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6. AUTHORS Philip LeDuc			5d. PROJECT NUMBER				
			5e. TASK NUMBER				
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14. ABSTRACT We worked on an experimental testing platform, called the "Nerve Integrated Tissue on a Chip (NITC)" to which we would apply mechanical force in a precisely controlled and measured system. Our focus was on measuring and modeling trauma-induced injuries to the network of neurons embedded in tissue for use in the diagnosis of innervated tissue injuries and with applications in developing future healing methods and treatment protocols. Through the experimental platform, our goals were to simulate the mechanical stimulation through the innervated tissues and relate this to neuron function in response to mechanical stimulation. We also are working toward							
15. SUBJECT TERMS Final Report							
16. SECURITY CLASSIFICATION OF:		17. LIMITATION OF ABSTRACT		15. NUMBER OF PAGES		19a. NAME OF RESPONSIBLE PERSON	
a. REPORT	b. ABSTRACT					c. THIS PAGE	Philip LeDuc
UU	UU	UU	UU			19b. TELEPHONE NUMBER 412-268-2505	

## Report Title

Final Report: Probing Response and Future Repair of Neural Function to Impact through a Nerve Integrated Tissue on a Chip (NITC) Platform and Mechanical Approach

### ABSTRACT

We worked on an experimental testing platform, called the "Nerve Integrated Tissue on a Chip (NITC)" to which we would apply mechanical force in a precisely controlled and measured system. Our focus was on measuring and modeling trauma-induced injuries to the network of neurons embedded in tissue for use in the diagnosis of innervated tissue injuries and with applications in developing future healing methods and treatment protocols. Through the experimental platform, our goals were to simulate the mechanical stimulation through the innervated tissues and relate this to neuron function in response to mechanical stimulation. We also are working toward predicting the level of neuronal injury, regenerative growth, and to understand how neuronal injuries are associated with injuries of tissues. We also would measure the healing of nervous system under a variety of manipulations, which may assist the therapy of disorders without the need for significant animal or human testing. Our expertise in the areas of mechanics, micromachining, micromolding and neurobiology was used to synthesize mimetic tissues with embedded neurons. In the future, finite element and mechanical analysis will be combined to develop a predictive model for improved healing and treatment of innervated tissue injuries, which will be very applicable to military personnel.

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**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

Received      Paper

**TOTAL:**

**Number of Papers published in peer-reviewed journals:**

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**(b) Papers published in non-peer-reviewed journals (N/A for none)**

Received      Paper

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

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**(c) Presentations**

Number of Presentations: 0.00

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**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

---

**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

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**(d) Manuscripts**

Received      Paper

**TOTAL:**

Number of Manuscripts:

---

**Books**

Received      Book

**TOTAL:**

Received

Book Chapter

**TOTAL:**

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**Patents Submitted**

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**Patents Awarded**

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**Awards**

William J. Brown Professor  
Treasurer/Board of Directors, American Institute for Medical and Biological Engineering  
Fellow, American Society of Mechanical Engineering  
Fellow, Biomedical Engineering Society  
Fellow, American Institute for Medical and Biological Engineering  
Member, National Research Council BEMA

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**Graduate Students**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Adam Wood	1.00	
<b>FTE Equivalent:</b>	<b>1.00</b>	
<b>Total Number:</b>	<b>1</b>	

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**Names of Post Doctorates**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

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**Names of Faculty Supported**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

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**Names of Under Graduate students supported**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

**Student Metrics**

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

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**Names of Personnel receiving masters degrees**

<u>NAME</u>
<b>Total Number:</b>

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**Names of personnel receiving PHDs**

<u>NAME</u>
<b>Total Number:</b>

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**Names of other research staff**

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

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**Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

## **Technology Transfer**

Manoj Puthenveedu, Biological Sciences (neurobiologist), Carnegie Mellon University

**Final Progress Report - ARO-W911NF15101  
(Reporting Period: April 2015 – January 2016)**

**Probing Response and Future Repair of Neural Function to Mechanical Impact through a Nerve Integrated Tissue on a Chip (NITC) Platform and Mechanical Approach**

Philip LeDuc  
Mechanical Engineering  
Carnegie Mellon University, Pittsburgh, Pennsylvania, 15213

