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TITLE: Adult Stem Cell-Based Enhancement of Nerve Conduit for Peripheral Nerve Repair

PRINCIPAL INVESTIGATOR: Rocky S. Tuan, PhD

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, PA 15213

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6. AUTHOR(S)

Rocky Tuan, Peter Alexander

E-Mail: rst13@pitt.edu

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

University of Pittsburgh,
450 Technology Drive, Rm 239
Pittsburgh, PA, 15219

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Musculoskeletal trauma is frequently accompanied by injuries to peripheral nerves; if not repaired, the trauma can lead to significant dysfunction and disability. While nerves have the ability to regenerate and to reconnect across a limited gap, surgical intervention is often required to assist them in bridging a larger gap. Typically, surgeons will transplant a less important nerve from elsewhere in the body to the site of injury to provide a patch for the injured nerve. However, acceptable donor nerves are often not available for this purpose, particularly in patients suffering multiple extremity injuries or faced with traumatic amputations. Alternatives include the use of a blood vessel graft or a synthetic nerve guide, although these devices are only effective over distances less than 3 cm, mainly because of their lack of appropriate nerve-enhancing biological activities. In our current work, we have identified and isolated stem cells from the injured tissue site that have wound healing promoting activities. In this application, we propose to use these cells, which may be obtained autologously from the patient, in conjunction with a biodegradable scaffold tube to form bioactive nerve conduits that may be grafted to provide better guidance for the microstructure of the nerve to bridge the injury gap. Our Specific Aims are as follows: (1) optimize the neurotrophic bioactivity of stem cell-seeded nanofibrous scaffolds; (2) design and fabricate stem cell activated nerve conduits with optimal neurotrophic and neuroconductive activities that are compatible with point-of-care nerve repair; and (3) perform proof-of-concept functional tests of stem cell-activated nerve conduits in small animal models of nerve repair. Based on our previous and current findings, we expect that we will have positive outcomes from these studies, which will be used to develop testing in a large, clinically relevant animal model, as a basis for future clinical trial. Our long-term goal is to develop efficient and effective strategies to repair and restore function to peripheral nerve injuries resulting from battlefield trauma.

15. SUBJECT TERMS

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

Peripheral nerve damage is a challenging complication of combat-related orthopaedic trauma. Given the severity of the orthopaedic injuries sustained during battlefield trauma, an acceptable donor nerve is often not available to serve as an autograft, particularly for patients with injuries in multiple extremities or traumatic amputations, and currently available nerve guide devices are often insufficient. We propose the use of a novel stem-cell activated nerve conduit graft with an aligned nanofiber scaffold and neurotrophic enhancement using cells clinically available at the site of surgery. Upon completion, these proposed studies will provide sufficient information to move to MPC-NC technology to testing in a large, clinically relevant animal model, which will be designed after consultation with the FDA. Favorable outcomes will form the basis for future clinical trials. The technologies described here are tailored for a "point-of-care" approach that could lead to improvements in overall functional recovery, minimized disability, and increased quality of life for our wounded warriors.

2. KEYWORDS:

Stem Cell, Nerve Conduit, Peripheral Nerve Regeneration, Nanofiber, Neurotrophic Factor, Tissue Engineering, Multifunctional

3. ACCOMPLISHMENTS:

- **The major goals of this project are listed in the table below**

Aim 1: Optimization of the neurotrophic bioactivity of MPC-based nanofibrous scaffold

Task 1: Produce nanofibrous scaffolds of controlled thickness comprised of laminated woven and aligned nanofibrous sheets

Milestone 1: Formation of bi-layered nanofibrous scaffolds (9/30/16, 100%)

Task 2: Optimize the neurotrophic activity of MPC-seeded nanofibrous scaffolds

Milestone 2: Validation of a cell-seeded nanofibrous scaffold with neurotrophic activity in vitro (12/30/16, 80%)

Aim 2: Fabrication of MPC-impregnated nerve conduit (MPC-NC)

Task 3: Construction of devices to assist in MPC-NC preparation

Milestone 3: Design and production of devices that aid in the preparation of MPC-NC that accommodate specific conduit dimensions (09/30/16, 100%)

Task 4: Biomechanical and biological testing of final cell-laden MPC-NC

Milestone 4: An assembled cell-laden MPC-NC that promotes neurite outgrowth in vitro (5/30/17, 0%)

Aim 3: Perform proof-of-concept functional test of the MPC-NC constructs in small animal models (rat/rabbit) of sciatic nerve repair

Task 5: Proof-of-concept functional test of the MPC-NC constructs in rats

Milestone 5: Demonstration of nerve repair in vivo using cell-laden MPC-NC (9/30/17, 0%)

Task 6: Proof-of-concept functional test of the MPC-NC constructs in rabbits

Milestone 6: Demonstration of nerve repair in vivo using cell-laden MPC-NC prepared in a point-of-care single-step procedure (9/30/18, 0%)

- **The major accompolishments achieved to date with respect to each proposed task are:**

Task 1: Produce nanofibrous scaffolds of controlled thickness comprised of laminated woven and aligned nanofibrous sheets

Objective 1: Purchase chemicals and polymeric materials for nanofibrous scaffold
- All chemicals and polymeric agents acquired for fabrication of scaffold

Objective 2: Purchase electrospinner instrumentation with custom-designed specifications and components
- Spinner has been acquired.

Objective 3: Fabricate sheath/tube constructs (months 4-8) of various PCL/PEO ratios
- Fabrication complete. PCL/methacrylated gelatin utilized (See Section 3). **See Methods 2**.

Objective 4: Mechanical testing of the nanofibrous constructs: Tensile testing and suture retention assays
- Suture testing and tensile testing have been completed. **See Figure 1.**

Objective 5: Imaging of nanofibrous constructs (SEM): Ensure uniform alignment, fiber size, and porosity
- Imaging complete. **See Figure 2.**

Objective 6: Optimize the spinning conditions as needed.
- Optimization in progress. **See Methods 3** for current parameters.

Task 2: Optimize the neurotrophic activity of MPC-seeded nanofibrous scaffolds

Objective 7: Acquire MPCs, ECs and MSCs (control cell type)
- MSCs and ECs have been acquired. We are on schedule to acquiring MPCs.

