AWARD NUMBER: W81XWH-15-1-0600

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

PRINCIPAL INVESTIGATOR: Rocky S. Tuan, PhD

CONTRACTING ORGANIZATION: University of Pittsburgh

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Oct 2019	Annual Report	09/30/2018 - 09/29/2019

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0600

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Rocky Tuan, Peter Alexander

E-Mail: rst13@pitt.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

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: Provide a brief list of keywords (limit to 20 words).

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

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the USAMRAA Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

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, 100%)

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, 100%)

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, 80%)
- 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, 40%)

What was accomplished under these goals?

Task 1: Produce nanofibrous scaffolds of controlled thickness comprised of laminated woven and aligned nanofibrous sheets (All objectives completed in previous year 9/30/15 – 9/30/16)

Specific objective 1: Purchase chemicals and polymeric materials for nanofibrous scaffold
Specific objective 2: Purchase electrospinner instrumentation with custom-designed specifications and components
Specific objective 3: Fabricate sheath/tube constructs (months 4-8) of various PCL/PEO ratios
Specific objective 4: Mechanical testing of the nanofibrous constructs: Tensile testing and suture retention assays
Specific objective 5: Imaging of nanofibrous constructs (SEM): Ensure uniform alignment, fiber size, and porosity
Specific objective 6: Optimize the spinning conditions as needed.

Task 2: Optimize the neurotrophic activity of MPC-seeded nanofibrous scaffolds (All objectives except specific objective 14 completed in previous year 9/30/15 – 9/30/16)

Specific objective 7: Acquire MPCs, ECs and MSCs (control cell type)
Specific 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
Specific objective 9: Purchase reagents and materials for methacrylation of ECM hydrogel
Specific objective 10: Create photocrosslinkable hydrogel mixtures
Specific objective 11: Acquire embryonated chick eggs, dorsal root ganglia (DRG) and PC-12 cells.
Specific 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.
Specific 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 in previous year 9/30/15 – 9/30/16. Additional data generated this cycle to elucidate mechanisms of observed nerve conduit effects**. **See Figure 1**.

Specific objective 14: Test the effect of EC cell co-encapsulation with MPCs within hydrogels in clean or conditioned medium-fabricated hydrogels

- **Completed in this cycle 9/30/2016 – 9/30/2017**. See Section 5: Changes/Problems.

Task 3: Construction of devices to assist in MPC-NC preparation (All objectives completed in previous year 9/30/15 – 9/30/16)

Specific objective 15: Design and fabricate device to assist in the formation of MPC-NC assembly
Specific objective 16: Test and optimize devices to assist in the formation of components of the MPC-NC assembly.
Specific objective 17: Optimize devices to assist in the formation of the full MPC-NC

Task 4: Biomechanical and biological testing of final cell-laden MPC-NC (All objectives completed in this cycle: 9/30/15 – 9/30/16)

Specific objective 18: Preparation of completed acellular and cell-laden MPC-NC

- Complete. **See Figure 2**.

Specific objective 19: Scanning electron microscopy to assess structural uniformity

- Complete. **See Figure 3.**

Specific objective 20: Tensile testing and suture retention assays

- Complete. **See Figure 4**.

Specific objective 21: Assess biodegradation of the scaffold (mass and volume)

- Complete. **See Figure 5.**

Specific objective 22: Cell viability assay for biocompatibility

- Complete. **See Figure 6.**

Specific objective 23: Immunohistochemistry for biopermeability (with respect to neurotrophic factors)

- Complete. **See Figure 7**.

Specific objective 24: Neurotrophic activity assay (PC-12/DRG neurite extension)

- Complete. **See Figure 8 (2D equivalent of 3D scaffold)** and Section 5: Changes/Problems.

Task 5: Proof-of-concept functional test of the MPC-NC constructs in rats (started and on-going in the current cycle: 9/30/16 – 9/30/17)

Specific objective 25: Local IRB/IACUC approval for rat model and ACURO approval.

