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

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT</b> 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.									
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## TABLE OF CONTENTS

	<u>Page No.</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	
a. Accomplishments by Task List (SOW)	5
b. Figures	7
c. Methods	18
4. Impact	21
5. Changes/Problems	21
6. Products	22
7. Participants & Other Collaborating Organizations	23
8. Special Reporting Requirements	29
9. Appendices	29

**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 through 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 2019.

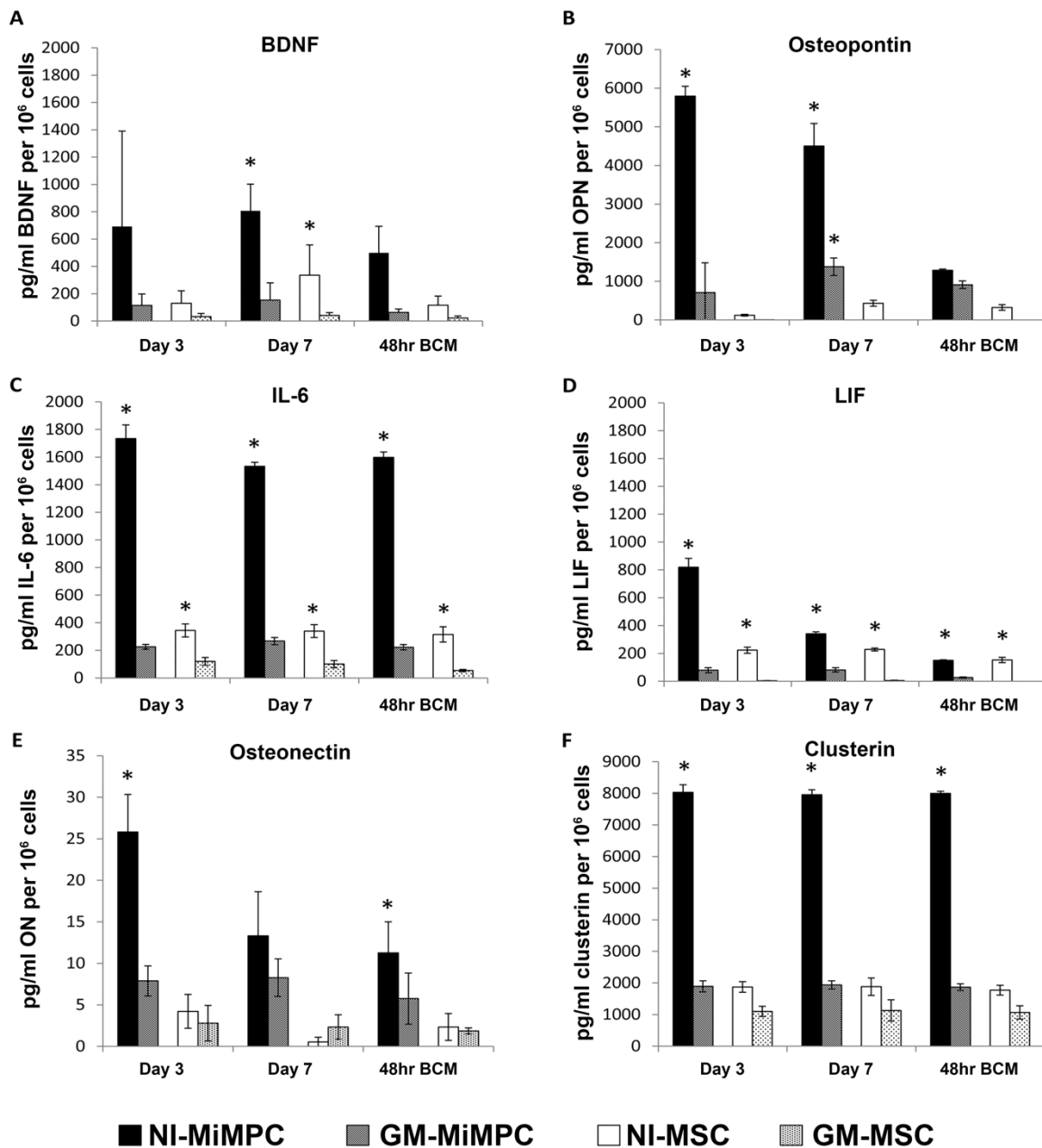
**Specific objective 35:** Harvest of samples at 6 month time point.

- **Estimated completion:** August 2019.

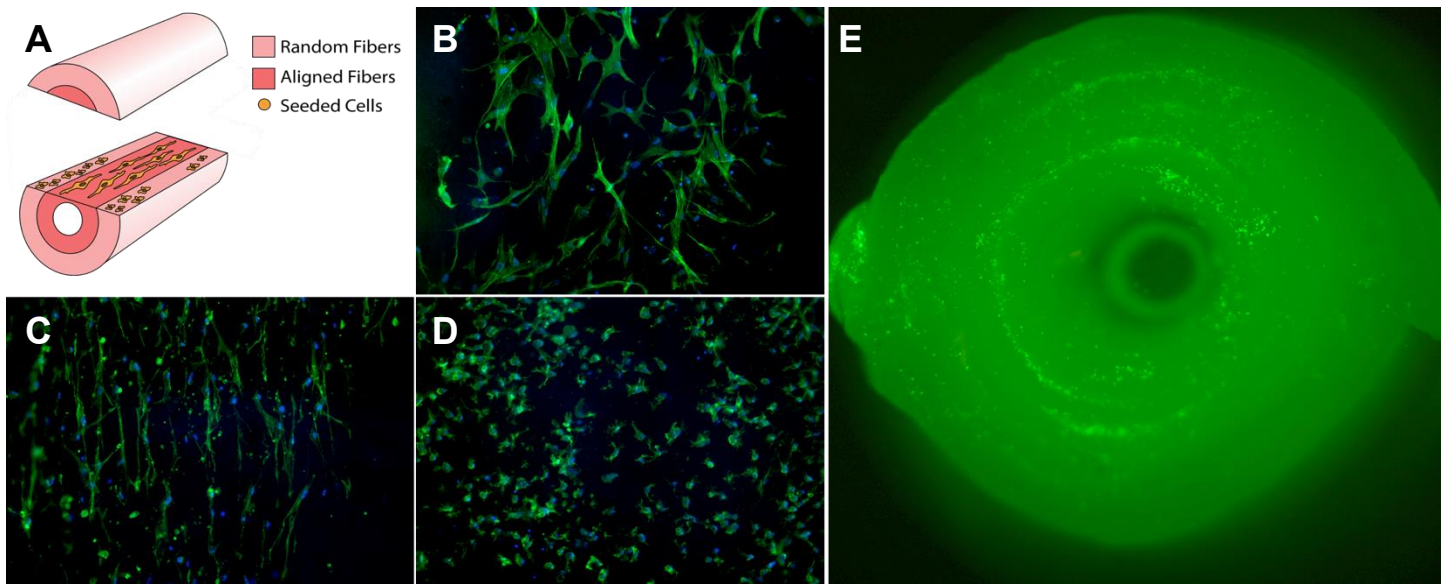
**Specific objective 36:** Macroscopic, histological and immunohistochemical assessment of nerve repair. At the 6-month time point,

- **Estimated completion:** November 2019.