Objective 8: Generation and verification of neurotrophically activated cell types and conditioned media (via RT-PCR and ELISA of neurotrophic factors), followed by cell storage
- MSCs have been verified to be neurotrophically activated in previous reports. MPCs have also been verified in previous reports. MSCs have been stored in cell bank.

Objective 9: Purchase reagents and materials for methacrylation of ECM hydrogel
- All necessary reagents and materials have been acquired

Objective 10: Create photocrosslinkable hydrogel mixtures
- Photocrosslinkable methacrylated gelatin and hyaluronic acid have been created. **See Methods 1**.

Objective 11: Acquire embryonated chick eggs, dorsal root ganglia (DRG) and PC-12 cells.
- Embryonated chick eggs and DRG have been acquired

Objective 12: Test the ability of the hydrogel-encapsulated MSCs adherent to the random fiber side of scaffold mats of different porosities to enhance neurite outgrowth on the aligned nanofibers in vitro.
- Completed. **See Figure 3** and **Methods 4-8**.

Objective 13: Test the effect of ECM coating, using clean or conditioned media as solvent, on neurite outgrowth (PC-12/DRG neurite extension assays) in 2D culture (using scaffold mats and ECM coating on the aligned fiber surface)
- Completed. **See Figure 4.**

Task 3: Construction of devices to assist in MPC-NC preparation

Objective 14: Design and fabricate device to assist in the formation of MPC-NC assembly
- A preliminary model has been designed. See **Figure 5.**

Objective 15: Test and optimize devices to assist in the formation of components of the MPC-NC assembly.
- Designs and construction of next generation device have been completed. **See Figure 6**.

Objective 16: Optimize devices to assist in the formation of the full MPC-NC
- Completed.

Task 5: Proof-of-concept functional test of the MPC-NC constructs in rats

Objective 17: Local IRB/IACUC approval for rat and rabbit models, and HRPO/ACURO for rat

- IRB approval has been acquired. IACUC/ACURO approval has been acquired for rat. IACUC for rabbit in progress.

- **Figures representing major accomplishments during the past year (see methods developed below)**

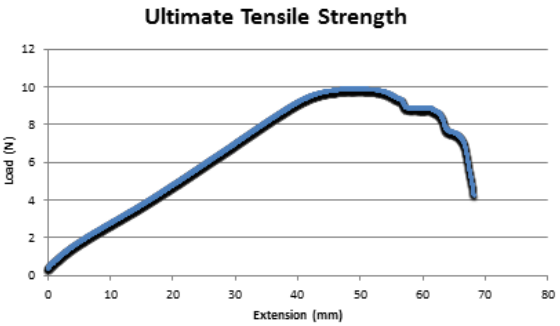


Figure 1. Suture Retention and Tensile Strength. Nerve conduit withstood up to 10N of tensile force applied with suture before elongating and failing. This is well above the necessary force for the application of a nerve conduit.

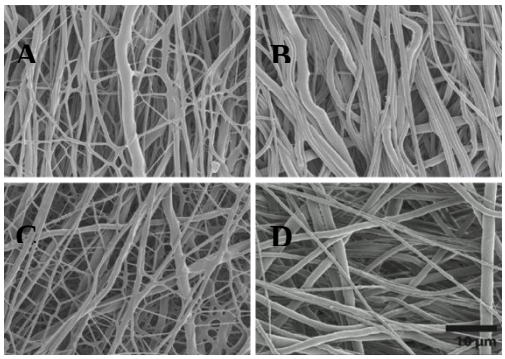


Figure 2. SEM images of composite GelMA / PCL electrospun scaffolds. SEM images of **(A)** aligned composite scaffold, **(B)** aligned scaffold with GelMA removed, **(C)** random composite scaffold, and **(D)** random scaffold without GelMA.

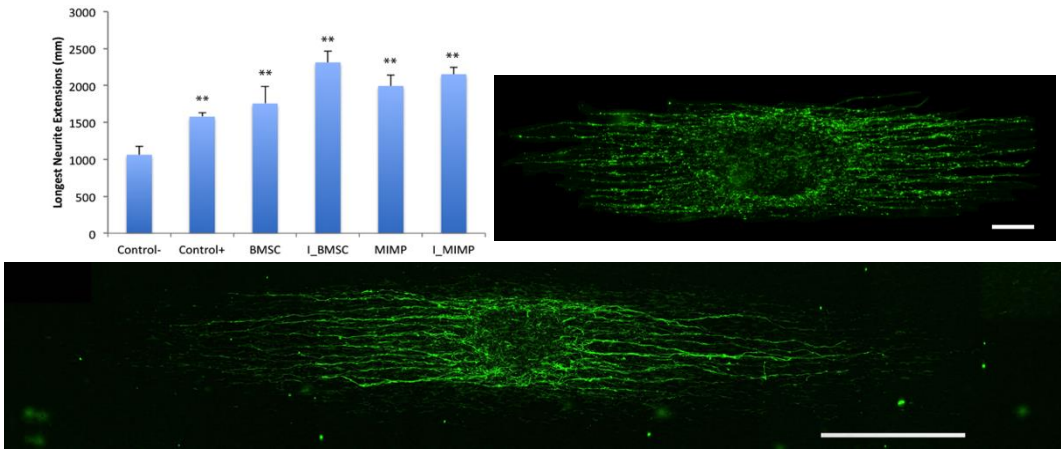


Figure 3. Cell-seeded scaffolds significantly enhance nanofiber-guided neurite extensions from cultured DRGs. (Top-Left) Average of 10 longest neurite extensions from 4 DRGs in each group. **(Top-Right)** Image of negative control - DRG on non-cell seeded scaffold. **(Bottom)** Image of DRG from I_BMSC group. Note remarkably increased neurite extension lengths in the cell-seeded groups. **, $p<0.001$ with respect to negative control. BMSC=Bone Marrow Stem Cell, MIMP = Induced Mesenchymal Progenitor, I_ = Neurotrophically Induced. Control- = Cultured on Non-Cell Seeded. Control+=10 ng/mL FGF, EGF, NGF supplementation. Top Scale Bar = 250 um. Bottom Scale Bar = 800 um.

