along the lamina. The devices were placed on either side of the spine (Fig. 3A) and loaded with OGP (200 ng/kg body weight) or vehicle (Phosphate-Buffered Saline (PBS)). The amounts loaded were calculated for release treatments expected to last for ~2 months. Treatment groups consisted of 1) OGP:OGP group (two devices releasing OGP), 2) PBS:PBS group (two devices releasing vehicle PBS), 3) OGP:PBS group (a mix treatment with right device releasing OGP and left device releasing PBS). The fascial incisions were closed with sutures (Fig. 3B). The skin was re-approximated with sutures and skin glue (Fig. 3C). After surgery the animals were monitored at least twice daily by the veterinary staff throughout the study period. The rabbits were sacrificed 56 days after fracture. The device site was assessed (Fig. 3D), the implants removed, and the area surrounding the implant was checked for scar tissue. Bone was collected and processed for H&E staining.

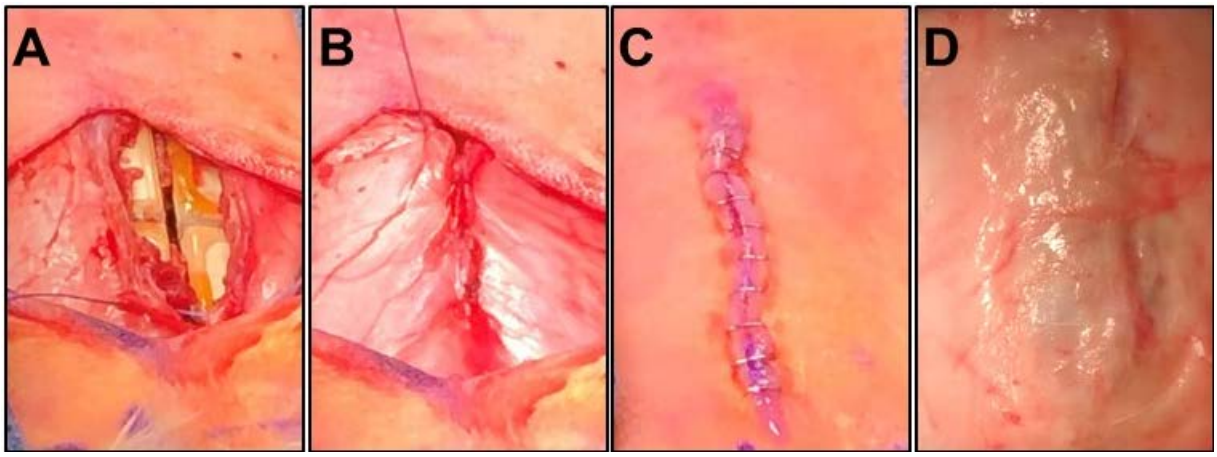

Figure 3. Surgical procedure. (A) Device implantation, (B) incision suture, (C) closed skin incision immediately post-surgery, and (D) device site 56 days after surgery.

Blood analyses results were obtained across the course of the experiment (Table 1). In the OGP:OGP group, the mean WBC count was lower than reported literature values (5.2-10.6 $x10^3/\mu L$)[1] for NZ white rabbits throughout all timepoints except 7 days post-implant. In the PBS:PBS group, the WBC was similarly lower at all timepoints. In the OGP:PBS group, WBC values were within reported values except on Day 42 post-implant, where the value was lower. This indicates no clinical sign of infection. In all groups, the percentage of eosinophils remained within the reported values (0.8-3.2 %)[1] throughout the studies, indicating the rabbits did not experience an allergic or adverse reaction to the drug, device or both. In the OGP:OGP group, the mean AST value was higher than the reported range (7.00-19.00 U/L)[2] at all timepoints except Days 28 and 42. Similarly, in the PBS:PBS group, the value was elevated at all time points except at Day 42, and the OGP:PBS group was elevated at all timepoints except for Day 7. Given that

the values were elevated before the implant, this is likely not due to the drug or device. The mean ALT was elevated in all groups at all timepoints when compared to the reported range (5.00-8.00 U/L)[2], suggesting this is not due to the drug or device but normal rabbit physiology. Similarly, in all groups and across all timepoints, creatinine levels remained within the range of reported values (0.68-1.58 mg/dL)[2]. Overall, there were no clinical signs of infection. Body weights were monitored and were within error for all three treatment groups dropping slightly from 3.0 Kg to 2.9 Kg one week after surgery but increasing to 3.5 Kg at the end of the study period.