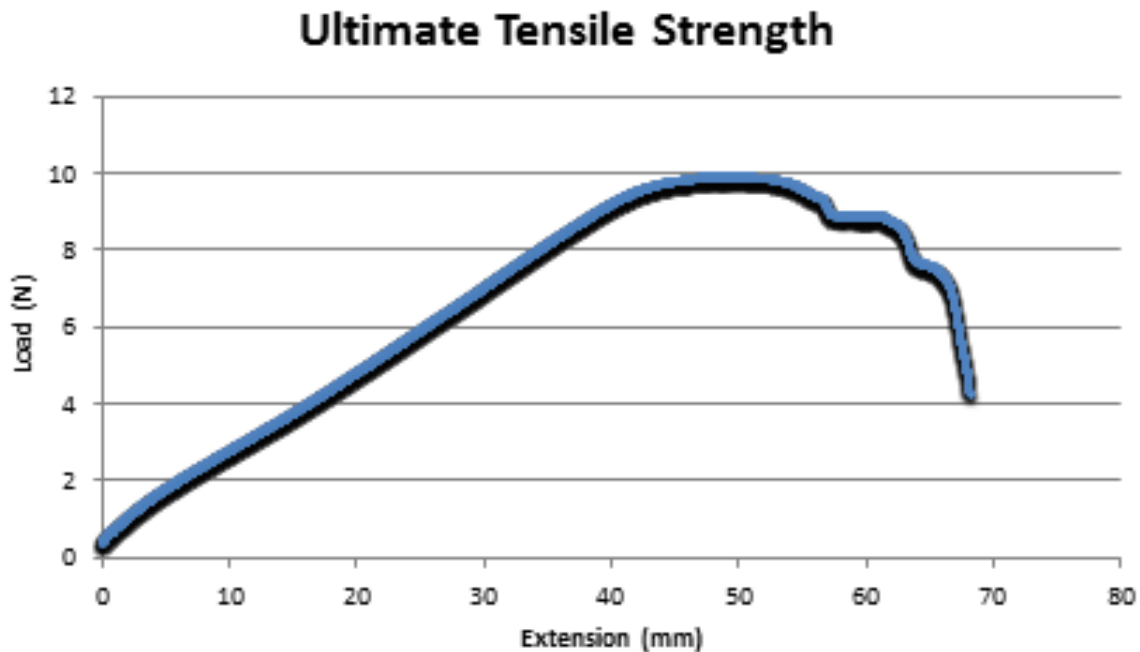
**Figures**  
**Figure 1.**



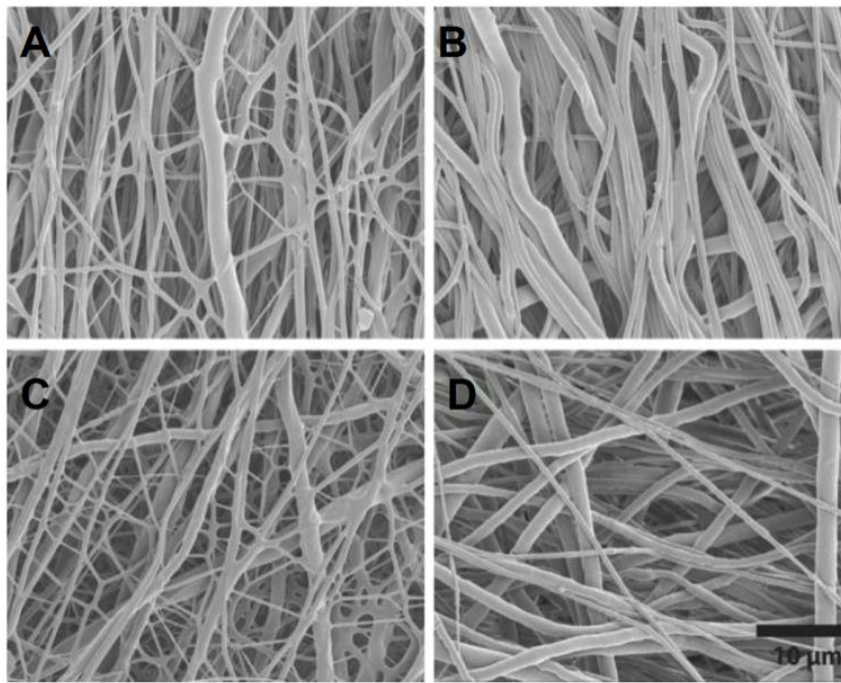
**Production of neurotrophic factors by Neurotrophically Induced – Induced Mesenchymal Progenitor Cells (NI-MiMPCs) and Mesenchymal Stem Cells (MSCs), quantified via ELISA.** MiMPCs and MSCs were cultured in neurotrophic induction medium (NI) or growth medium (GM). Conditioned media taken from days 3 and 7 of induction culture, and basal conditioned medium taken from cultures 48 hours post-induction (48hr BCM). The conditioned media assayed include those from MiMPCs (induced, NI-MiMPCs; uninduced, GM-MiMPCs), and from MSCs (induced, NI-MSCs; uninduced, GM-MSCs), as well as basal medium (controls). Medium was assayed for levels of (A) BDNF, (B) Osteopontin, (C) IL-6, (D) LIF, (E) osteonectin, and (F) clusterin. All ELISA results are expressed in pg/ml or ng/ml produced per million cells. Medium taken from NI-MiMPC cultures contained high levels of all assayed factors compared to GM-MiMPCs, NI-MSCs, and GM-MSCs. BDNF, IL-6, osteonectin and clusterin exhibited relatively constant secretion levels, while osteopontin and LIF showed decreased expression levels during induction treatment and the 48-hour post-induction period. \*,  $p < 0.05$ , compared to GM controls,  $n \geq 3$  for all assays.



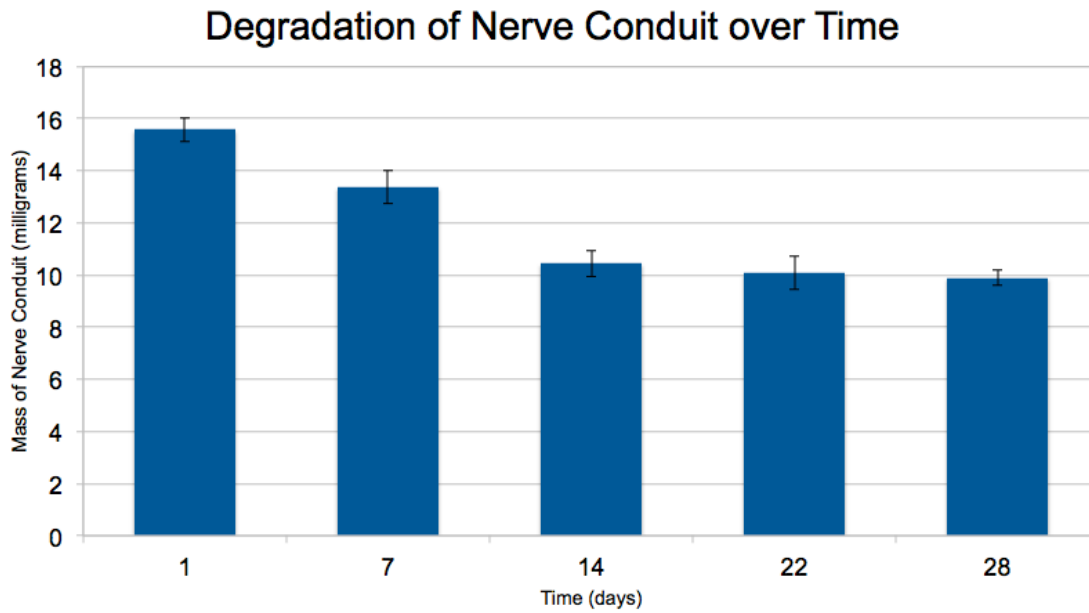
**Figure 2. Phalloidin and Calcein-AM staining of cells demonstrating morphology and distribution within scaffold.** (A) Simplified view of constructed conduit without concentric layers showing location of aligned and randomly oriented cells. (B-D) Phalloidin/DAPI staining of cells within an unraveled conduit showing cells with randomly oriented processes, cells with aligned processes, and cells trapped in gelatin with retracted processes, respectively. (E) Calcein-AM live cell staining of cross-section of constructed scaffold demonstrating concentric distribution of cells.



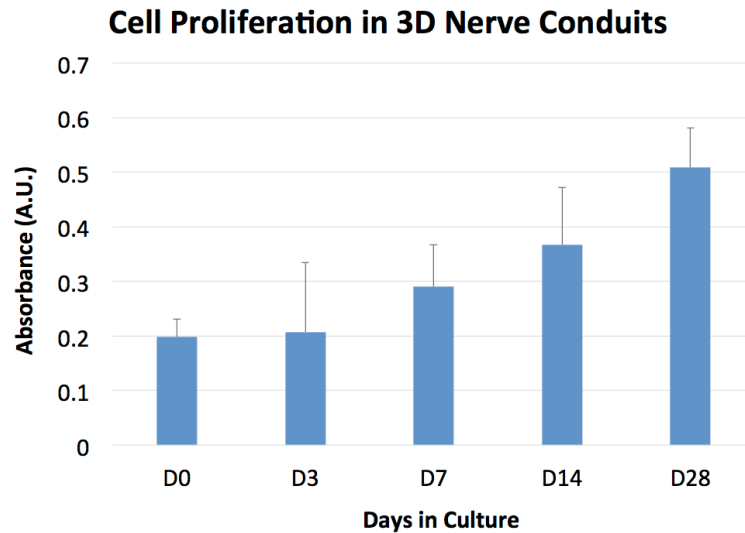
**Figure 3. Suture Retention and Tensile Strength.** Nerve conduit withstood up to 10N of tensile force applied with suture before elongating and failing. This is well above the necessary force for the application of a nerve conduit.



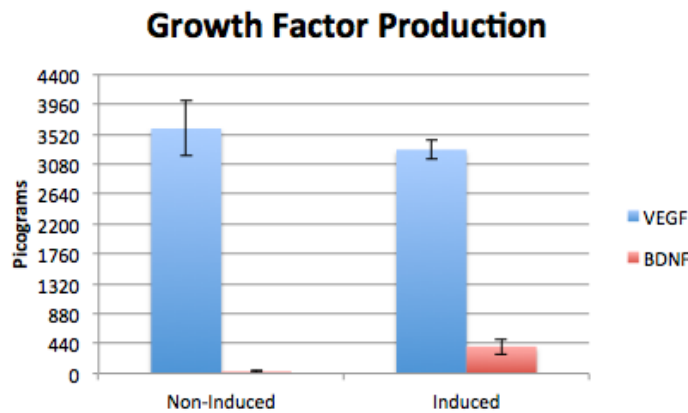
**Figure 4.** SEM images of composite GelMA / PCL electrospun scaffolds. SEM images of (A) aligned composite scaffold, (B) aligned scaffold with GelMA removed, (C) random composite scaffold, and (D) random scaffold without GelMA.