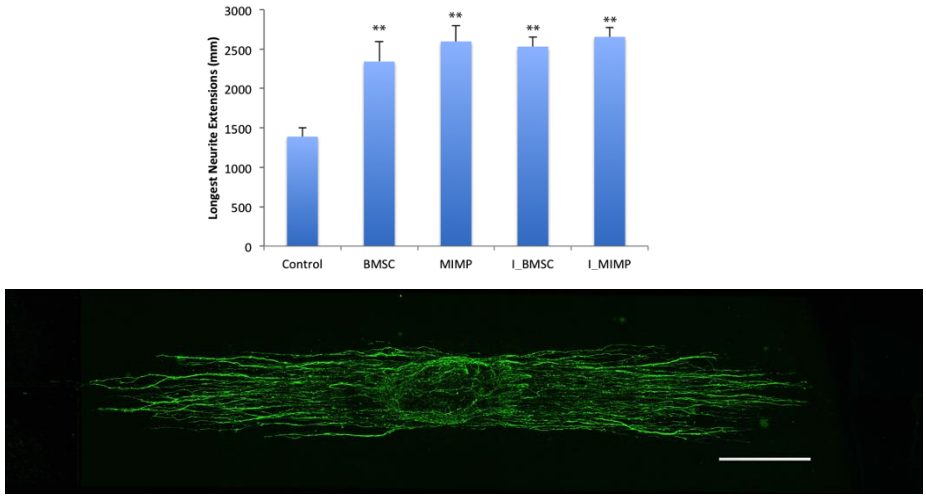


Figure 4. Conditioned medium significantly enhances nanofiber-guided neurite extensions from cultured DRGs. (Top) Average of 10 longest neurite extensions from 4 DRGs in each group. **(Bottom)** Image of DRG from I_MIMP group. Note remarkably increased neurite extension lengths in the conditioned medium groups. **, $p<0.001$ with respect control. BMSC=Bone Marrow Stem Cell, MIMP = Induced Mesenchymal Progenitor, I_ = Neurotrophically Induced. Control = Basal Medium. Scale Bar = 800 um.

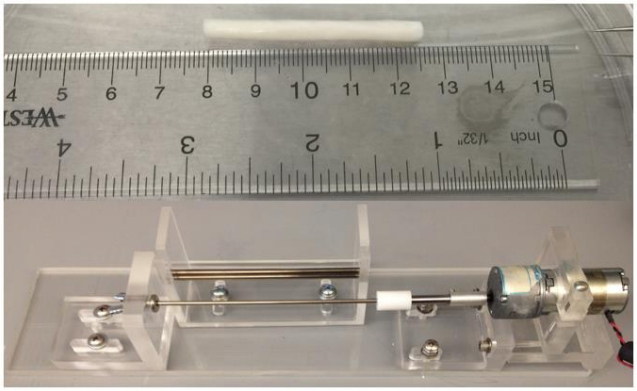


Figure 5. Preliminary device designed for MPC-NC fabrication. (Bottom) Preliminary device used to construct trial nerve conduits and **(top)** macroscopic view of constructed conduit.

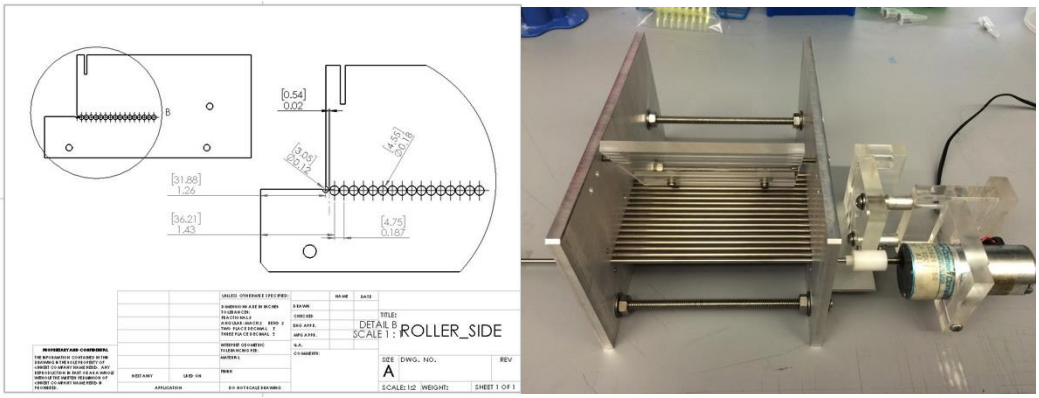
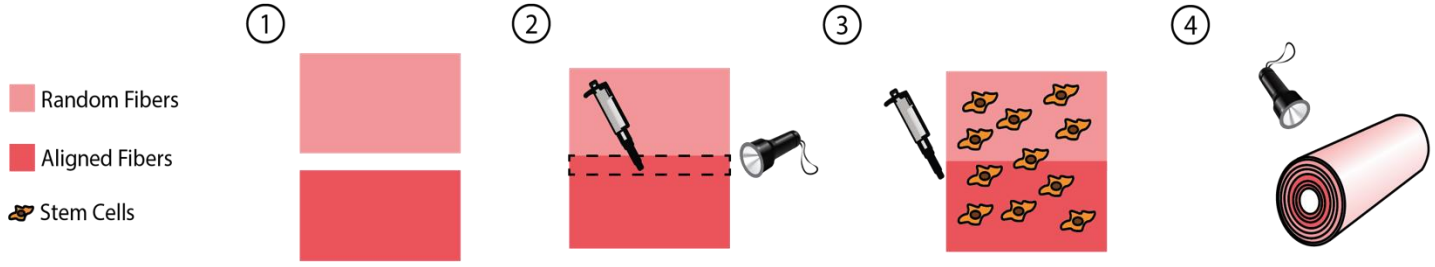


Figure 6. Optimized device designed for MPC-NC fabrication. (Left) Schematic for optimized device, and **(Right)** constructed optimized nerve conduit fabrication device