Blood Parameter	Timepoint	Treatment group		
		PBS: PBS	OGP:PBS	OGP:OGP
WBC Count ($x10^3/\mu L$)	Baseline	5.24	5.98	5.61
	Day 7	5.82	6.11	5.96
	Day 28	5.35	5.86	5.60
	Day 42	4.36	4.89	4.63
	Day 56	5.18	7.21	6.20
% EOS	Baseline	1.33	1.67	1.30
	Day 7	1.95	2.14	2.35
	Day 28	1.03	1.62	1.25
	Day 42	1.10	1.43	1.03
	Day 56	1.03	1.62	0.99
AST (U/L)	Baseline	26.00	20.75	54.00
	Day 7	19.50	13.50	24.50
	Day 28	27.50	23.00	15.50
	Day 42	17.50	24.00	14.25
	Day 56	26.25	24.50	45.25
ALT (U/L)	Baseline	29.00	24.50	22.75
	Day 7	41.50	34.25	25.75
	Day 28	28.25	23.75	15.00
	Day 42	25.00	24.50	15.25
	Day 56	31.33	26.25	20.50
Creatinine (mg/dL)	Baseline	0.89	0.87	1.14
	Day 7	0.72	0.75	0.83
	Day 28	0.75	0.77	0.89
	Day 42	0.72	0.81	0.88
	Day 56	0.76	0.70	0.94

Table 1. Average serum parameters for n=12 rabbits for the different treatment groups.

Under the effect of sedation, bone images were assessed for all animals (n=12) 14 days before surgery and at 7, 28, 42, and 56 days after device implantation by an Axiom Artis system (Siemens Medical Solutions, USA). Figure 4 shows lateral view x-ray images for three different OGP:OGP treated rabbits, pre-implantation (Fig. 4 A, C, E) and 56 days post-implantation (Fig. 4. B, D, F). The membranes inside the devices (two membranes on each side of the spine) can be seen in the post-implantation images. Black arrows highlight observed areas of ossification as seen by thickening near the articular processes.

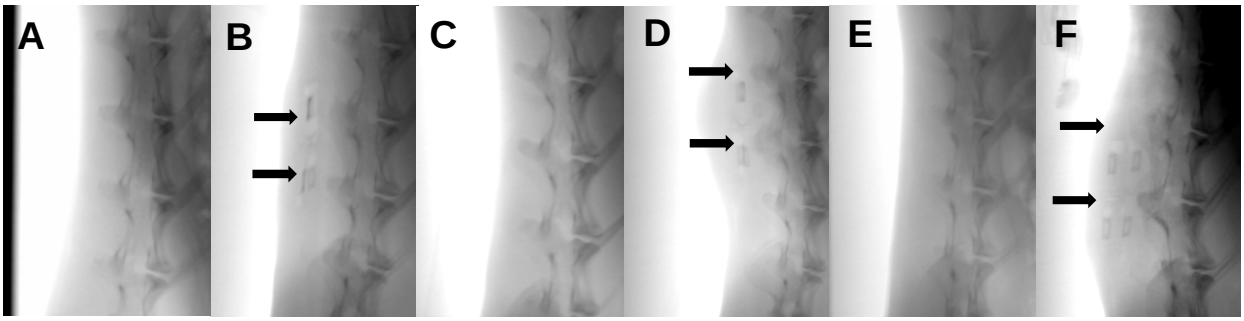

Figure 4. X-ray images of three different rabbit spines from the OGP:OGP treated group, lateral views. (A, C, E) pre-implant and (B, D, F) 56 days post-implantation. Black arrows indicate areas of ossification observed near the articular processes.