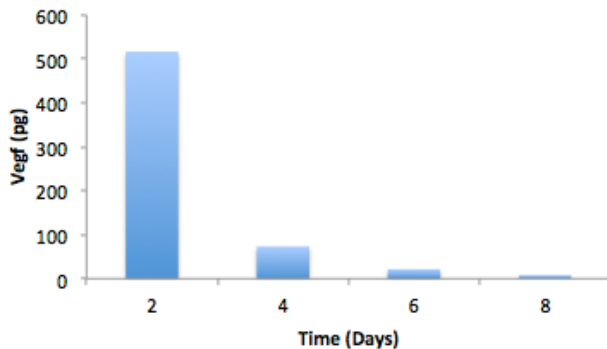
**Figure 5.** Degradation of 3D nerve conduit over 28 days. Mass of conduit measured over a 28 day span. As expected, the gelatin component degrades relatively quickly with the hydrophobic polycaprolactone component remaining behind for structural stability. All conduits maintained structural stability over this period.



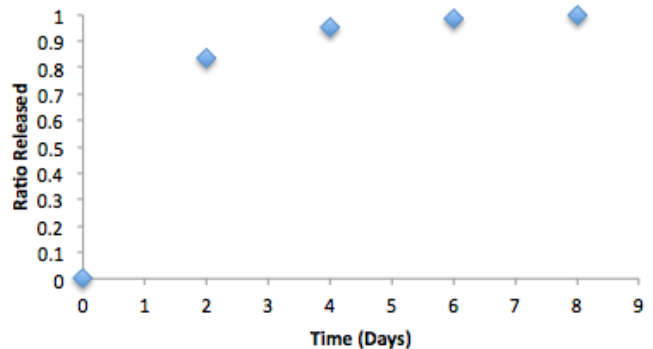
**Figure 6.** MTS assay for cellular metabolism within 3D nerve conduits over a 28 day period. Metabolism of cells within conduit significantly increases over a 28 day period, indicating cellular proliferation and biocompatibility of this nerve conduit system.



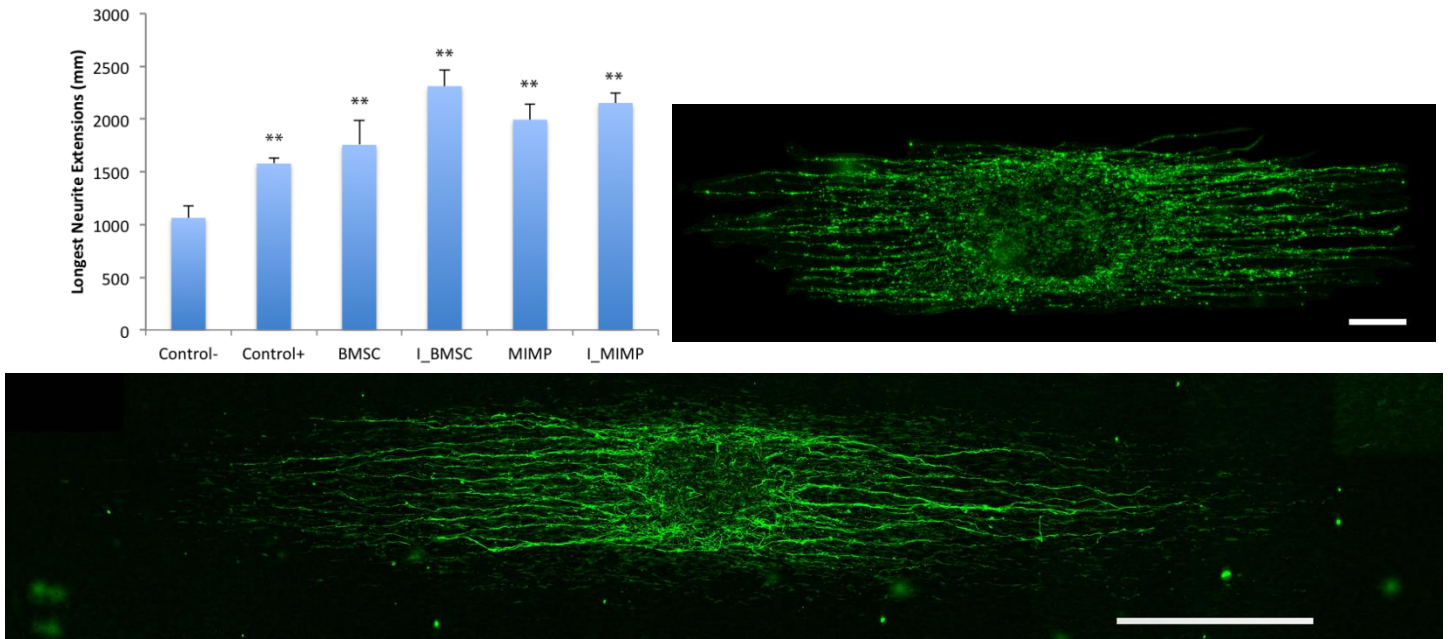
### Vegf Release



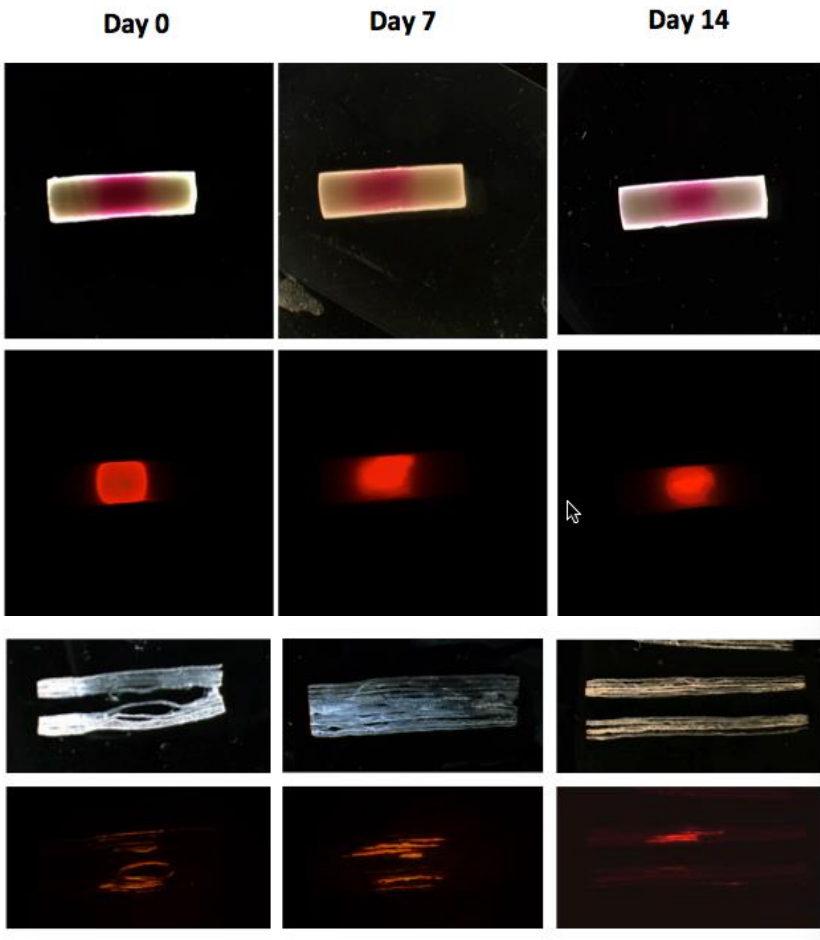
### Cumulative VEGF Release



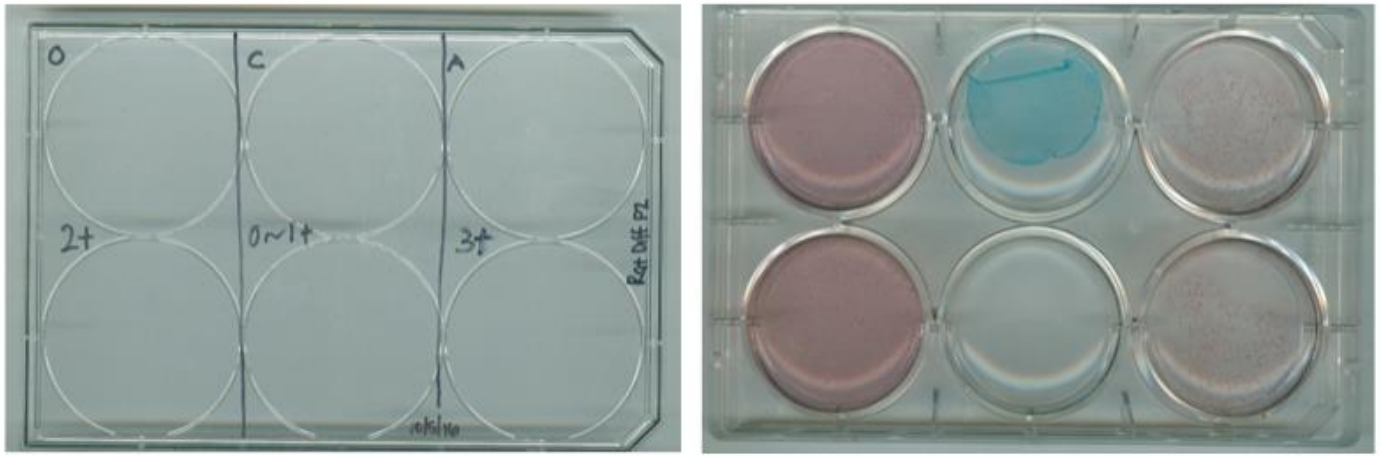
**Figure 7.** Growth factor release from 3D nerve conduits. **(Top)** Secretion of BDNF and VEGF from non-induced and neurotrophically-induced MSCs seeded in 3D nerve conduits. **(Left)** VEGF measured in medium after bolus loading during conduit fabrication. **(Right)** Cumulative VEGF release from conduit over a 9 day period. Permeability of growth factors and slow release are observed, as expected due to the slow release capabilities of methacrylated gelatin.