Methods 1. Synthesis of methacrylated gelatin. 15 g gelatin (type B) (Sigma-Aldrich; St. Louis, MO) was dissolved in 500 mL water and placed in a 37° C shaker at 106 rpm for 2h or until dissolved. Subsequently, 12 mL methacrylic anhydride (Sigma-Aldrich; St. Louis, MO) was added to the solution and it was placed back into the shaker overnight. The resulting solution was dialyzed against deionized water using 2000 NMWCO dialysis tubing (Sigma-Aldrich)for a total of 3 days with at least 10 water changes. This was then lyophilized to obtain a foamy solid. **Synthesis of methacrylated hyaluronic acid.** Methacrylic anhydride was added to a solution of 1% w/v sodium hyaluronate (research grade, MW ~70 kDa, Lifecore, Chaska, MN) in deionized (DI) water, adjusted to a pH of 8 with 5 N NaOH, and reacted on ice for 24 h. The macromer solution was purified via dialysis (MW cutoff 2k) against deionized water for a minimum of 48 h with repeated changes of water. The final product was obtained by lyophilization.

Methods 2. Fabrication of PCL/Methacrylated gelatin conduit. In order to create a tubular structure, we utilize electrospun nanofibrous mats of co-spun PCL and gelatin methacrylate (GelMA). Gelatin is the hydrolyzed form of collagen, a native extracellular matrix molecule, and GelMA has been widely applied in hydrogels for its ability to be photopolymerized as well as its excellent biocompatibility. Despite the hydrophobicity of PCL, the presence of GelMA within the nanofiber mat allows it to readily incorporate aqueous solutions, which instantly dissolve the GelMA and create pores allowing for cell penetration between the PCL fiber.



Thus, we first apply a very thin film of aqueous solution with cells to the scaffold, immediately hydrating it. Subsequently, this hydrated mat consisting of half aligned fibers and half random fibers is rolled around a hypodermic needle of desired diameter to create the layered tubular structure. Finally, the entire conduit is photopolymerized with visible light to bond the layers together. The result is an elastic conduit that is bioactivated with cells, contains microstructural guidance cues for growing nerves, and has mechanical properties suitable for suture retention. This process is illustrated below.

Methods 3. Electrospinning of composite scaffolds for conduit fabrication. To create electrospun scaffolds, two separate solutions of (1) 14.0% w/v PCL (80 kDa; Sigma-Aldrich, St. Louis, MO) in 2,2,2-trifluoroethanol (Sigma Aldrich; St. Louis, MO) and (2) 18% methacrylated-gelatin (mGelatin) in 95% 2,2,2-triflurorethanol in water. To fabricated aligned scaffolds, a custom-designed electrospinning device was utilized to generate a 40:60 composite scaffold consisting of mGelatin and PCL fibers. Two 10-mL syringes were separately filled with the PCL and mGelatin electrospinning solution, and they were fitted with a stainless steel 22G blunt-ended needle that served as a charged spinneret, and directed at a single central rotating mandrel (surface velocity of 10 m/s). The speed of the mandrel was sufficiently fast to align the collected fibers in a single direction. A flow rate of 2 mL/h was maintained with a syringe pump (Harvard Apparatus, Holliston, MA). A power supply (Gamma High Voltage Research, Inc., Ormond Beach, FL) applied a +15-20 kV potential difference between the needles and grounded mandrel to obtain a taylor cone for mGelatin and a +7-10 kV potential difference for PCL. Additionally, two aluminum shields charged to +5kV were placed perpendicular to and on either side of the mandrel to better direct the electrospun fibers toward the grounded mandrel. The distance between the mandrel and the needle was 15 cm for mGelatin fibers and 15 cm for the PCL fibers. The composite electrospun scaffold was generated with a final thickness of 100µm. The procedure was the same for creating randomly aligned scaffolds, except that the mandrel surface velocity was 0.75 m/s.

Methods 4. Preparation of 2D Scaffolds and Seeding of DRG The composite scaffold described previously was utilized for this procedure. A 3.0 x 5.5 cm sheet of aligned scaffold and a 3.0 x 5.5 cm sheet of random

scaffold was cut for each group. Approximately 540 uL of photointiator solution (8% methacrylated gelatin, 0.3% photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) in HBSS) was used to wet both scaffolds. Following this, each scaffold was folded lengthwise (along the 5.5 cm side) into halves. The remaining 60 uL of solution was then evenly applied on top of the folded random scaffold, and then the folded aligned scaffold was placed on top of that. The 4 layered construct was then exposed to visible light radiation for 3 minutes (1.5 minutes on each side) to photopolymerize the construct. After construction of the completed multilayer scaffold, four cylinders of 8 mm diameter were punched out with a punch biopsy. A DRG was then placed on the aligned side of each circular scaffold and placed in a 12 well plate. For cell-seeded versions, 100 uL photointiator solution was used to suspend 6 million cells, which was applied to the random and aligned scaffolds before folding, and between the scaffolds after folding, allowing for encapsulation of cells.
Methods 5. DRG-seeded Scaffolds Culture Day 9 embryonic DRG-seeded scaffolds were cultured in either basal medium (5% FBS, 1x Pen-Strep in Basal Medium Eagle), supplemented basal medium (basal medium with 10 ng/ml Nerve Growth Factor, Epidermal Growth Factor, Fibroblast Growth Factor-2), or conditioned medium drawn from cultured cells for a total of 5 days before fixation and imaging. Medium changes were performed on days 2 and 4 with 2 mL per well. Cell-encapsulated groups were treated with basal medium while control negative groups consisted of non cell-seeded scaffolds with basal medium and control positive groups had non cell-seeded scaffolds with supplemented basal medium.

Methods 6. Fixing and immunohistochemistry. Wash DRGs with wash buffer (0.05% Tween 20 in PBS) and fix in paraformaldehyde for 20 minutes. Hot 10mM cyclic acid with 10% ethanol was added for 1 hour. DRGs were blocked with 5% FBS for 1 hour at room temperature or overnight at 4˚C. DRGs were washed with wash buffer and anti-heavy neurofilament primary antibody (Abcam) was added at a 1:10,000 dilution. DRGs were washed at least 2-3 times with wash buffer, and secondary antibody (Invitrogen) was added at a dilution of 1:300. Cells were washed with wash buffer and imaged.