Under the effect of sedation, lumbosacral cone-beam computed tomography (DynaCT®) scans were acquired for all animals (n = 12) 14 days before surgery and at 7, 28, 42, and 56 days after device implantation. The images were obtained with an Axiom Artis C-arm (d)FC (Siemens Medical Solutions, USA). Scanning was performed with a 48 cm × 36 cm flat-panel integrated detector. Acquisition parameters for DynaCT were as follows: 70 kV tube voltage, automatic tube current of 107 mA, 20 s scan. Each scan entailed 200° of rotation, with 1 image taken every 0.5° for a total of 444 images (each digital acquisition had a matrix of 512 × 512 pixels) per acquisition. Cone-bean CT images across different time points were reconstructed in 3D using cinematic rendering technique (Syngo.Via, Siemens Medical Solutions, USA) (Fig. 5). White arrows highlight areas of ossification which were found to progress and thicken over the course of the study.

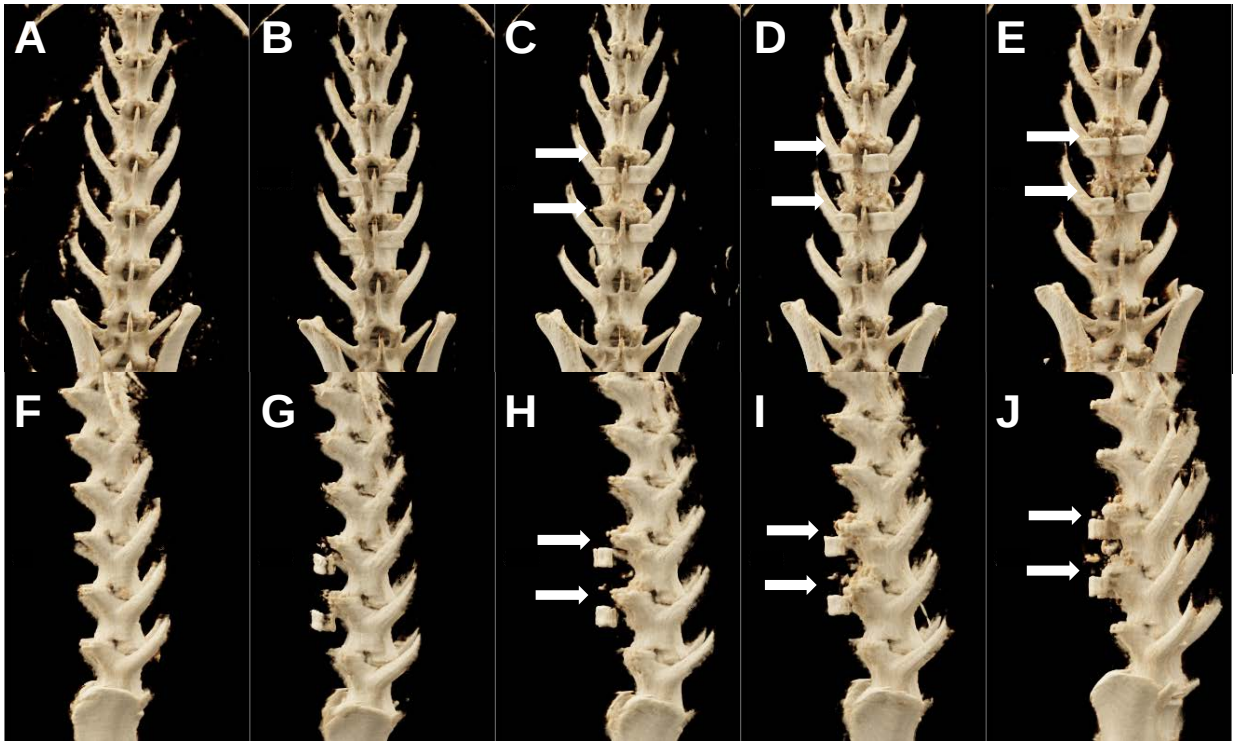

Figure 5. Cone-beam CT images reconstructed in 3D using cinematic rendering technique of rabbit spine from the OGP:OGP treated group. (A-E) Posterior views and (F-J) lateral views obtained (A, F) pre-implant, (B, G) 7 days, (C, H) 28 days (D, I) 42 days and (E, J) 56 days post-implantation. White arrows indicate areas of ossification observed near the articular processes.

