NDnano
Center for Nano Science and Technology

NDnano Summer Undergraduate Research 2019 Project Summary

1. Student name & home university:
Adriana Archilla Fraticelli, Syracuse University

2. ND faculty name & department:
Donny Hanjaya-Putra, Aerospace and Mechanical Engineering Department

3. Summer project title:
Controlling Lymphatic Tube Formation Using Synthetic Hydrogels

4. Briefly describe new skills you acquired during your summer research:

During my research this summer, I learned how to synthesize hydrogels, the culture and maintenance of lymphatic endothelial cells and endothelial colony forming cells, RNA isolation techniques, synthesizing cDNA for PCR analysis and how to transfect lymphatic endothelial cells with siRNA. I also learned how to use the ImageJ software to edit fluorescent images and measure fluorescent intensities. Not only did I acquire technical skills this summer, but also effective communication and organizational skills. This experience was a great insight into what graduate school is like and how to prepare for it.

5. Briefly share a practical application/end use of your research:

Currently, there's no cure for diseases that arise from lymphatic system complications, such as lymphedema. Some of the treatment options available are compressive sleeves, diet and exercise, all of which are minimal, short-term solutions. Investigating the molecular controls of lymphangiogenesis and regulating lymphatic endothelial cell (LEC) behavior will allow the synthesis of an implantable gel to promote lymphatic vessel formation in the body as a therapeutic option.

6. 50- to 75-word abstract of your project:

Here, we utilize hyaluronic acid hydrogels to investigate mechanical and biomechanical cues that regulate lymphangiogenesis. Using different substrate stiffnesses as mechanical stimuli and a supplemental growth factor as biochemical stimuli, lymphatic endothelial cell growth and migration is promoted, causing lymphatic vessel formation. Real-Time PCR analysis suggests several genes, which are associated with matrix remodeling and cell migration, are directly linked to these findings and may be the underlying mechanism of improved lymphatic vessel development.

7. References for papers, posters, or presentations of your research:
[1] Alderfer et al., *Journal of Biological Engineering*, 2018.
[2] Vanderhooft et al., *Macromolecular Bioscience*, 2009.
[3] Arnaoutova et al., *Nature Protocols*, 2010.
[4] Cho et al., *Circulation Research*, 2018.
[5] Hanjaya-Putra, et al., *Blood*, 2011

One-page project summary that describes problem, project goal and your activities / results:

Approximately 250 million people worldwide suffer from lymphedema, a life-long disease that causes swelling due to abnormal lymphatic vessel development or disorders. Currently, there is no cure for this disease and only minimal, short-term treatments are available. Lymphangiogenesis, the formation of new lymphatic vessels from pre-existing ones, has been hypothesized to be the first long-term treatment option for lymphedema, which can arise from multiple diseases or cancers. Here, we utilize hyaluronic acid (HA) hydrogels to investigate mechanical and biomechanical cues that regulate lymphangiogenesis. Collectively, investigating the molecular controls of lymphangiogenesis and regulating lymphatic endothelial cell (LEC) behavior will allow us to create an implantable gel to promote lymphatic vessel formation as a therapeutic option.

Lymphatic endothelial cells are mechanically stimulated by seeding them on three different stiffnesses of HA hydrogels. The hydrogel is composed of three factors, thiol modified hyaluronic acid that contains a heparin group, thiol modified gelatin, and Poly (ethylene glycol) diacrylate (PEGDA). The PEGDA serves as the crosslinker that solidifies the hydrogels. The percentage of PEGDA used is directly proportional to the gel stiffness. On this experiment, 2%, 1% and 0.25% PEGDA hydrogels were used. LECs are also biochemically stimulated by supplying them with high, 50ng/mL, and low, 0.50ng/mL, concentrations of vascular endothelial growth factor C (VEGF-C), a supplemental growth factor. The best vessel formation has been found to occur in the softest substrate at the highest concentration of VEGF-C supplied (Figure 1).

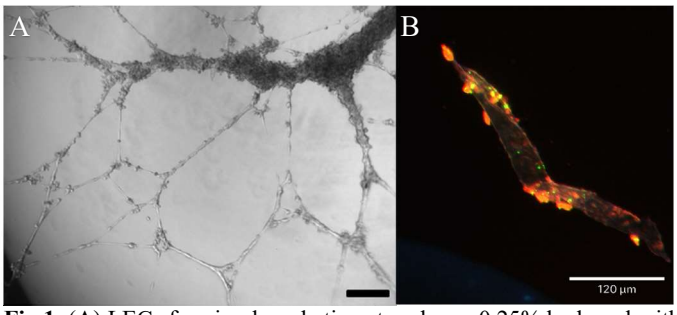

Fig 1. (**A**) LECs forming lymphatic networks on 0.25% hydrogel with 50ng/mL of VEGF-C supplemented media. Cells are seeded at 150,000 cells/mL [3] and network formation is observed 12-16 hours after seeding. Scale bar is 250µm. (**B**) Immunofluorescently stained LECs to label nuclei (DAPI), YAP (green) and TAZ (red) on 0.25% hydrogel with 0.50ng/mL of VEGF-C supplemented media. Scale bar is 120 µm.

The impact gene expression has on vessel formation is also being investigated. Real-Time PCR analysis suggests several genes, associated with matrix remodeling and cell migration, may be the underlying mechanism of improved lymphatic vessel development. Flt4, a receptor on the cell surface for VEGF-C, and MMP14, an enzyme on the cell membrane, seem to express more on the softest gel at the highest concentration of VEGF-C supplied, which are the conditions for best vessel formation. To observe what impact MMP14 has on vessel formation, I silenced its expression by transfecting LECs with MMP14 siRNA. MMP14 activates proteins MMP1 and MMP2 that are responsible for degrading and remodeling the extracellular matrix. Cells migrate to these sites and lymphatic vessel formation commences. We hypothesize that silencing this gene will negatively impact the formation of lymphatic vessels since there will be less MMPs degrading the extracellular matrix and thus less migration sites for cells will be present. Future steps include seeding the transfected LECs on gels and quantifying vessel formation, and quantifying total and activated Flt4 signaling using an ELISA analysis.

YAP and TAZ are two other genes being investigated. These are mechanoreceptors that help regulate growth by signaling the cells when to start or stop growing. They are being investigated because they have an important role in the formation of blood vessels, so we hypothesize they may be important for the formation of lymphatic vessels. I have been immunofluorescently staining LECs for YAP and TAZ to localize their expression in a single cell using ImageJ (Figure 1). Future steps include normalizing the percent localization of YAP and TAZ to the number of cells per image. Localizing and quantifying their expression will hopefully further our understanding of their role in lymphatic vessel formation.