Methods 6. Differentiating iPS cell cultures into mesenchymal-parentage induced mesenchymal progenitor cells (MiMPCs). Two lines of induced pluripotency stem (iPS) cells were piloted in this study – the first were reprogrammed from human amniotic epithelial cells, and was a generous gift from Dr. Gerald Schatten's lab at the University of Pittsburgh. The second iPS cell we tested was reprogrammed via lentivirus from human bone marrow mesenchymal stem cells (MSCs) by University of Pittsburgh's Stem Cell Core. When iPS cell cultures were confluent and ready for differentiation, mTeSR medium was aspirated, cells were washed with 1xPBS, and then medium was replaced with fresh mesenchymal stem cell (MSC) growth medium (aMEM, 10% FBS, 1ng/ml FGF2, 1xPSF). They were allowed to incubate for 3 days before another medium change, and allowed another 3 days to incubate. MiMPCs were trypsinized and seeded onto gelatin coated flasks. Cells were passaged and expanded following conventional methods for MSCs.

Methods 7. Neuroinductive treatment for MiMPs and MSCs. Culture cells on gelatin-coated flasks, and maintain culture to approximately 70% confluency in normal MSC growth medium. Once cells have reached 70% confluency, begin neuroinductive pre-treatment – 24 hours incubation in pre-treatment 1 medium (aMEM, 10% FBS, 1xPSF, 1mM BME). After 24 hours, change medium to pre-treatment 2 medium (aMEM, 10%FBS, 1xPSF, 1mM BME, 20ng/ml IL-1B, and 35ng/ml all-trans retinoic acid [RA]) every 24 hours for the next 48 hours. After a total of 72 hours in pre-treatment, change the culture medium for neuroinductive treatment medium (DMEM/F12, 5%FBS, 1xPSF, 6ug/ml all-trans RA, 10ng/ml IL-1B, 10ng/ml FGF, 20ng/ml EGF, 1xB-22 supplement, 0.5mM IBMX, 5ng/ml PDGF, 10uM Forskolin, 50ng/ml hNRG). This medium should be changed every 72 hours for a total of 2 medium changes for the duration of the NIM treatment.

Methods 8. Culturing DRGs with conditioned media. Allow neurotrophically induced cells (or un-induced cells) to condition basal medium for 48 hours prior to use with DRGs. After 48 hours, collect conditioned media from cells and use in place of DRG medium.

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

There have been multiple training opportunities involving transfer of knowledge/skills from senior mentors Rocky Tuan and Peter Alexander to graduate students: Aaron Sun, Rachel Brick and Kelsey Gloss.

- **How were the results disseminated to communities of interest?**

An oral presentation at the Military Health System Research Symposium was given in August 2016. See Section 6 below.

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

We plan to adhere to the schedule proposed in the statement of work in order to accomplish the goals we have set forth. We have assembled our surgical teams and debriefed animal facility veterinarians in preparation for our animal studies. Strong leadership from senior personnel will continue to be practiced.

4. IMPACT:

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

We have reported here the first method for spatially controlled immediate seeding of cells during the fabrication of a nerve conduit that contains cells within the conduit walls. This allows us to control the interaction between the cells and the regenerating nerve by limiting their cross-talk to diffusible factor signaling (cytokines). Our results show that this technique allows for greatly enhanced neurite extensions in *in vitro* chicken dorsal root ganglion peripheral nerve injury models with significant contributions from factors secreted by the cells. We believe this technique can be employed with optimized materials to achieve even more effective synthetic conduits in the field of nerve tissue engineering.

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**

We are moving forward with PCL/methacrylated gelatin composite electrospun sheets in lieu of PCL/PEO. Recent data from our lab (Yang et. al, 2016) demonstrates the ability of PCL/methacrylated gelatin sheets to provide porous structures like PCL/PEO, which allow for cell infiltration, with the added benefit that the sheets can be made porous instantly when coming into contact with aqueous solution unlike PCL/PEO which requires a series of ethanol washes. The advantages of this new composite scaffold allow us to integrate cells into the nerve conduit directly as opposed to the former PCL/PEO method which relies on cell migration from the outside in. This change will greatly benefit the project in creating stem cell bioactivated nerve conduits, and *in vitro* results are very promising.

Yang G, Lin H, Rothrauff BB, Yu S, Tuan RS. Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering. *Acta Biomaterialia*. 2016; 35:68-76

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

Nothing to report.

- **Changes that had a significant impact on expenditures**

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

No changes occurred during the reporting period that had a significant impact on expenditures.

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

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  A.X. Sun, G. Yang, R. Brick, P.G. Alexander, R.S. Tuan, "Assisted Nerve Regeneration Utilizing Novel Human Bone Marrow Stem Cell-Seeded Conduits." *Military Health System Research Symposium*, Kissimmee, FL. Oral Presentation. August, 2016

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

  The technique to create a stem cell-seeded nerve conduit that allows for immediate incorporation during fabrication as well as spatially controllable cell distribution within the walls of the conduit has been described in this reporting period. This technique will be disseminated to the research community through conferences and future publications.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Nothing to Report

The following individuals have worked on the project

Name:	Rocky S. Tuan
Project Role:	PI
Research Identifier:	University Employee ID# 124200
Nearest person month worked:	11% effort (1.32 Person Months)
Contribution to Project:	Dr. Tuan will have direct responsibility for the overall design and conduct of the study, oversight of data analysis and writing of publications and research reports. Dr. Tuan will supervise the day-to-day research activities of all personnel.
Funding Support:	N/A
Name:	Peter Alexander
Project Role:	Co-Investigator
Research Identifier:	University Employee ID# 124097
Nearest person month worked:	20% effort (2.40 Person Months)
Contribution to Project:	Dr. Alexander's responsibilities will include cell isolation, propagation, activation, biomaterial scaffold fabrication, histological, biochemical and histological analyses, and animal surgeries. He will work under close supervision of Dr. Tuan and will be involved in experimental design, data analysis, and the training of graduate students and residents. He will also be involved in data analysis, and presentation of research findings in manuscripts and at scientific meetings.
Funding Support:	N/A
Name:	John Fowler
Project Role:	Co-Investigator
Research Identifier:	University Employee ID# 150464
Nearest person month worked:	0% effort (0.00 Person Months)
Contribution to Project:	Dr. Fowler will be involved with this project during the midpoint of year 2 (April 2017)
Funding Support:	N/A
Name:	MaCalus Hogan
Project Role:	Co-Investigator
Research Identifier:	University Employee ID# 152173
Nearest person month worked:	0% effort (0.00 Person Months)
Contribution to Project:	Dr. Hogan will be involved with this project during the midpoint of year 2 (April 2017)