Upon euthanasia, the implants were removed and the area surrounding the implant was checked for scar tissue. Bone as well as the fibrotic capsule tissues were collected and processed for Hematoxylin and Eosin (H&E) staining. Staining was performed using the ST Infinity H&E Staining System (Leica Biosystems) in Leica Autostainer ST5010 XL. Paraffin was melted prior to staining by heating the slides at 60˚C for 30 minutes, then deparaffinized by performing 3 x 2 minute washes in xylene, 3 x 1 minute washes in 100% ethanol, 1 x 1 minute washes in 95% ethanol before rinsing in tap water. Slides were incubated for 30 seconds in Hemalast, for 5 minutes in Hematoxylin, and were rinsed for 1 minute in tap water. Next, slides were incubated for 30 seconds in Differentiator and 1 minute in Bluing agent, with each step followed by a tap water rinse for 1 minute. Then 95% Ethanol for 1 minute. Slides were stained with Eosin for 30 seconds, dehydrated in 95% Ethanol for 1 x 1 minute, 1 x 4 minute in 100% Ethanol, 2 x 1 minute in 100% ethanol and cleared in 3 x 2 minutes in xylene. Every step after the initial heating of the slides was performed at room temperature.

Figure 6 shows a comparison of spine morphology obtained from H&E histological examination from the PBS:PBS (Fig. 6A) and OGP:OGP (Fig. 6B) treated groups. Morphology was assessed using an Olympus FLUOVIEW FV3000 Confocal Laser Scanning Microscope (Waltham, Massachusetts, USA). In the treated animals, we found evidence of endochondral ossification, the process by which growing cartilage is systematically replaced by bone to form the growing skeleton, as well as reactive bone formation with osteoblastic rimming. Reactive bone formation is a periosteal reaction resulting in the formation of new bone in response to injury or other stimuli of the periosteum surrounding the bone.

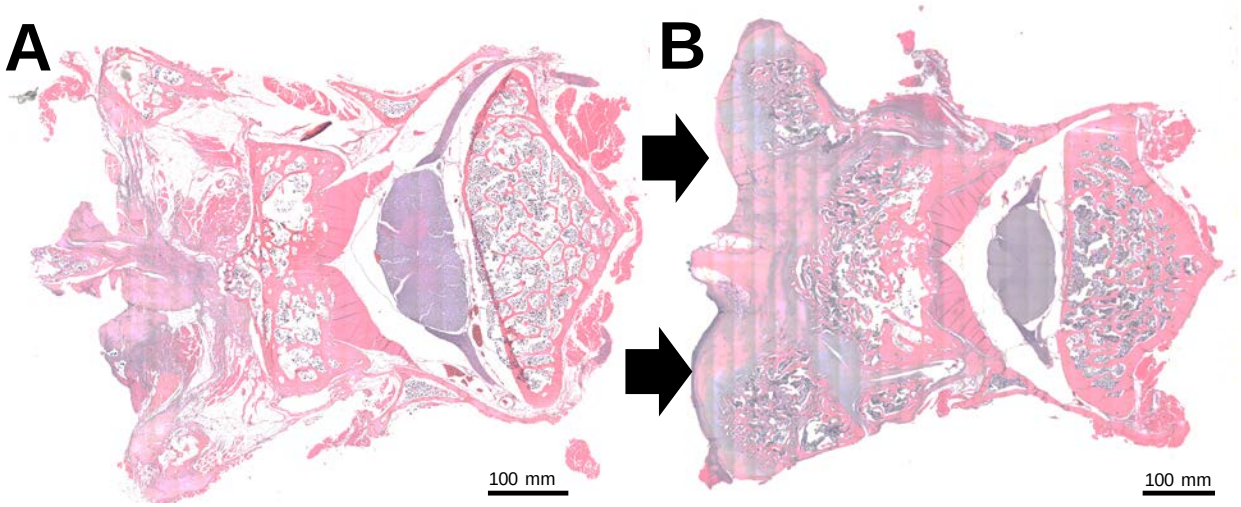

Figure 6. Representative images of spine histology from rabbits treated with (A) PBS:PBS and (B) OGP:OGP devices (10x magnification, 100 mm scale bar). Black arrows indicate areas of endochondral ossification.