- **Complete.**

Specific objective 26: Acquisition of Thy1-GFP rats

- **Complete:** Non-transgenic Lewis rats were obtained. See Section 5: Changes/Problems.

Specific objective 27: Optimization of implant technique using cadaveric samples

- **Completed** by Orthopaedic surgeon Dr. John Fowler.

Specific objective 28: RFP Lenti-viral transduction of MPC

- **Complete:** DiI labeling and use of transgenic GFP lewis rat used instead of RFP Lenti-viral transduction. See Section 5: Changes/Problems.

Specific objective 29: Implantation of MPC-NC scaffold variants in sciatic nerve defects

- **Complete:** A total of 60 rats have been implanted thus far – 30 for a 6-week time point and 30 for a 16-week time point. We plan to implant a total of 36 more rats to determine the mechanism of action of our nerve conduit. See Section 5: Changes/Problems

Specific objective 30: Functional testing of nerve repair at experimental day 3 and the experimental end point

- **Complete:** Functional testing following sciatic nerve transection and 16 weeks of healing has been completed. Results show that the wall-encapsulated MSCs enhanced Schwann cell migration thorugh the lumen and function of the muscle as shown through gait analysis (SFI) carried out at 16 weeks (ongoing). See Section 5: Changes/Problems

Specific objective 31: Harvest of samples

- **Complete:** Harvest of samples at 6-week and 16-week time points were completed in February 2018.

Specific objective 32: Macroscopic, histological and immunohistochemical assessment of nerve repair.

- **Complete:** Analysis of samples at 6 and 16-week time points were completed in July of 2018. **See Figures 9-17.** Functional analysis of nerve repair the sciatic function index (SFI). **See Figure 18.**

Task 6: Proof-of-concept functional test of the MPC-NC construct in rabbits: (to be carried out in F

Specific objective 33: Local IRB/IACUC approval for rabbit model and ACURO approval.

- **Complete**

Specific objective 34: Optimization of implant technique using cadaveric samples

- **In progress:** Training and practice on cadavers completed in July and August of 2018. Surgeries are scheduled for January 2020.

Specific objective 35: Harvest of samples at 6 month time point.

- **Estimated completion:** June 2020.

Specific objective 36: Macroscopic, histological and immunohistochemical assessment of nerve repair. At the 6-month time point,

- **Estimated completion:** June 2020.

Figures

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

This year, the main training opportunity involved transfer of knowledge/skills from senior mentors Rocky Tuan and Pete Alexander to the graduate student Aaron Sun.

How were the results disseminated to communities of interest?

A publication was published in Biomaterials.

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

We plan to stick to the schedule proposed in the statement of work, as we have in the past year, 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.

4. IMPACT: This component is used to describe ways in which the work, findings, and specific products of the project have had an impact during this reporting period. Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- the development of the principal discipline(s) of the project;
- other disciplines;
- technology transfer; or
- society beyond science and technology.

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. In addition, initial *in vivo* results are very promising for our nerve conduit system. 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: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change

None to report

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

Space for the in vivo study of nerve repair in the rabbit has not been available. A No-Cost extension was requested and granted 11/4/2019. We anticipate the study beginning in January, 2020.

Gait analysis for rabbit is not established. Electrophysiology will replace gait analysis in the rabbit. Electrophysiology will be undertaken with the assistance of Dr Bing Wang (Molecular Therapy Group) and in collaboration with the Translational Neureoscience Labs.

Changes that had a significant impact on expenditures

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: List any products resulting from the project during the reporting period. Examples of products include:

- publications, conference papers, and presentations;
- website(s) or other Internet site(s);
- technologies or techniques;
- inventions, patent applications, and/or licenses; and
- other products.

If there is nothing to report under a particular item, state "Nothing to Report."

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

Report only the major publication(s) resulting from the work under this award. There is no restriction on the number. However, agencies are interested in only those publications that most reflect the work under this award in the following categories:

Journal publications.