	N/A
Funding Support:	
	Alessandro Pirosa
Name:	Postdoctoral Associate
Project Role:	University Employee ID# 160892
Research Identifier:	45% effort (5.40 Person Months)
Nearest person month worked:	Alessandro responsibilities will include cell isolation and culture,
Contribution to Project:	nanofiber scaffold fabrication, histological and immunohistochemical evaluation of tissue and cell phenotype, ELISA, tissue imaging, and production of the MPC-NC constructs.
	N/A
Funding Support:	
	Jian Tan
Name:	Research Specialist
Project Role:	University Employee ID# 124708
Research Identifier:	35% effort (4.20 Person Months)
Nearest person month worked:	Jian will assist in the execution of the experiments in this project for all
Contribution to Project:	the proposed tasks. Jian will be trained by Dr. Alexander, and will be supervised directly by Dr. Tuan and Dr. Alexander in all of her research activities, including experimental design, assays, and data analyses. Jian will also be responsible for safety requirement, material acquisition, protocol development, and handle reporting duties according to Department of Defense protocols.
	N/A
Funding Support:	
	Rachel Brick
	Graduate Student Researcher
Name:	University Employee ID# 138267
Project Role:	100% effort (12.00 Person Months)
Research Identifier:	Rachel's responsibilities will include cell isolation and culture,
Nearest person month worked:	histological and immunohistochemical evaluation of tissue and cell
Contribution to Project:	phenotype, gene expression analysis by RT-PCR, ELISA, tissue imaging, and in vitro functional testing of the MPC-seeded nanofibrous constructs. She will work under close supervision of Dr. Tuan and Dr. Alexander.
	N/A
Funding Support:	Kelsey Gloss
	Graduate Student Researcher
	University Employee ID# 171537
Name:	100% effort (12.00 Person Months)
Project Role:	Kelsey's responsibilities will be involved in general laboratory protocol
Research Identifier:	development and optimization, preparation of research reports,
Nearest person month worked:	presentations and manuscripts. She will work under close supervision of
Contribution to Project:	Dr. Tuan and Dr. Alexander.
Funding Support:	N/A

The following changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period have occurred:

Rocky Tuan:

The following previously active grants have closed:

Title: "Development of Novel Point-of-Care Treatment for Articular Cartilage Injury"
Grant#: W81XWH-10-1-0850
Time Commitment: 0.60 calendar months (5% effort)
Role: Co-PI (PI: Dr. Hyun Joon Paek, Tissue Genesis Inc.)
Supporting Agency: Department of Defense, Susan Dellinger, 820 Chandler St, Ft. Detrick MD, 21702
Performance Period: 9/30/2010 – 9/29/2014
Level of Funding: $680,330
Goals/Aims: The overall objective of this project is to develop a point-of-care treatment option for articular cartilage injury, utilizing adipose stem cells and nanofibrous biomaterial scaffold, evaluated in both in vitro and in vivo animal models.
Overlap: None

Title: "AFIRM - Wake Forest / University of Pittsburgh Consortium"
Grant#: W81XWH-08-2-0032
Time Commitment: 2.60 calendar months (21.66% effort)
Role: Co-Principal Investigator and Co-Director (PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense, Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 3/10/2008 – 6/30/2014 (No cost extension requested)
Level of Funding: $18,300,000
Goals/Aims: The overall objective of this multi-institutional project is to accelerate regenerative solutions for the treatment of battlefield injuries. Administrative role.
Overlap: None

Title: "3-D Osteochondral Micro-tissue to Model Pathogenesis of Osteoarthritis"
Grant#: 5U18 TR000532-02
Time Commitment: 0.60 calendar months (5% effort)
Role: PI
Supporting Agency: National Institutes of Health, Ashley M. Norwood, 6001 Executive Boulevard, Bethesda, MD 20892
Performance Period: 7/1/2012 – 6/30/2015 (No cost extension)
Level of Funding: $721,450
Goals/Aims: For this project, we propose to construct an *in vitro* 3-dimensional microsystem that models the structure and biology of the osteochondral complex of the articular joint. Osteogenic and chondrogenic tissue components will be produced using adult human mesenchymal stem cells (MSCs) derived from bone marrow and adipose seeded within biomaterial scaffolds photostereolithographically fabricated with defined internal architecture.
Overlap: None

Title: "Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes"
Grant#: W81XWH-08-2-0032
Time Commitment: 0.60 calendar months (5% effort)
Role: Project Leader (PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense (AFIRM Seed project), Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 11/1/2012 – 6/30/2015 (No cost extension requested)
Level of Funding: $294,276

Goals/Aims: This project will examine the potential efficacy of using a combination of adult stem cells and chondrocytes in the presence of platelet-rich plasma to repair articular cartilage defects.
Overlap: None

Title: "Development of Novel Bioartificial Ligament Using Autologous Biological Scaffold and Cells"
Grant#: W81XWH-13-2-0030
Time Commitment: 0.60 calendar (5% effort)
Role: Co-Investigator (PI: Dr. Hyun Joon Paek, Tissue Genesis Inc.)
Supporting Agency: Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 8/1/13 – 4/28/15
Level of Funding: $400,000
Goals/Aims: The ultimate goal of this project is to develop novel cell-based therapies to treat injured ligaments and tendons that affect millions of Americans each year, using autologous biological materials.
Overlap: None