Upon retrieval, the PEEK devices were surrounded by fibrous capsules (Fig. 7A). After removal of the device, the shape of the fibrotic capsule was the same as the implant (Fig. 7B). Capsules were fixed in 10% neutral buffered formalin (Statlab), embedded in paraffin, sectioned and stained with H&E, and morphology assessed (Fig. 7C) using an Olympus FLUOVIEW FV3000 Confocal Laser Scanning Microscope (Waltham, Massachusetts, USA). The inset in Fig. 7C shows the tissue protrusion into the drug eluting port of the device with no significant signs of lymphocyte infiltrate. The thickness of the fibrotic capsule is estimated here to be approximately 1.6 mm.

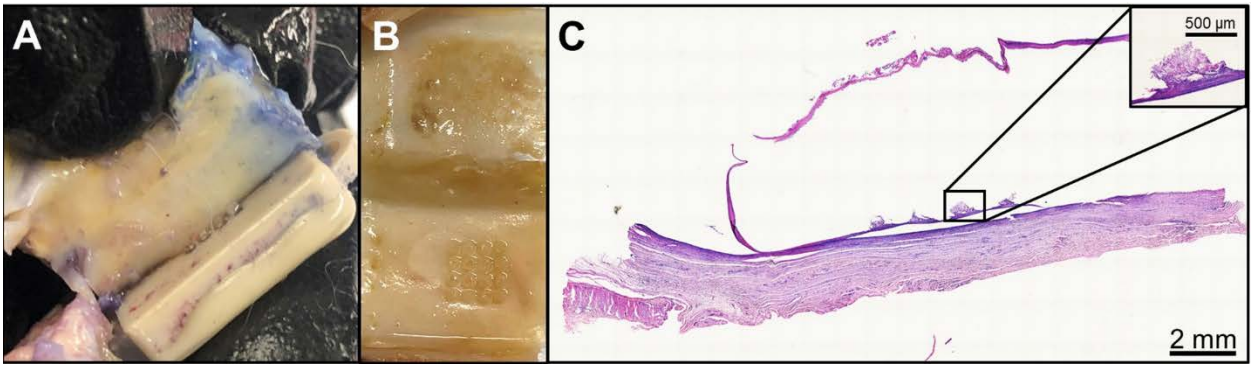

Figure 7. Device retrieval and histology. (A) Inspection and removal of the fibrotic capsule around the device, (B) macroscopic view of the capsule formed around the device, and (C) microscopic view of a fibrotic capsule taken at 10x magnification and inset showing a tissue protrusion into the drug eluting port taken at 4x.

Overall, the implants were well-tolerated with minimal inflammation and migration in the rabbits. The devices remained in position for the duration of the study and non-invasive imaging through X-ray and cone beam CT provided evidence of new bone formation near the sites of implantation. Comparative views of the spine reconstructed in 3D using cinematic rendering technique gave visual evidence of the new bone growth process. Histopathological comparison showed robust endochrondral ossification. Collectively, our findings demonstrate that our novel nanochannel implant offers sustained, low dose and constant administration of osteogenic growth peptide providing a valuable approach to induce bone formation in a target area.

References.

1. Moore DM, Zimmerman K, Smith SA, Hematological assessment in pet rabbits: blood sample collection and blood cell identification. Vet. Clin. North Am. Exot. Anim. Pract. 2015 Jan;18(1): 9-19.
2. Özkan C, Kaya A, Akgül Y Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. World Rabbits Sci. 2012, 20: 253-259.

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?

A manuscript is in preparation.

4. IMPACT

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

Our major accomplishments were: 1) sustained release in vitro of OGP for over two months using 3.5 nanochannel membranes, 2) the ability to custom fabricate our devices in PEEK, and 3) demonstration of a rabbit study whereby the tolerability and efficacy of OGP loaded devices were assessed and compared with vehicle releasing devices for 56 days. These accomplishments 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

Nothing to report

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:	6
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 maintained the NIH 3T3 cells and assembled the implants for the in vivo rabbit

	studies. During these studies Dr. Filgueira monitored 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 performed the implant surgery in rabbits.

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.