Conduits harnessing spatially controlled cell-secreted neurotrophic factors improve peripheral nerve regeneration. Sun AX, Prest TA, Fowler JR, Brick RM, Gloss KM, Li X, DeHart M, Shen H, Yang G, Brown BN, Alexander PG, Tuan RS. Biomaterials. 2019 May;203:86-95. doi: 10.1016/j.biomaterials.2019.01.038.

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

  Nothing to report.

- **Technologies or techniques**

  The technique to create a stem cellseeded 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

Provide the following information on participants:

- what individuals have worked on the project?
- has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?
- what other organizations have been involved as partners?

Nothing to Report

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

- Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	Mary Smith
Project Role:	Graduate Student
Researcher Identifier (e.g., ORCID ID):	1234567
Nearest person month worked:	5
Contribution to Project:	Ms. Smith has performed work in the area of combined error-control and constrained coding
Funding Support:	The XYZ Foundation (Complete only if the funding support is provided from other than this award.)

Name:	Rocky S. Tuan
Project Role:	PI
Research Identifier:	University Employee ID# 124200
Nearest person month worked:	7.75% effort (0.93 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:	18.33% effort (2.20 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:	Alessandro Pirosa
Project Role:	Postdoctoral Associate
Research Identifier:	University Employee ID# 160892
Nearest person month worked:	60% effort (7.20 Person Months)
Contribution to Project:	Alessandro responsibilities will include cell isolation and culture, nanofiber scaffold fabrication, histological and immunohistochemical evaluation of tissue and cell phenotype, ELISA, tissue imaging, and production of the MPC-NC constructs. He also is involved in general laboratory protocol development and optimization, preparation of research reports, presentations and manuscripts.
Funding Support:	N/A

Name:	Jian Tan
Project Role:	Research Specialist
Research Identifier:	University Employee ID# 124708
Nearest person month worked:	25.41 % effort (3.05 Person Months)
Contribution to Project:	Jian will assist in the execution of the experiments in this project for all 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.
Funding Support:	
Name:	
Project Role:	
Research Identifier:	N/A
Nearest person month worked:	
Contribution to Project:	

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

Rocky Tuan:

The following previously active grants have closed:

Title: "Exploring the Mechanisms of Sarcoma-Associated Cachexia"
Grant#: 1R21 CA199472-01A1
Role: Co-Investigator (PI: Weiss); Time Commitment: 0.12 calendar (1% effort)
Supporting Agency: National Institutes of Health
Performance Period: 4/1/17 – 3/31/19
Level of Funding: $625,000
Goals/Aims: We hypothesize that the TNF-α and Notch pathways are important to the biology of sarcoma-associated cachexia (SAC), muscle differentiation can be rescued with targeted inhibition of these pathways, and SAC can be investigated with patient-derived sarcoma cells lines. To test these hypotheses, we propose the following: Specific Aim 1: Investigate correlations between human sarcoma cell line gene expression, protein production, and MDSC suppression with the clinical incidence of SAC. We will evaluate human sarcoma cell lines to determine if correlations exist between clinical SAC and the sarcoma cells' gene expressions, protein expressions, and abilities to inhibit MDSC differentiation. Specific Aim 2: Determine if the manipulation of specific factors or pathways causes an alteration in the ability of sarcoma cell lines to suppress myogenesis. We will employ the TNF-α inhibitor etanercept and the Notch inhibitor MK-0752 to determine if the suppression of MDSC differentiation can be rescued with one or both of these agents. We will also evaluate their ability to alter gene expression and protein production. Specific Aim 3: Use tumor xenografts to evaluate the ability of patient-derived sarcoma cell lines to induce SAC, and test the efficacy of TNF-α and Notch inhibition to reverse the cachectic phenotype. We will generate tumor xenografts from human sarcoma cell lines and test their capacities to induce SAC, and the ability of TNF-α and Notch

inhibition to rescue the cachectic phenotype in vivo.
Overlap: None

Title: "Cell-Based Meniscal Repair Using an Aligned Bioactive Nanofibrous Sheath"
Grant#: W81XWH-15-1-0104
Role: PI; Time Commitment: 0.12 calendar (1% effort)
Supporting Agency: Department of Defense
Performance Period: 5/15/15 – 12/14/17
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

Previously pending grants that are now active:

None.