<u>Previously pending grants that are now active:</u>

Title: "Customized Fabrication of Osteochondral Tissue for Articular Joint Surface Repair"
Grant#: OR130296
Time Commitment: 1.20 calendar (10% effort)
Role: PI
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 9/1/14 – 8/31/16
Level of Funding: $770,000
Goals/Aims: This study aims to test the hypothesize that cell-laden scaffolds loaded with microparticles designed to provide temporospatially specific differentiation cues for chondrogenesis and osteogenesis may be constructed by the 3D printing method of projection stereolithography (PSL) to produce functional osteochondral constructs using adipose stem cells.
Overlap: None

Title: "Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology (VPROMPT)"
Grant#: Identifier Pending
Time Commitment: 0.90 calendar (7.5% effort)
Role: Project Principal Investigator (PI: Dr. Shane Hutson, Vanderbilt University)
Supporting Agency: Environmental Protection Agency, Grants Officer – TBN, 1200 Pennsylvania Ave NW, Washington, DC 20004
Performance Period: 11/1/14 – 10/31/18
Level of Funding: $1,000,000
Goals/Aims: Our objective is to develop two robust in vitro three-dimensional (3D) organotypic microculture models (OCMs) based on human mesenchymal stem cells (hMSCs) to examine three critical phenomena of embryonic limb development that are prime targets of limb teratogenesis, and their susceptibility to perturbation by candidate toxicants/teratogens.
Overlap: None

<u>Newly funded grants (not listed on previous Other Support Document from initial Proposal):</u>

Title: Why Don't Lizards Regenerate Perfect Tails Like Salamanders?
Grant#: 1R01 GM115444-01A1
Role: Co-Investigator (PI: Thomas Lozito); Time Commitment: 1.35 calendar (11.26% effort)
Supporting Agency: National Institutes of Health
Performance Period: 4/1/16 – 3/31/21

Level of Funding: $1,083,868
Goals/Aims: Study of lizard tail regeneration as a bone/cartilage wound healing model.
Overlap: None

Title: A Microphysiological 3D Organotypic Culture System for Studying Degradation and Repair of Composite Skeletal Tissues in a Microgravity Environment?
Grant#: GA-2016-236
Role: PI, Time Commitment: 0.60 calendar (5% effort)
Supporting Agency: Center for the Advancement of Science in Space
Performance Period: 4/30/16 – 12/31/17
Level of Funding: $364,188
Goals/Aims: Developing a 3D microphysiological system of skeletal tissues to implement on the Int Space Station.
Overlap: None

Title: "Cholesterol Sensitivity and Mechanisms of MSC Responses to 3D Substrate Rigidity"
Grant#: 1R01EB019430-01A1
Role: PI; Time Commitment: 0.91 calendar (7.6% effort)
Supporting Agency: National Institutes of Health
Performance Period: 4/1/15 – 3/31/19
Level of Funding: $1,487,932 Goals/Aims: We propose a mechanistic study of the biological responses of adult human bone marrow-derived mesenchymal stem cells to 3D substrate rigidity using gelatin scaffolds of photocrosslink-controllable stiffness. Specifically, our focus is on the role of membrane cholesterol and caveolae subdomains, and focal adhesion signaling in these responses.
Overlap: None

John Fowler:

Newly funded grants (not listed on previous Other Support Document from initial Proposal):

5R01AR062066 (PI: Chung) 8/1/13 – 7/31/17* 1.2 cal mo
University of Michigan (NIH) $37,330
A Clinical Trial for the Surgical Treatment of Elderly Distal Radius Fractures

The specific aim of this 21-center randomized controlled trial is to compare outcomes of these three surgical techniques in treating unstable DRFs in the elderly. The secondary aim is to follow a cohort of elderly patients who choose not to have surgery to evaluate outcomes following treatment by close reduction and casting alone. This clinical trial is the most ambitious study in hand surgery by assembling most of the leading centers in North America to collect evidence-based data to guide future treatment of this prevalent injury in the growing elderly population.
*Extension

N/A 8/1/15 – 7/31/17 1.2 cal mo
American Foundation for the Surgery of the Hand $40,000
Ultrasound of the Median Nerve for Diagnosis of Carpal Tunnel Syndrome

We hypothesize that ultrasound will replace electrodiagnostic testing as the confirmatory test of choice for diagnosis of carpal tunnel syndrome.

AD2016-1765-15 7/1/16 – 6/30/17 0.12 cal mo
The Pittsburgh Foundation $5,000
Changes in Median Nerve Cross-Sectional Area after Carpal Tunnel Release

The current project proposes the use of ultrasound to track changes in nerve morphology after carpal tunnel release and to compare these changes to patient reported outcomes.

AD2015-1765-24 7/1/15 – 6/30/17* 0.12 cal mo

The Pittsburgh Foundation $5,000
Evaluation of Scaphoid Fracture Union-A Comparision between CT and Plain Radiographs

We believe that this study will determine if plain radiographs are an appropriate method to confirm healing of scaphoid fractures. If this study finds that plain radiographs are not accurate, there would be a paradigm shift away from ordering X-rays at 10-12 weeks and only ordering a CT scan. It has the potential to change the standard of care.
*Extension

AD2015-1765-17 7/1/15 – 6/30/17* 0.12 cal mo
The Pittsburgh Foundation $5,000
Prospective, Randomized Placebo Controlled Trial of Activated versus Non-Activated Platelet Rich Plasma, Corticosteriods and Saline for the Treatment of Lateral Epicondylitis

We hypothesize that leukocyte-poor PRP (PRP-R) treatment will result in superior long-term outcomes to placebo and corticosteroids and similar longterm outcomes to L-PRP, without the early increase in pain.

MaCalus Hogan:

Newly funded grants (not listed on previous Other Support Document from initial Proposal):

3R01AR061395-03 (PI: Wang) 8/1/14 – 6/30/17 3.0 cal mo
Co-PI: Hogan
NIH (Diversity Supplement) $25,400
Repair of tendinopathic tendons

In this supplemental project, we aim to determine the anti-inflammatory role of PRP in the treatment of tendinopathy. The end results of this project will not only help define the efficacy of PRP treatment but also enable the candidate of this supplemental award to gain abundant scientific training and as a result become an independent physician-scientist in the near future.