**Statement of problem studied**

We worked on an experimental testing platform, called the "Nerve Integrated Tissue on a Chip (NITC)" to which we would apply mechanical force in a precisely controlled and measured system. Our focus was on measuring and modeling trauma-induced injuries to the network of neurons embedded in tissue for use in the diagnosis of innervated tissue injuries and with applications in developing future healing methods and treatment protocols. Through the experimental platform, our goals were to simulate the mechanical stimulation through the innervated tissues and relate this to neuron function in response to mechanical stimulation. We also are working toward predicting the level of neuronal injury, regenerative growth, and to understand how neuronal injuries are associated with injuries of tissues. We also would measure the healing of nervous system under a variety of manipulations, which may assist the therapy of disorders without the need for significant animal or human testing. Our expertise in the areas of mechanics, micromachining, micromolding and neurobiology was used to synthesize mimetic tissues with embedded neurons. In the future, finite element and mechanical analysis will be combined to develop a predictive model for improved healing and treatment of innervated tissue injuries, which will be very applicable to military personnel.

**Approach**

- Creating a Nerve Integrated Tissue on a Chip (NITC) system through fabricating a multilayer mutable polymer molded system with a single channel structure embedded with nerve cells surrounded by skeletal myoblasts in a collagen matrix.
- Integrating mechanical stimulation to understand the response of the NITC innervated tissue for future healing and therapies.

**Relevance to Army**

Mechanically related injuries to nerve systems and associated tissues are critical in diverse areas such as the brain, knee, hand, ankle and shoulder. The type of mechanical loading experienced by different axon bundles varies from sudden mechanical impact in battle and during explosions such as improvised explosive devices. Here in this initial 9 month project, we worked to develop an experimental platform to allow us to assess healing of nerves and their connections following a variety of mechanical loading paradigms with future work to integrate a computational approach for predictive capabilities. This project worked toward providing a tremendous advantage in reducing the need for numerous animal or human tests. This approach also has potential applications in future therapy of injured innervated tissues that would benefit military personnel and society in general.

**Collaborations and Technology Transfer**

- Manoj Puthenveedu, Biological Sciences (neurobiologist), Carnegie Mellon University