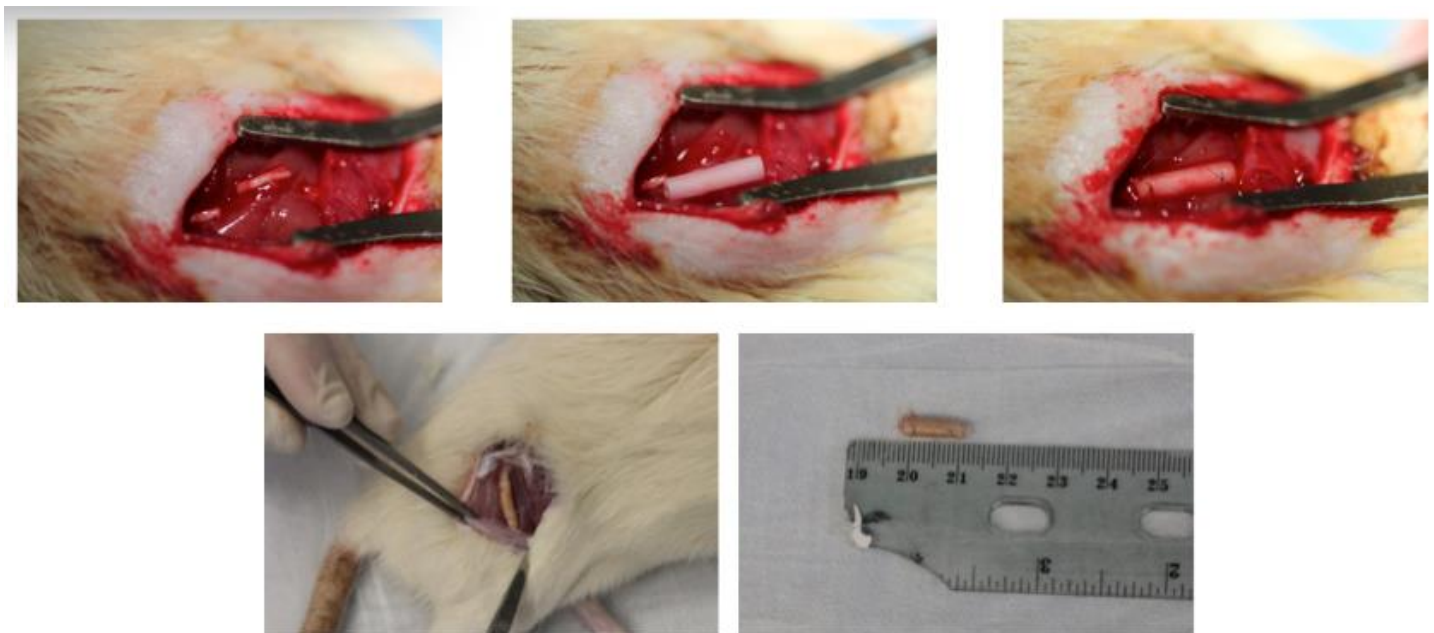
**Figure 8. Cell-seeded scaffolds significantly enhance nanofiber-guided neurite extensions from cultured Dorsal Root Ganglia (DRGs).** (Top-Left) Average of 10 longest neurite extensions from 4 DRGs in each group. (Top-Right) Image of negative control - DRG on non-cell seeded scaffold. (Bottom) Image 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  $\mu$ m. Bottom Scale Bar = 800  $\mu$ m.



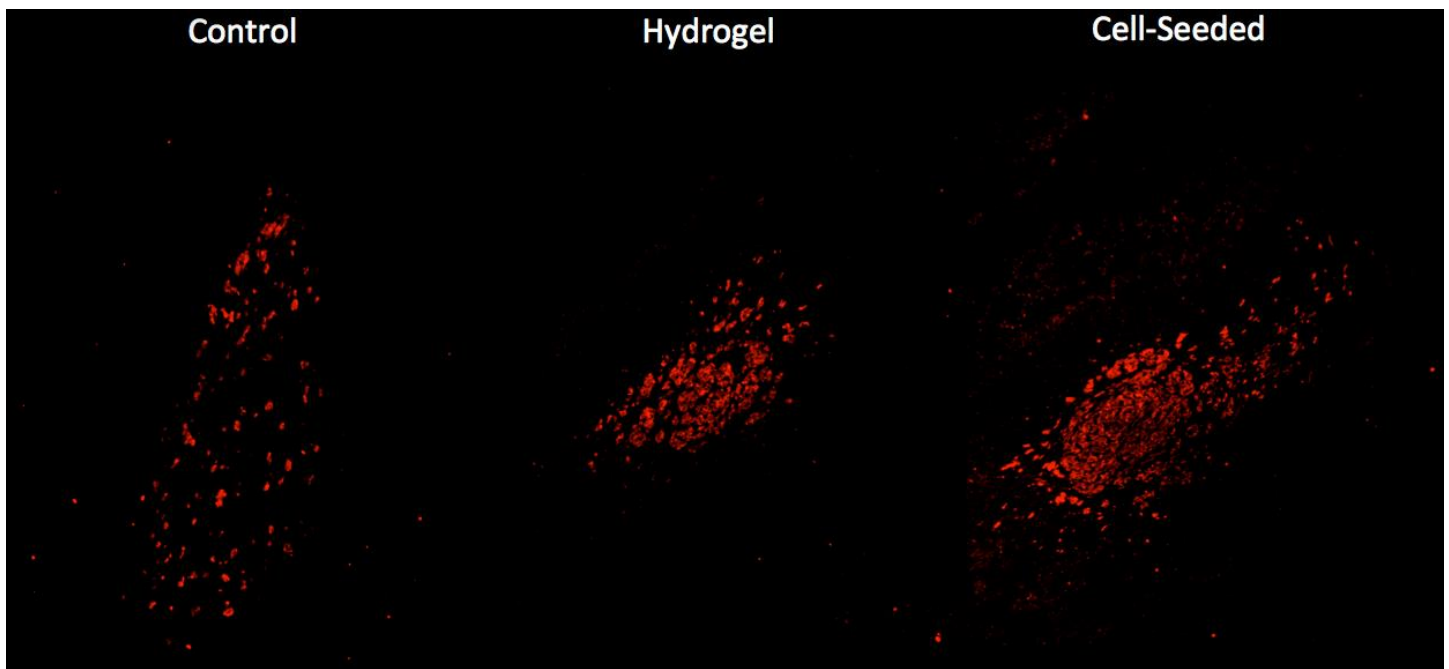
**Figure 9. DiI tracking of seeded cells within 3D nerve conduits.** Day 0 (left column), day 7 (middle column), and day 14 (right column) macroscopic and longitudinal sections of conduits with accompanying fluorescent images of DiI labeled cells. As seen from the images, cells that are encapsulated within the walls of the conduit tend to stay within the location they are seeded at day 0 over a 14 day course. This allows spatial control of cell seeding for *in vivo* conduits which utilize cells seeded in the middle of the conduit to provide a neurotrophic gradient for growing axons.