7/1/15 – 3/31/17* 2.76 cal mo
Amniox Medical Inc. $30,000
Determination of the role of human amniotic membrane and umbilical cord membrane materials in musculoskeletal tissue repair and regeneration

The goal of this study is to assess the effect of cryopreserved AM/UC tissues vs. competitor products on the regeneration of musculoskeletal tissues in an *in vitro* three-dimensional, mechanically loaded system. Tissues will be assessed for the presence of regenerative markers, expression of inflammatory cytokines, as well as histological and biomechanical assessment of regenerated tissues.
*NCE extension

OR140390 (PI:Tuan) 7/1/15 – 6/30/18 0 cal mo (yr 1); 1.2 (yr 2); 1.2 (yrs 3-4)
Co-I: Hogan
Department of Defense $1,557,090
Adult Stem Cell-Based Enhancement of Nerve Conduit for Peripheral Nerve Repair

The goal of this application is to use traumatized hand derived multipotent progenitor cells (mpcs) obtained autologously from the patient, in conjunction with a biodegradable scaffold tube, to form bioactive nerve conduits that may be grafted to provide better guidance for the microstructure of the nerve to bridge the injury

Pete Alexander:

The following previously active grant has closed:

Title: "3-D Osteochondral Micro-tissue to Model Pathogenesis of Osteoarthritis"
Grant#: 5U18 TR000532-02

Time Commitment: 0.72 calendar months (6% effort)
Role: Data Analyst Scientist (PI: Dr. Rocky S. Tuan)
Supporting Agency: National Institutes of Health, Tijuana Decoster, 6001 Executive Boulevard, Bethesda, MD 20892
Performance Period: 7/1/2012 – 6/30/2015 (No cost extenstion)
Level of Funding: $721,450
Goals/Aims: For this project, we propose to construct an *in vitro* 3-dimensional microsystem that models the structure and biology of the osteochondral complex of the articular joint. Osteogenic and chondrogenic tissue components will be produced using adult human mesenchymal stem cells (MSCs) derived from bone marrow and adipose seeded within biomaterial scaffolds photostereolithographically fabricated with defined internal architecture.
Overlap: None

Title: "Regenerative Repair of Traumatic Articular Cartilage Injuries: Point-of-Care Application of Mesenchymal Stem Cells and Chondrocytes" – Project in AFIRM I
Grant#: W81XWH-08-2-0032
Time Commitment: 0.90 calendar months (7.5% effort)
Role: Data Analyst Scientist (Project Leader: Dr. Rocky Tuan; PI: Dr. Anthony Atala, Wake Forest University)
Supporting Agency: Department of Defense (AFIRM Seed project), Philip Huff, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 11/1/2012 – 6/30/2015 (No cost extension)
Level of Funding: $294,276
Goals/Aims: This project will examine the potential efficacy of using a combination of adult stem cells and chondrocytes in the presence of platelet-rich plasma to repair articular cartilage defects.
Overlap: This project will end in 3 months, with primary focus on the biological activity of platelet-rich plasma and cartilage fragments. No scientific overlap.

<u>Previously pending grant that are now active:</u>

Title: "Customized Fabrication of Osteochondral Tissue for Articular Joint Surface Repair"
Grant#: OR130296
Time Commitment: 4.80 calendar (40% effort)
Role: Data Analyst Scientist (PI: Dr. Rocky Tuan)
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 9/1/14 – 8/31/16
Level of Funding: $770,000
Goals/Aims: This study aims to test the hypothesize that cell-laden scaffolds loaded with microparticles designed to provide temporospatially specific differentiation cues for chondrogenesis and osteogenesis may be constructed by the 3D printing method of projection stereolithography (PSL) to produce functional osteochondral constructs using adipose stem cells.
Overlap: None

Title: "Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology (VPROMPT)"
Grant#: Identifier Pending
Time Commitment: 2.40 calendar (20% effort)
Role: Data Analyst Scientist (Project PI: Dr. Rocky Tuan; Principal Investigator: Dr. Shane Hutson, Vanderbilt University)
Supporting Agency: Environmental Protection Agency, Grants Officer – TBN, 1200 Pennsylvania Ave NW, Washington, DC 20004
Performance Period: 11/1/14 – 10/31/18
Level of Funding: $1,000,000
Goals/Aims: Our objective is to develop two robust in vitro three-dimensional (3D) organotypic microculture models (OCMs) based on human mesenchymal stem cells (hMSCs) to examine three critical phenomena of embryonic limb

development that are prime targets of limb teratogenesis, and their susceptibility to perturbation by candidate toxicants/teratogens.
Overlap: None

Title: "Cell-Based Meniscal Repair Using an Aligned Bioactive Nanofibrous Sheath"
Grant#: W81XWH-15-1-0104
Time Commitment: 0.6 calendar (5% effort)
Role: Co-Investigator (PI: Dr. Rocky Tuan)
Supporting Agency: Department of Defense, Grants Officer – TBN, USAMRAA, 820 Chandler St., Fort Detrick, MD 21702
Performance Period: 4/1/15 – 9/30/16
Level of Funding: $200,000
Goals/Aims: Develop a stem cell-seeded, nanofibrous scaffold-based tissue engineering approach for the repair of meniscal tear.
Overlap: None

Title: A Microphysiological 3D Organotypic Culture System for Studying Degradation and Repair of Composite Skeletal Tissues in a Microgravity Environment?
Grant#: GA-2016-236
Role: Co-Investigator (PI: Dr. Rocky Tuan), Time Commitment: 1.80 calendar (15% effort)
Supporting Agency: Center for the Advancement of Science in Space
Performance Period: 4/30/16 – 12/31/17
Level of Funding: $364,188
Goals/Aims: Our long-term goal is to develop new capabilities in studying biology, medicine, pharmacology, physiology, and related toxicology of skeletal tissues by combining tissue engineered organotypic microphysiological cultures with human stem cells and non-invasive, real-time analytical techniques.
Overlap: None

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission.

Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS: The Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted as an appendix.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix 1: Quad Chart