**Resulting Journal Publications**

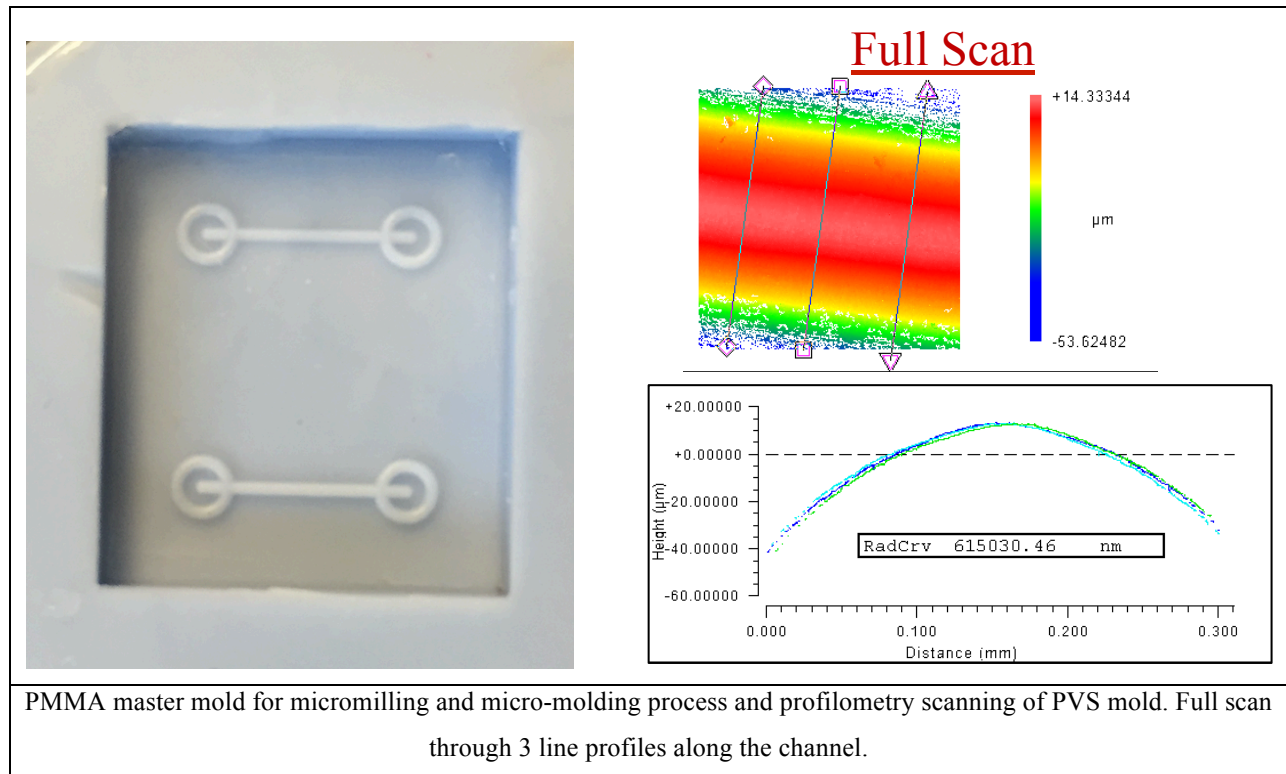
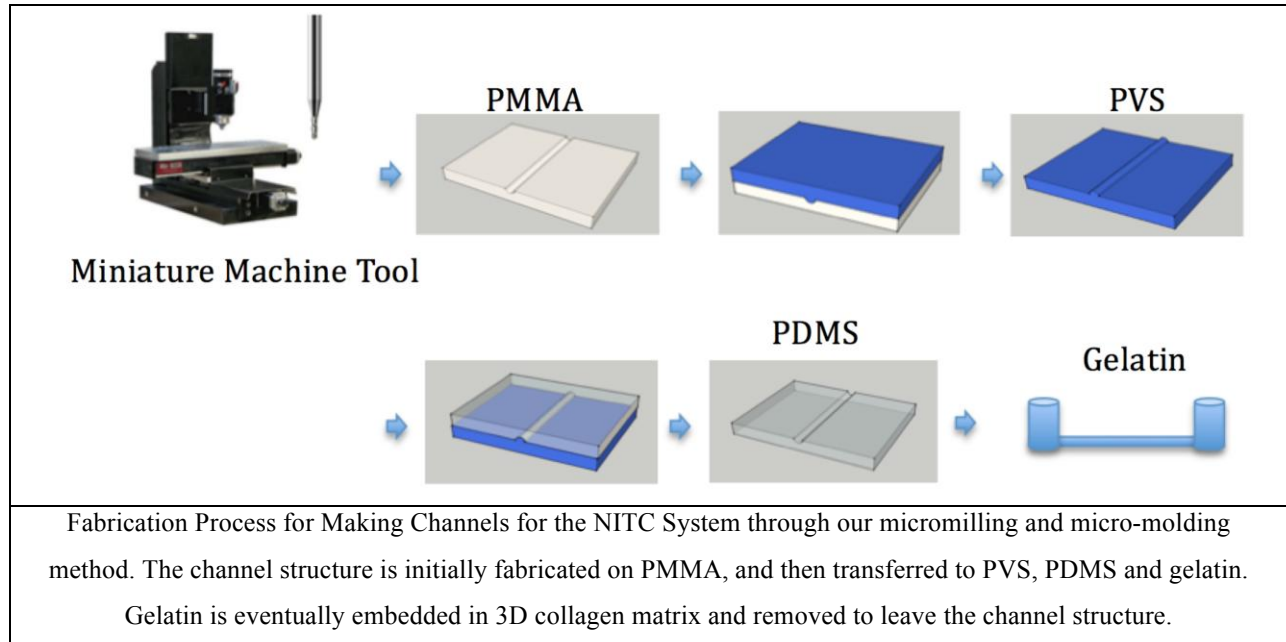
- Hazar, M., Kim, Y.T., Song, J., LeDuc, P.R.\* Davidson, L.A.\*, Messner, W.C.\* 3D Bio-Etching of a Complex Composite-Like Embryonic Tissue. Lab-on-a-Chip 15, 3293-3299 (2015).
- Warren, K.M., Mpagazehe, J.N., LeDuc, P.R.\* , Higgs, C.F.\* Geometric Effects In Microfluidics on Heterogeneous Cell Stress Using an Eulerian–Lagrangian Approach. Lab-on-a-Chip 16, 593-598 (2016).
- Naserifar, N., LeDuc, P.R.\* , Fedder, G.K.\* Material Gradients in Stretchable Substrates toward Integrated Electronic Functionality. Advanced Materials 28, 3584-3591(2016)
- Warren, K.M., Islam, M.M., LeDuc, P.R.\* , Steward, R.\* 2D and 3D Mechanobiology in Human and Nonhuman Systems. ACS Appl Mater Interfaces (in press)
- Other data from this work is being integrated into manuscripts for submission.