Name:	**Dennis Wang**
Project Role:	Research Assistant
Nearest person month worked:	7 (Year 1)
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:	**Silvania Teixeira**
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0002-9967-1693
Nearest person month worked:	3 (Year 2)
Contribution to Project:	Dr. Teixeira assisted with loading the implants, taking photos during the surgical procedure, transferring x-ray and CT data, and processing the spine for histological assessment.

Name:	**Yareli Carcamo-Bahena**
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	0000-0001-9998-9696
Nearest person month worked:	1 (Year 2)
Contribution to Project:	Ms. Carcamo assisted with imaging the histology slides.

Name:	**Rossana Terracciano**
Project Role:	Visiting Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0001-5026-0589
Nearest person month worked:	1 (Year 2)
Contribution to Project:	Ms. Terracciano assisted with analyzing the DynaCT images and image processing.

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)

Ongoing Research Support

W81XWH1810438 (Filgueira) 08/01/2018-1/31/2021 7.8 calendar
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: Principal Investigator
Point of Contact: Dr. Zachary Zabarsky, zachary.k.zabarsky.ctr@mail.mil, Scientific Officer

Grattoni/Butler/**Filgueira** 09/01/2016-12/31/2020 0.24 calendar
Golfer's Against Cancer $80,000
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.
Specific Aims: 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, tlpolk@houstonmethodist.org

Butler/**Filgueira** 09/01/2018-12/31/2020 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.
Specific Aims: 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, tlpolk@houstonmethodist.org

Butler/**Filgueira** 03/22/2019-12/31/2020 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.
Specific Aims: 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, tlpolk@houstonmethodist.org

Filgueira/Hafner 03/01/2020-02/28/2021 0.24 calendar
Houston Methodist Research Institute/Rice University
A Field-Deployable, Small Molecule Nanosensor with Specificity Based on Lipophilicity
The project will test a mobile chemical sensing platform that uses our recent advances in solution-phase surface-enhanced spectroscopy from lipid-coated gold nanoparticles.
Specific Aims: 1) Utilize a surface-enhanced Raman spectroscopy (SERS) platform of gold nanorods coated with lipid membranes to address reproducibility in defense and medical sensing applications, 2) Calculate specific fingerprint modes of the simulant methyl salicylate with time-dependent density functional theory
Role: Co-Principal Investigator
Point of Contact: TBD, StrategicResearchOSRI@houstonmethodist.org

OVERLAP: None

Previous Research Support

(REMOVED)
Grattoni/**Filgueira**/Bruckner/Igo 02/01/2017-01/1//2020
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.
Specific Aims: 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: TBD, StrategicResearchOSRI@houstonmethodist.org

(REMOVED)
Grattoni/**Filgueira**/Bruckner/Igo 03/01/2019-02/29/2020
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.
Specific Aims: 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: TBD, StrategicResearchOSRI@houstonmethodist.org

GRATTONI, A. (Co-I)

Previous Support

(REMOVED)
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.

(REMOVED)
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.

Ongoing Research Support

(NEW)
BC191397 (PI: Grattoni) 6/1/2020-5/31/2023 15% effort, 1.8 calendar
DoD $942,867
Transforming triple negative breast cancer treatment through intratumoral immunotherapy via nanofluidic drug eluting seed
The goal is to evaluate an intratumoral nanofluidic technology for the sustained delivery of immunotherapeutics to enhance efficacy of radio-immunotherapy in triple negative breast cancer murine models.
Specific Aims: 1) To evaluate the effect of NDES-mediated sustained intratumoral delivery kinetics on immunotherapy biodistribution and tumor immune microenvironment modulation. 2) To evaluate efficacy and toxicity of intratumoral NDES immunotherapy alone or in conjunction with RT for local and systemic tumor control.
Role: Co-Investigator
Contact: Jamie A. Shortall
There is no scientific or budgetary overlap.

(NEW)
Grattoni 5/29/2020-10/1/2021 23% effort, calendar
CASIS GA-2020-145 $105,243
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.

(NEW)
Chua/**Grattoni** 08/26/2019-6/19/2021 2% effort, calendar
CASIS GA-2019-953 $94,339
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.

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/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 07/01/2017-09/30/2020 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.

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.

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-18-1-0438 08/01/2018-1/31/2021 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

Previous Research Suuport

(REMOVED)
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

Overlap
No overlap