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

Title: "Multi-Scale In Vitro 3D Tissue Model of Vascularized Bone-Cartilage Interactions"
Grant#: 1R43 AR072169-01
Role: PI, Time Commitment: 0.60 calendar (5.0% effort)
Supporting Agency: National Institutes of Health
Performance Period: 6/1/18 – 5/31/19
Level of Funding: $74,250
Goals/Aims: The overall objective of this study is to develop an in vitro 3D tissue model for understanding of vascularized bone-cartilage interactions.
Overlap: None

Title: "MicroJoint Tissue Chip to Study Osteoarthritis Pain: Mechanistic Analysis, Therapeutic Targets, and Impact of Opiods"
Grant#: 3UG3TR002136-02S1
Role: PI, Time Commitment: As this is an administrative supplement effort reported on master grant ((1.8 calendar (15% effort)) • Home

Supporting Agency: National Institutes of Health
Performance Period: 9/1/18 – 6/30/19
Level of Funding: $274,725
Goals/Aims: The goal is to differentially interrogate the relative contribution of any and/or all four of the joint tissue compartments as the source of mediators responsible for OA-associated pain.
Overlap: None

John Fowler:

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

None.

MaCalus Hogan:

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

None.

Pete Alexander:

The following previously active grant has closed:

None.

Previously pending grants that are now active:

None

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

Title: "Tissue Chip Modeling of Synovial Joint Pathologies: Effects of Inflammation and Adipose-Mediated Diabetic Complications"
Grant#: 1UG3 TR002136-02
Role: PI; Time Commitment: 1.35 calendar (11.26% effort)
Supporting Agency: National Institutes of Health/NCATS
Performance Period: 7/15/17 – 6/30/22
Level of Funding: $4,273,066
Goals/Aims: We propose engineering a 3D human micro-joint chip (mJoint), physiologically analogous to the native joint and capable of modeling pathogenesis of joint diseases for disease-modifying medications screening/development.
Overlap: None

Title: "Exploring the Mechanisms of Sarcoma-Associated Cachexia"
Grant#: 1R21 CA199472-01A1
Role: Co-Investigator (PI: Weiss); Time Commitment: 0.24calendar (2% effort)
Supporting Agency: National Institutes of Health
Performance Period: 4/1/17 – 3/31/19
Level of Funding: $625,000
Goals/Aims: We hypothesize that the TNF-α and Notch pathways are important to the biology of sarcoma-associated cachexia (SAC), muscle differentiation can be rescued with targeted inhibition of these pathways, and SAC can be investigated with patient-derived sarcoma cells lines. To test these hypotheses, we propose the following: Specific Aim 1: Investigate correlations between human sarcoma cell line gene expression, protein production, and MDSC suppression with the clinical incidence of SAC. We will evaluate human sarcoma cell lines to determine if correlations exist between clinical SAC and the sarcoma cells' gene expressions, protein expressions, and abilities to inhibit MDSC differentiation. Specific Aim 2: Determine if the manipulation of specific factors or pathways causes an alteration in the ability of sarcoma cell lines to suppress myogenesis. We will employ the TNF-α inhibitor etanercept and the Notch inhibitor MK-0752 to determine if the suppression of MDSC differentiation can be rescued with one or both of these agents. We will also evaluate their ability to alter gene expression and protein production. Specific Aim 3: Use tumor xenografts to evaluate the ability of patient-derived sarcoma cell lines to induce SAC, and test the efficacy of TNF-α and Notch inhibition to reverse the cachectic phenotype. We will generate tumor xenografts from human sarcoma cell lines and test their capacities to induce SAC, and the ability of TNF-α and Notch inhibition to rescue the cachectic phenotype in vivo.
Overlap: None

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

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.