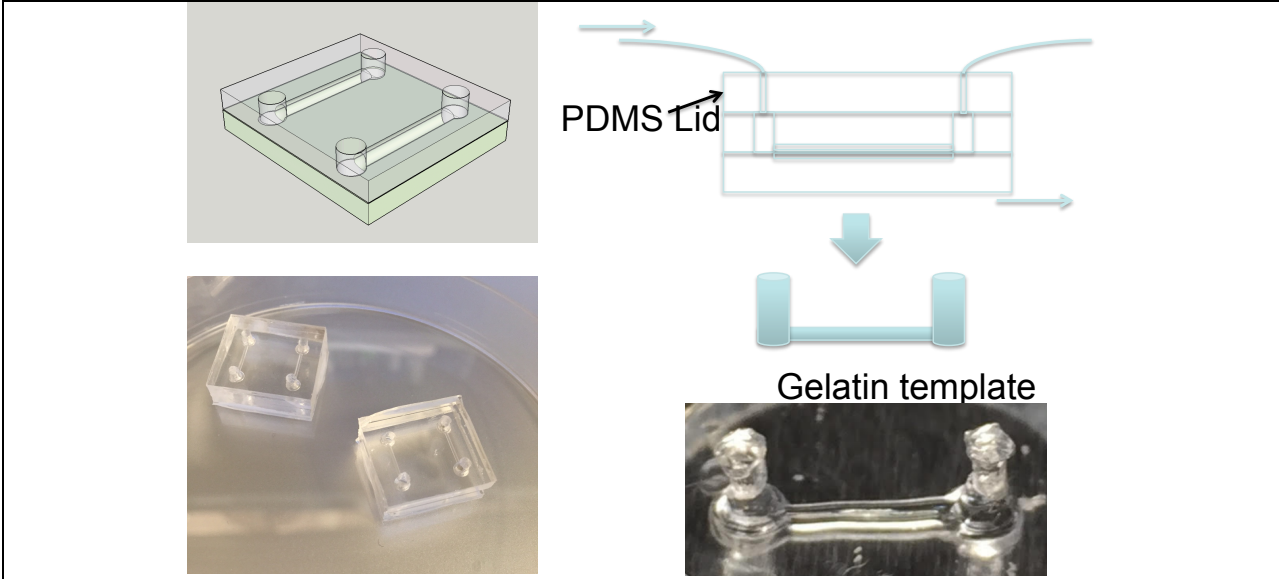
\*Corresponding authors

### **Awards, Honors and Appointments**

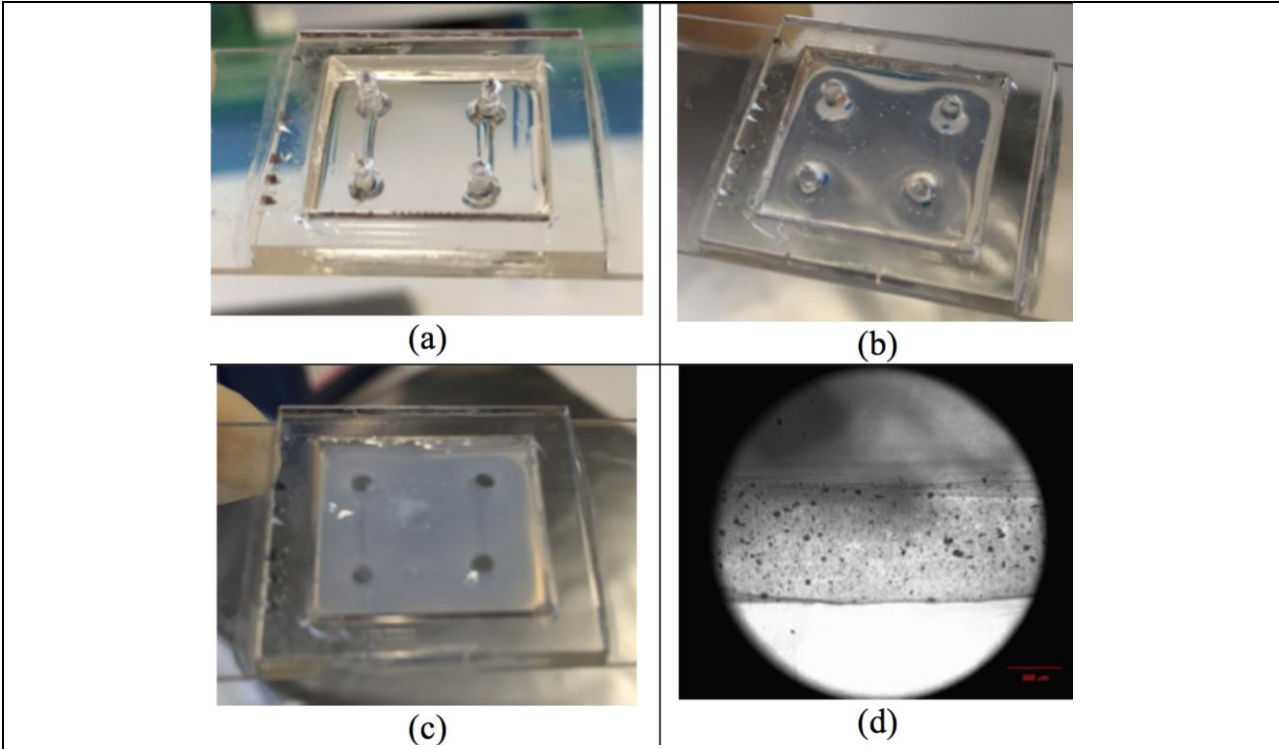
- William J. Brown Professor
- Treasurer/Board of Directors, American Institute for Medical and Biological Engineering
- Fellow, American Society of Mechanical Engineering
- Fellow, Biomedical Engineering Society
- Fellow, American Institute for Medical and Biological Engineering
- Member, National Research Council BEMA