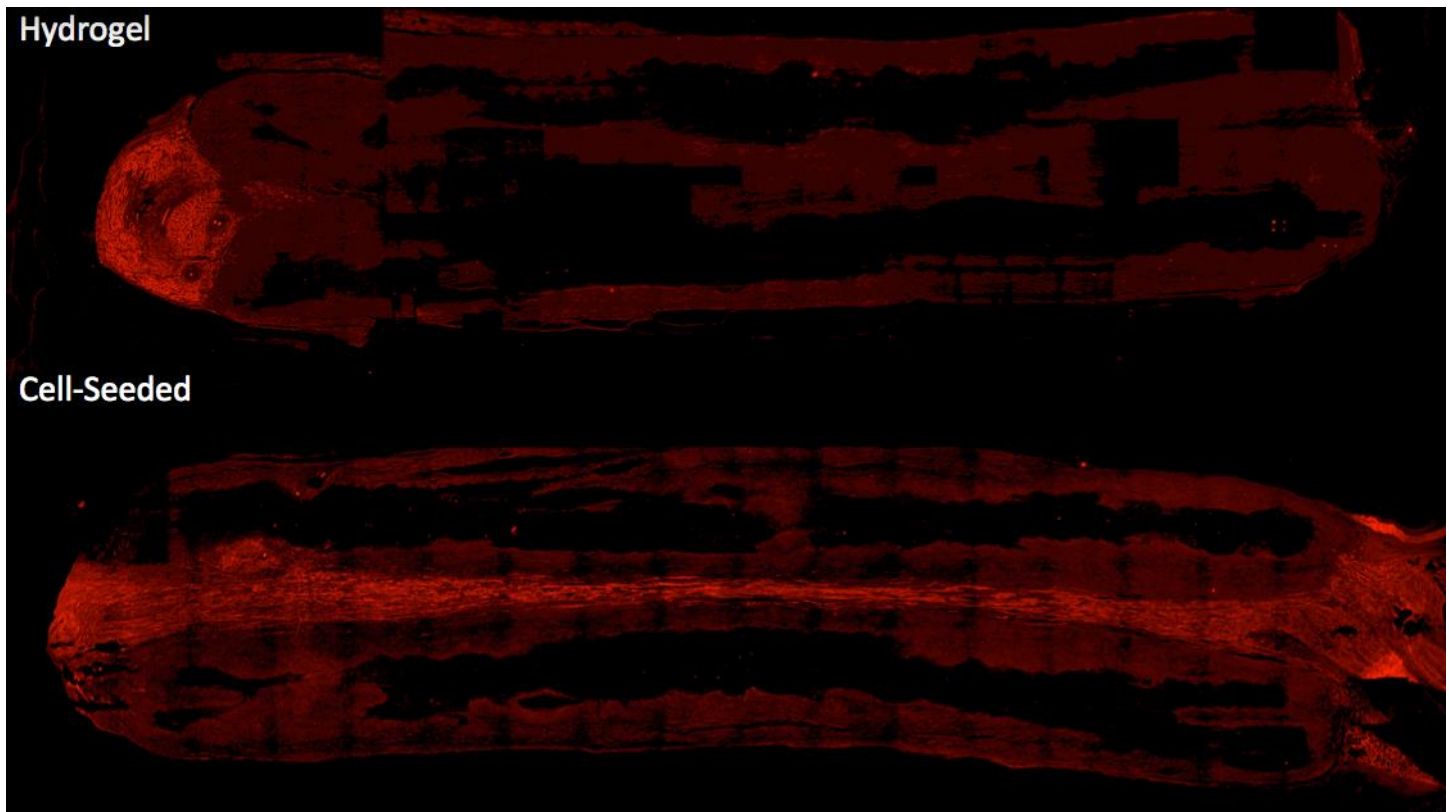
**Figure 10. Verification of multipotency of isolated rat MSCs.** Rat adipose stem cells were isolated and underwent a differentiation protocol to demonstrate multipotency. Here, osteogenic, chondrogenic, and adipogenic differentiation are shown in the left, center, and right wells, respectively along with their corresponding scores (0-3+) on the left hand side.



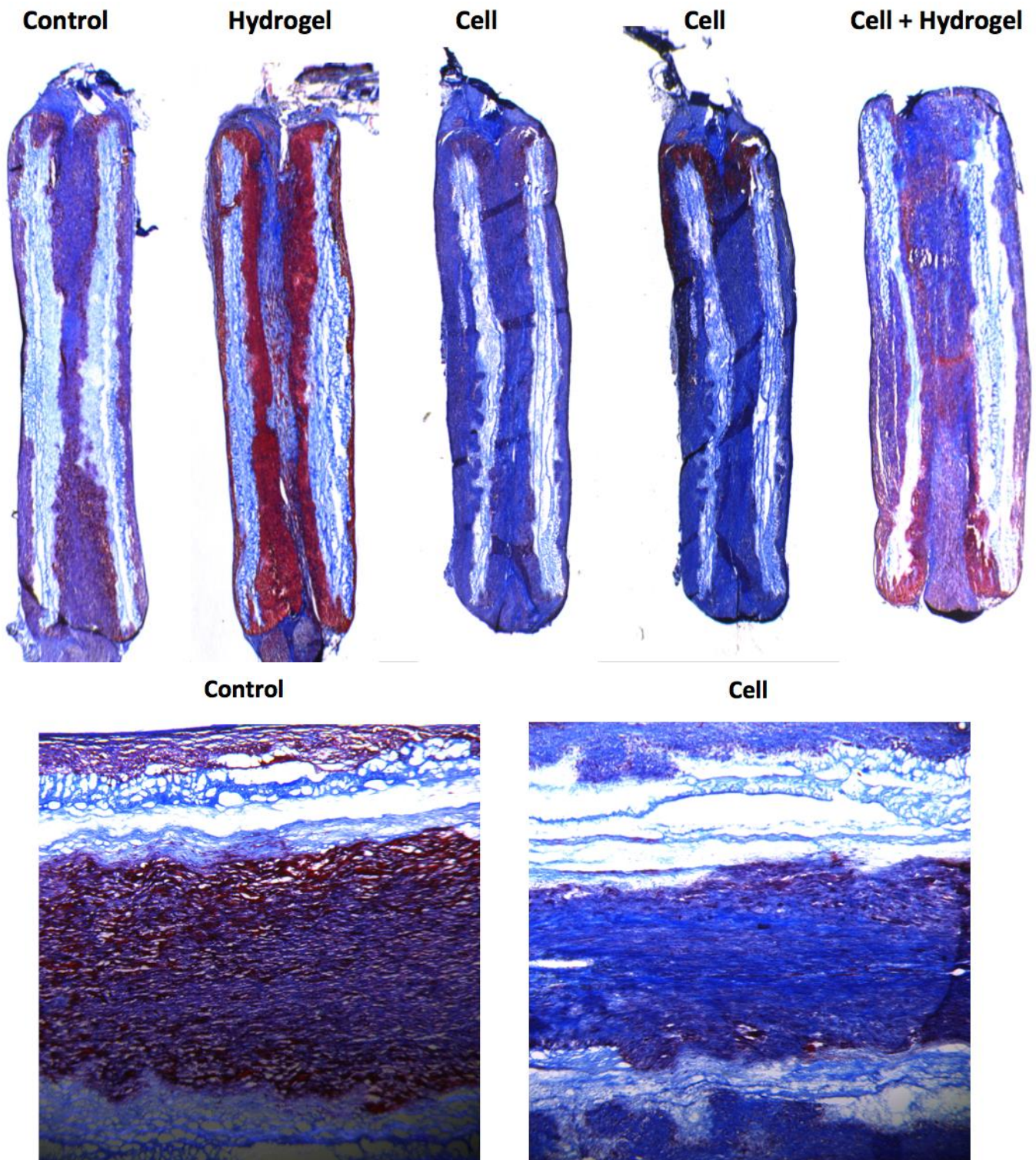
**Figure 11. Surgical implantation of scaffold within rats and macroscopic conduit view after 6 weeks.** Removal of 1cm sciatic nerve followed by implantation of scaffold into the defect site (**Top panels**). Images of conduit during harvest after 6 weeks (**Bottom panels**). As seen from the conduits at harvest, structural integrity is maintained and there is no foreign body reaction to the conduit itself.



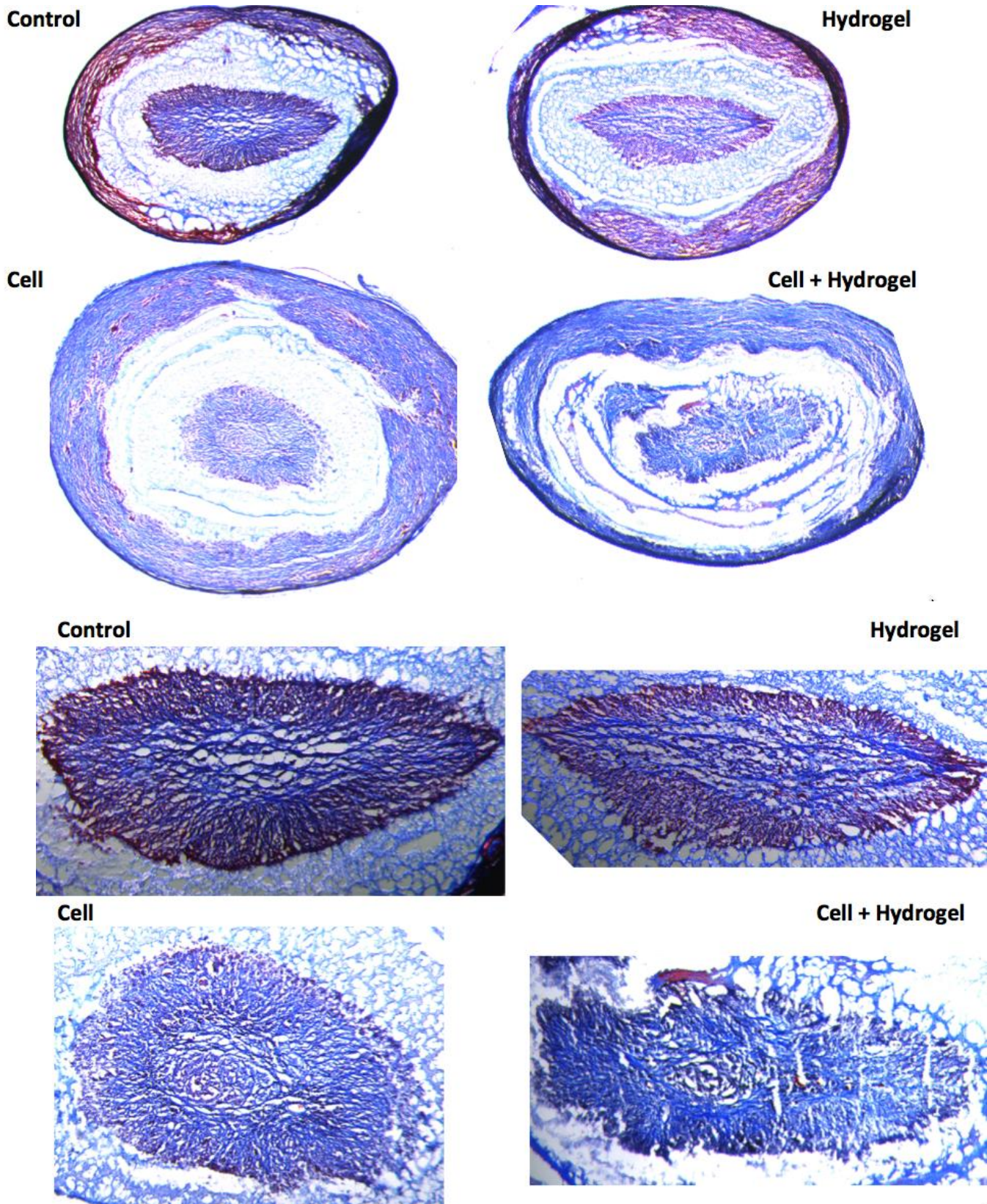
**Figure 12. S100 staining of tranverse sections at midpoint of conduit after 6 weeks.** Immunohistochemistry for S100 schwann cell staining was used to observe schwann cell infiltration into the growing nerve. As seen from the staining, control conduits (with no encapsulated cells or hydrogel lumen filler) yielded spotty staining with little organization. In contrast, cell-seeded and hydrogel scaffolds displayed dense S100 staining with fascicular distribution – especially cell-seeded groups.