### Summary of the most important results

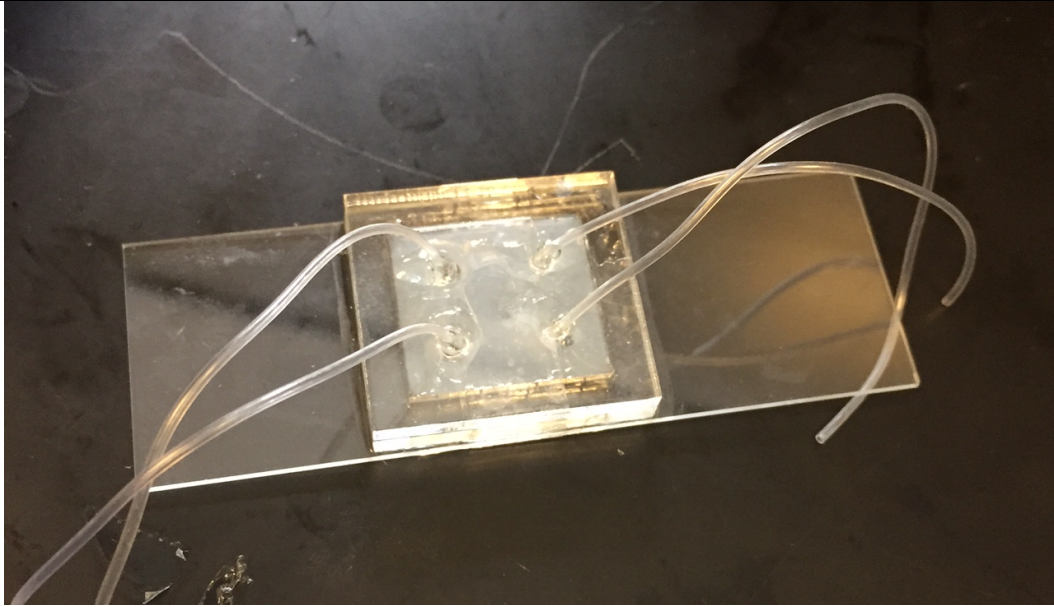




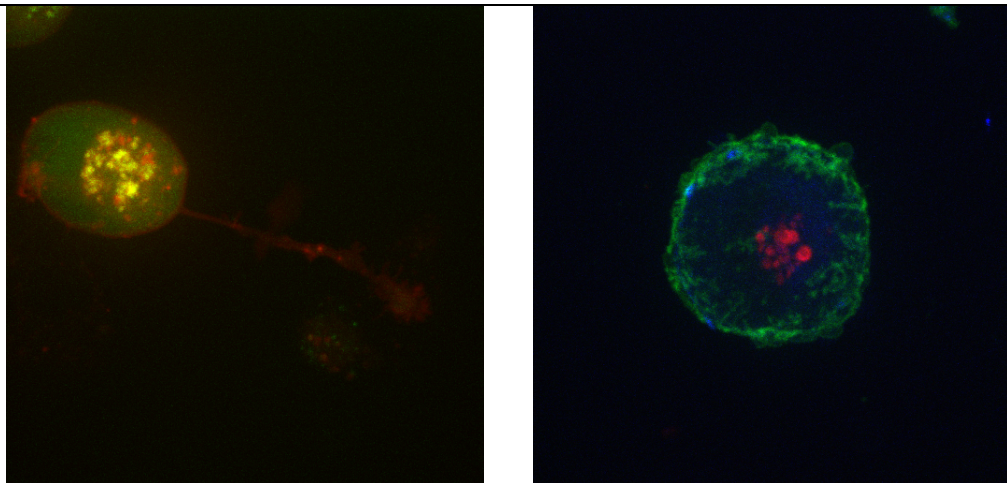
PDMS mold and gelatin template. Two PDMS molds are attached together to form full circular channel, with the inlet/outlet opened. A gelatin sacrificial template molded from the PDMS.



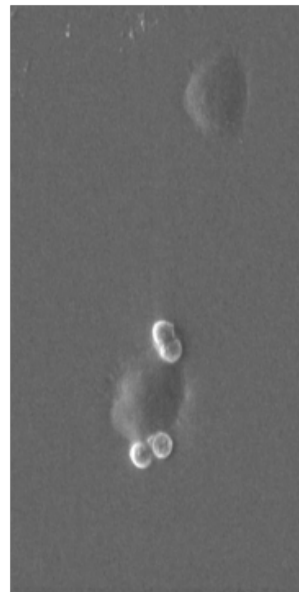
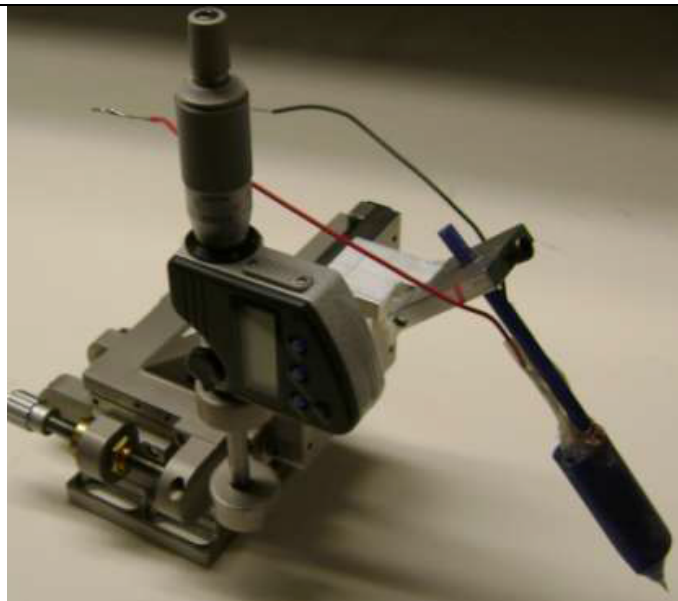
Collagen gel production: (a) gelatin template in our custom fabricated rectangular chamber; (b) chamber filled with collagen solution (c) after incubation in 37°C for 30 minutes, the gelatin template melted and the collagen crosslinked; (d) channel characterization by flowing beads.



Collagen NITC within PMMA custom built housing. The tubing allows controlled additional and removal of liquids.



Neural cells within our NITC system under high-resolution imaging. We are able to resolve cellular and subcellular protein distribution within neural cells growing in the collagen channels.



Our magnetic tweezer system for imposing mechanical force on cells through magnetic beads attached to cells.