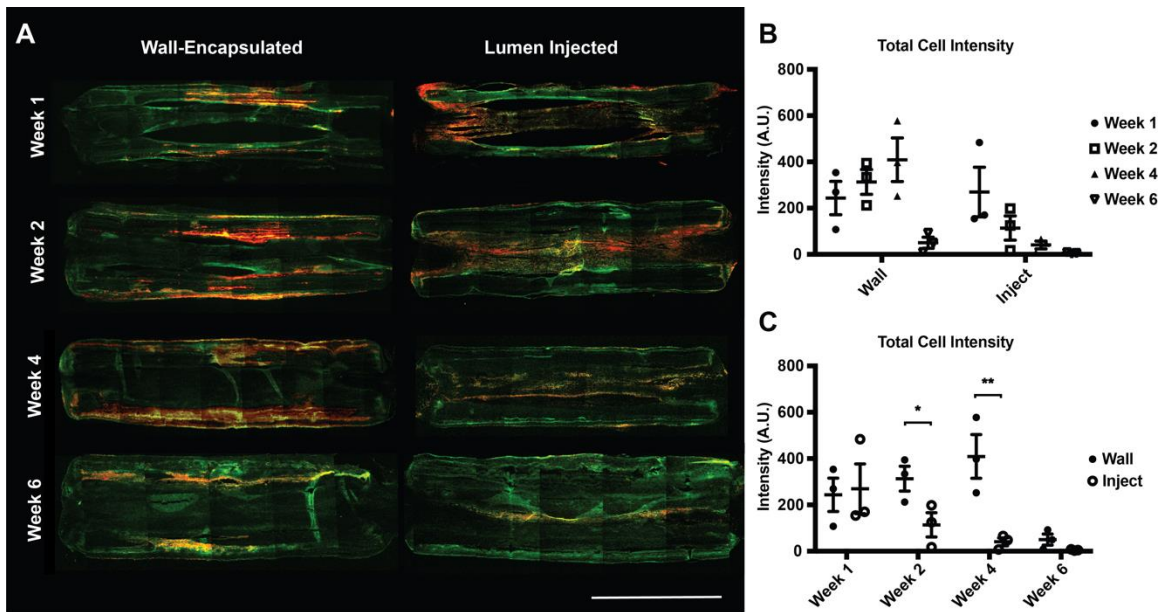
**Figure 13. Longitudinal S100 staining of conduit after 6 weeks.** Immunohistochemistry for S100 schwann cell staining was used to observe schwann cell infiltration into the growing nerve. As seen from the staining, cell-seeded conduits exhibit a remarkable ability to allow for S100 schwann cell migration into the growing nerve with complete continuity after only 6 weeks. This was not observed in either hydrogel or control conduits (not shown due to imaging equipment malfunction).



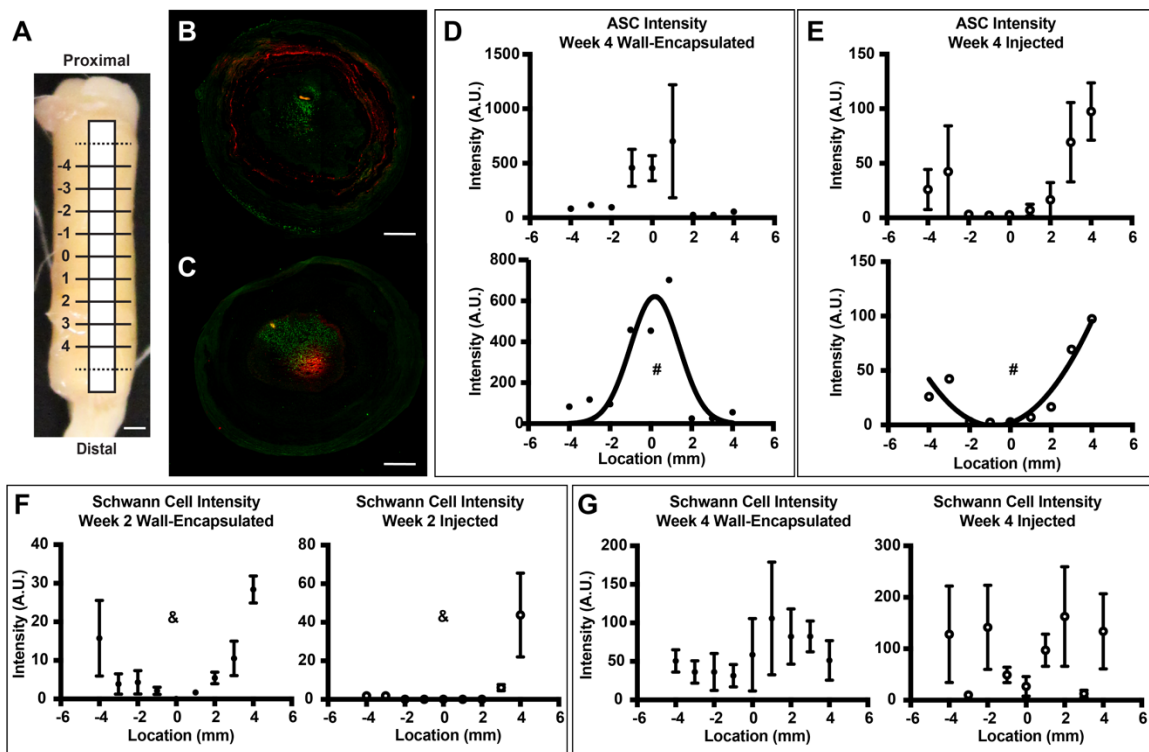
**Figure 14. Longitudinal Masson Trichrome staining of conduit after 6 weeks.** Masson trichrome staining was used to analyze longitudinal sections after 6 weeks growth (proximal oriented towards top and distal towards bottom). Pink is cellular cytoplasm while blue is presence of collagen. **(Top)** Strikingly, cell-seeded conduits display strong collagen staining (indicative of axon formation) in full continuity throughout the conduit – even at the distal end. This is in contrast to control and hydrogel-only conduits, which display weak staining at the distal portions. Hydrogel-only groups seem to promote cellular infiltration into the conduit without concurrent schwann cell migration or axon regrowth. In addition, blue staining is seen on the outer walls of the conduits, indicating cellular infiltration into only the random layers of the conduit while allowing nutrient perfusion through all layers as desired. **(Bottom)** Magnified view of distal portion of control and cell conduits demonstrating strong collagen staining in the cell group with weak blue staining in the control.



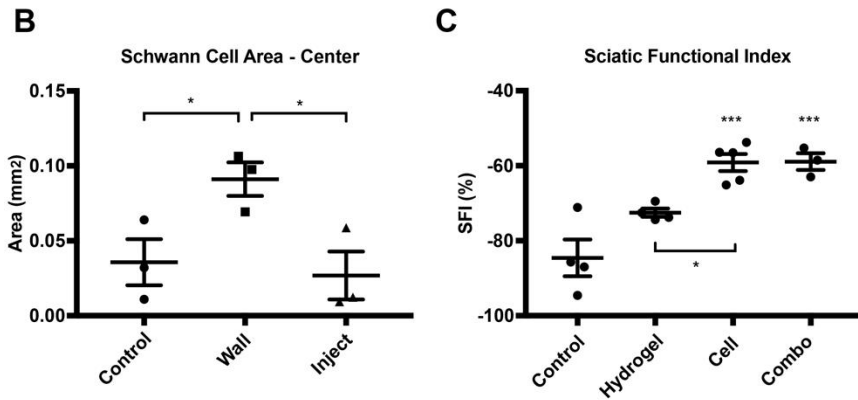
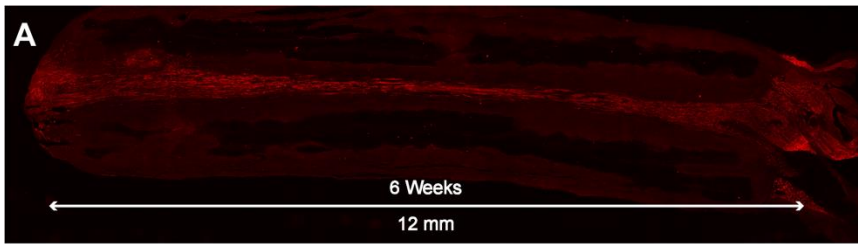
**Figure 15.** Transverse Masson Trichrome staining at midpoint of conduit after 6 weeks. Masson trichrome staining was used to analyze longitudinal sections after 6 weeks growth. Pink is cellular cytoplasm while blue is presence of collagen. **(Top)** The same observations hold true in these transverse sections as seen in the longitudinal sections in Figure 14. The cell-containing groups demonstrate dense collagenous stainin in the middle of the conduits (indicative of axon formation). In addition, cell-containing groups have much higher collagen deposition within the walls of the conduit – likely contributing to the structural stability of the conduit itself. **(Bottom)** Magnified views of the central portion of the conduits to better demonstrate the increased organization and collagen content in the cell based groups.



**Figure 16. Cells are retained longer *in vivo* with wall-encapsulation compared to lumen injection. (A)** Representative images of DiI labeled cells in conduits over 6 weeks. Red channel is DiI label while green is scaffold autofluorescence. **(B)** Total DiI-labeled cell intensity across 6 weeks for wall-encapsulated vs. cell groups showing trends within groups. **(C)** Total DiI-labeled cell intensity across 6 weeks comparing between groups at each week. \*,  $p < 0.05$ . \*\*,  $p < 0.005$ . Scale bar = 5 mm.



**Figure 17. Conduits with centrally located wall-encapsulated ASCs drive directional Schwann cell migration. (A)** Schematic of locations sectioned along explanted nerve conduits (4 mm out from the center proximally and distally) from 1 cm rat sciatic nerve transection model. Scale bar = 1 mm. **(B,C)** Representative pictures of localization of Schwann cells and DiI-labeled wall-encapsulated or lumen-injected cells, respectively. Green channel equals Schwann cells while red channel equals ASCs. Scale bar = 500  $\mu\text{m}$ . **(D,E)** ASC intensity along the length of explanted conduits in wall-encapsulation vs injection, respectively. Wall-encapsulated ASCs fit a Gaussian distribution while lumen-injected ASCs fit a quadratic distribution. **(F,G)** Schwann cell intensity along the length of the explanted conduit at two weeks and four weeks, respectively. Schwann cells possess a stronger inwards migration at two weeks in wall-encapsulation groups and by four weeks resemble a single peaked distribution near the center of the conduit as opposed to lumen injection, which possess no clear distribution pattern. #,  $p < 0.005$  between distributions (Gaussian, fit probability = 84.74% and  $r^2 = 0.4463$ . Quadratic, fit probability = 91.17% and  $r^2 = 0.3280$ ). &,  $p < 0.05$  between distributions.

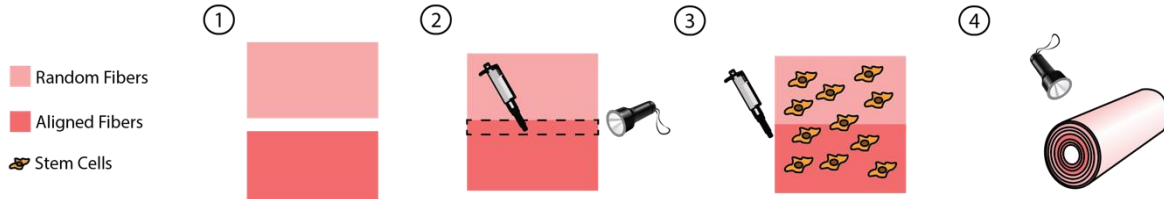


**Figure 18. Conduits with centrally located wall-encapsulated ASCs improve functional return.** (A) Image of Schwann cells bridging the nerve conduit with wall-encapsulated ASCs at 6 weeks in a rat 1 cm sciatic nerve transection model. (B) Schwann cell-positive area in cross-section of center of conduits in cell-free control, wall-encapsulated and lumen injected cell groups. (C) Sciatic functional index at 16 weeks for cell-free control (control), nerve conduits with ECM hydrogel lumen filler (hydrogel), nerve conduit with wall-encapsulated cells (cell), or nerve conduit with wall-encapsulated cells plus ECM hydrogel lumen filler groups (combo). \*,  $p < 0.05$ . \*\*\*,  $p < 0.001$  compared to Control.

## Methods

**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-trifluoroethanol in water. To fabricate 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 100 $\mu$ m. 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  $\mu$ L of photoinitiator 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  $\mu$ L 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  $\mu$ L photoinitiator 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 for 2D scaffolds and DRGs.** 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 MiMPCs 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.

**Methods 9. DiI labeling of cells.** Vybrant DiI cell labeling solution (Life Technologies) was used to label cells before seeding into nerve conduits. Manufacturers protocol was followed.

**Methods 10. Surgical implantation of nerve conduits within rats.** Animals (Lewis rats: male, 8-10 weeks of age, 275-300g) will be sedated with isoflurane and the right hindquarter will be shaved and depilated with nair. The surgical field will be prepared with alcohol and betadine. The skin will be incised 1 mm posterior and parallel to the femur. The biceps femoris will be split via blunt dissection to expose the underlying sciatic nerve. Bupivacaine will be administered locally and allowed to permeate the sciatic nerve. The incision will be extended distally and a 10 mm segment of the sciatic nerve will be removed prior to its bifurcation into the common peroneal and tibial nerves. Hemostasis will then be achieved with pressure and battery-powered cautery as needed. Nerve conduits of different compositions 12mm in length will be implanted. Nerve conduits are created in the lab, containing either an empty conduit, a conduit with nerve ECM hydrogel lumen filler, a conduit with wall-encapsulated cells, and a conduit with both ECM hydrogel lumen filler and wall-encapsulated cells. The conduit will be sutured to the proximal and distal ends of the nerve defect using 8-0 to 10-0 nylon sutures under loupe or overhead microscopy. 4-0 chromic gut sutures and/or skin glue will be used to close the biceps femoris and skin.

**Methods 11. Harvest of nerve conduits from rats.** Conduits were harvested from rats that had reached their timepoints and placed in formalin for 2 days. Following this, they were transferred to 10% sucrose solution for 2 hours, followed by 20% sucrose for 6 hours, and 30% sucrose overnight. They were then equilibrated in OCT compound for 2 hours followed by transfer to new OCT and subsequent freezing. Cryosections were obtained at 16 microns.

**Methods 12. Immunohistochemistry of rat frozen sections<sup>1</sup>.** Sections were incubated for 1 h at room temperature in a blocking solution of 4% goat serum (Gibco) in PBS, were incubated overnight at 4 °C in a mixture of primary antibody and blocking solution, and were washed and incubated for 1 h at room temperature in a solution of secondary antibody mixed in 0.5% Triton X-100 (Sigma) in PBS. Slides were washed twice more with PBS, incubated with DAPI for 10 min, and then dried and coverslipped for analysis. Primary antibody S100 (1:250, rabbit IgG; DakoCytomation), were used. The following secondary antibodies were used: goat anti-rabbit IgG Alexa 488/594 (1:220; Invitrogen) and goat anti-mouse IgG1 Alexa 488/594 (1:220; Invitrogen).

<sup>1</sup>This was adapted from: Mokarram et al. 10.1073/pnas.1705757114

**Methods 13. Histology of rat frozen sections.** Masson's Tri-crhome stain was purchased from IMEB and manufacturers protocol was followed utilizing microwave.

### **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 Pete Alexander to junior graduate students Rachel Brick and Kelsey Gloss. In addition, surgical technique and training has been acquired under Dr. John Fowler's guidance.

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

A publication is in revision at Stem Cell Translation Medicine. See Section 6 below.

### **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. Strong leadership from senior personnel will continue to be practiced.

**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**

Specific objective 14: Endothelial cells were not used in co-culture with MSCs *in vitro* because culture conditions necessary for survival of ECs would confound the results of neurite extension given the vast number of growth factors necessary to sustain EC viability *in vitro*. In addition, from a point-of-care translatability aspect the use of two cell types – especially difficult to isolate ECs – would greatly reduce its applicability.

Specific objective 24: A 2D equivalent of this 3D assay was used instead due to problems with imaging a DRG inside of the nerve conduit. We found that the results of this *in vitro* assay was still correlative with results demonstrated *in vivo*.

Specific objective 26: Thy-1 GFP rats were not used due to cost and difficulty in obtaining them. In addition, from a scientific standpoint there are plenty of immunohistochemical labels that can identify host axons and nerves without needing a Thy-1 transgenic GFP rat.

Specific objective 28: Instead of using a Lenti-viral construct to label cells, we chose a higher efficiency labeling method (DiI). In addition, with this membrane label there is minimal concern of altering the secretome of the MSC that a lenti-viral approach might do.

Specific objective 29: Given the very promising results we saw *in vivo* at the 6 week mark, we chose to do earlier time points as well in order to track cells and parse out the activity of these cells in the early nerve regeneration response.

Specific objective 30: Functional testing was not completed at 8 weeks because full functional capacity after a sciatic nerve defect returns at 16 weeks. We have modified this timepoint thus we are still waiting for resolution of this timepoint. We have already completed a 6 week timepoint to monitor early biological response of the nerve conduit system.

#### **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. We anticipate the study beginning in January, 2019.

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 Neuroscience 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.**

Brick RM, Sun AX, Tuan RS. Neurotrophically Induced Mesenchymal Progenitor Cells Derived from Induced Pluripotent Stem Cells Enhance Neuritogenesis via Neurotrophin and Cytokine Production. *Stem Cell. Trans Med.* In Revision.

Sun AX, Prest TA, Fowler JR, Brick RM, Gloss KM, Li X, DeHart M, Shen H, Yang G., Brown BN, Alexander PG, Tuan RS. Conduits Harnessing Spatially Controlled Cell-Secreted Neurotrophic Factors Improve Peripheral Nerve Regeneration. *Acta Biomaterialia* (in submission)

• **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**

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.)

<i>Name:</i>	Rocky S. Tuan
<i>Project Role:</i>	PI
<i>Research Identifier:</i>	University Employee ID# 124200
<i>Nearest person month worked:</i>	7.75% effort (0.93 Person Months)
<i>Contribution to Project:</i>	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.
<i>Funding Support:</i>	N/A
<i>Name:</i>	Peter Alexander
<i>Project Role:</i>	Co-Investigator
<i>Research Identifier:</i>	University Employee ID# 124097
<i>Nearest person month worked:</i>	18.33% effort ( 2.20 Person Months)
<i>Contribution to Project:</i>	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.
<i>Funding Support:</i>	N/A
<i>Name:</i>	John Fowler
<i>Project Role:</i>	Co-Investigator
<i>Research Identifier:</i>	University Employee ID# 150464
<i>Nearest person month worked:</i>	10% effort ( 1.20 Person Months)

<i>Contribution to Project:</i>	Dr. Fowler participates in the animal surgery aspects of this project, as well as in research design, outcome analysis, manuscript preparation, and preparation for future large animal trials.
<i>Funding Support:</i>	N/A
<i>Name:</i>	MaCalus Hogan
<i>Project Role:</i>	Co-Investigator
<i>Research Identifier:</i>	University Employee ID# 152173
<i>Nearest person month worked:</i>	7.5% effort ( 0.90 Person Months)
<i>Contribution to Project:</i>	Dr. Hogan participates in the animal surgery aspects of this project, as well as in research design, outcome analysis, manuscript preparation, and preparation for future large animal trials.
<i>Funding Support:</i>	N/A
<i>Name:</i>	Alessandro Piroso
<i>Project Role:</i>	Postdoctoral Associate
<i>Research Identifier:</i>	University Employee ID# 160892
<i>Nearest person month worked:</i>	60% effort (7.20 Person Months)
<i>Contribution to Project:</i>	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.
<i>Funding Support</i>	N/A
<i>Name:</i>	Jian Tan
<i>Project Role:</i>	Research Specialist
<i>Research Identifier:</i>	University Employee ID# 124708
<i>Nearest person month worked:</i>	25.41 % effort ( 3.05 Person Months)
<i>Contribution to Project:</i>	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.
<i>Funding Support:</i>	N/A
<i>Name:</i>	Aaron Sun
<i>Project Role:</i>	Graduate Student Researcher
<i>Research Identifier:</i>	University Employee ID# 149944
<i>Nearest person month worked:</i>	91.66% effort (11.0 Person Months)
<i>Contribution to Project:</i>	Aaron performs 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. N/A
<i>Funding Support:</i>	
<i>Name:</i>	Alyssa D. Falcione
<i>Project Role:</i>	Animal Technician
<i>Research Identifier:</i>	University Employee ID# 163911
<i>Nearest person month worked:</i>	15% effort (1.8 Person Months)
<i>Contribution to Project:</i>	Animal ordering, husbandry, pre-operative preparation, anesthesia and , post-operative care. Ms Falcione is an ALAT certified technician with experience in care, management and surgery in several experimental model animals including goats. In the 2 <sup>nd</sup> and 3 <sup>rd</sup> years, Ms Falcione will devote 25% (3.0 calendar months) of her time to this project.
<i>Funding Support::</i>	N/A

**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- $\alpha$  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- $\alpha$  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- $\alpha$  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- $\alpha$  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 Opioids”

Grant#: 3UG3TR002136-02S1

Role: PI, Time Commitment: As this is an administrative supplement effort reported on master grant ((1.8 calendar (15% effort))

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- $\alpha$  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 cell 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- $\alpha$  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- $\alpha$  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- $\alpha$  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.

# Adult Stem Cell-Based Neurotrophic Conduit Enhancement of Peripheral Nerve Repair



Log Number: OR140390

Award Number: W81XWH-15-1-0600

PI: Rocky S Tuan

Org: University of Pittsburgh

Award Amount: \$1,557,090

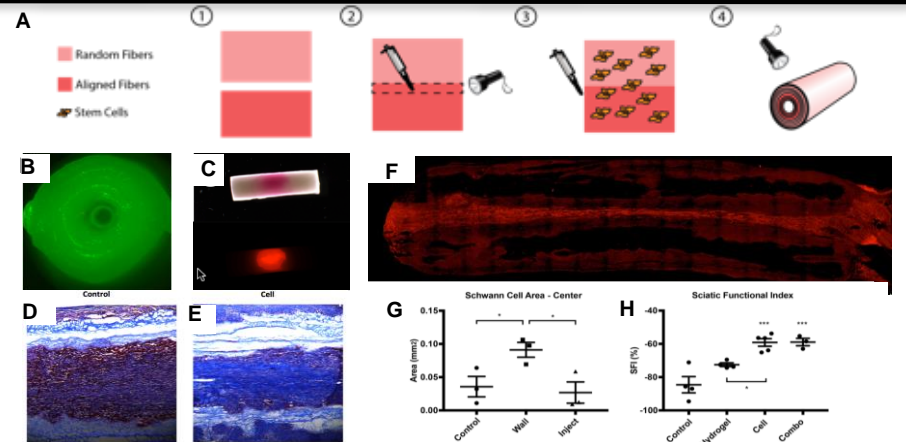
## Project Aims

The goal of this study is to develop a synthetic nerve conduit (NC) enhanced with bioactivated matrix and neuro-potentiating autologous stem cells (MPCs). The following aims are proposed:

1. Produce bi-layered nanofibrous scaffolds (NFS)
2. Optimize the neurotrophic (NT) activity of MPC-seeded NFS
3. Design and construct device for PoC NT-MPC-NC preparation
4. Biomechanical and biochemical testing of final NT-MPC-NC
5. Test NT-MPC-NC in small animal models (rat and rabbit)
6. Prepare and submit an IDE clinical trial protocol to FDA

## Approach

A bilaminar scaffold comprised of (1) inner aligned nanofibers to mediate contact-guidance axonal migration and (2) outer randomized fibers to provide tensile strength and suture retention may be filled with HA/gelatin or similar hydrogel if proven efficacious activated with conditioned medium from neurotrophically activated MPCs conditioned medium and coated externally with MPCs to provide extended, physiologic release of neurotrophic, immuno-modulatory cytokines to enhance nerve regeneration.



**Figure 1:** (A) Conduit assembly: (1) fabrication of aligned and random NFS, (2) adhesion by photocross-linked hydrogel (PXL-mGL) (3) application of MPC via PXL-mGL (4) rolling to size + PXL; (B) live cell localization by Calcein-AM (transverse section); (C) Cell localization to central third by DiI (longitudinal); (D&E) Trichrome staining in (D) mGL and (E) mGL+cells; (F&G) Schwann cell infiltration by (F) DiI and (G) IHC histomorphometry and (H) functional outcomes

## Timeline and Cost

\* 9/29 NCE

Activities	FY	16	17	18	19
1. Produce bi-layered NFS.		█	█		
2. Optimize NT-MPC-seeded NFS.		█	█		
3. P.o.C. NT-MPC NC prep.		█	█		
4. Final NT-MPC NC Biomech/Biochem testing			█	█	
5a. Test NT-MPC-NC in rats			█	█	
5b. Test NT-MPC-NC in rabbits				█	█
6. Submit IDE clinical trial protocol				█	█
<b>Budget (\$K)</b>		<b>\$473</b>	<b>\$506</b>	<b>\$577</b>	

Updated: Oct 29, 2018

Complete  
To be met

Total Cost: \$1,557K

## Goals/Milestones

**CY16 Goals** – Optimize MPC-NFS production

- Optimize biomaterial structure and composition of nerve conduit

**CY17 Goals:**

- Determine optimal neuroconductive ECM for nerve conduit.
- Construct device for point-of-care NT-MPC NC preparation
- Biomechanical and biological testing of MPC-NC.

**CY18 Goals:**

Test MPC-NC in small animal models of sciatic nerve repair

- Test biological activity of MPC-NC in a rat model
- Test surgical and regenerative activity of MPC-NC in a rabbit model
- Prepare and submit IDE protocol to the FDA

**Comments/Challenges/Issues/Concerns:** None

**Budget Expenditure to Date:**

Projected Expenditure: \$1,317,849; Actual Expenditure: \$1,241,330