NDnano
Notre Dame Nanoscience and Technology

NDnano Undergraduate Research
2020-21 Winter Session Project Summary

1. Student name: **Erin Neu**

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

3. Winter Session project title:
Coupled Cellular Polarization of Vascular Morphogenesis Patterns

4. Briefly describe new skills you acquired during your Winter Session research:
- **Analyzed Matrigel assays using Kinetic Analysis of Vasculogenesis (KAV), which analyzes vascular formation of different endothelial cell lines**
- **Further developed ability to passage endothelial cells**
- **Seeded various endothelial cell lines and expanded the cell lines in order to collect exosome-rich supernatant**
- **Filtered supernatant prior ultracentrifugation to pellet the exosomes to run Nanosight and quantify the concentration and size distribution of the exosomes**

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

Endothelial colony-forming cell (ECFC) are transfected with preeclampsia colony forming cell (PREC) exosomes, causing preeclampsia (PE) like tube formations. ECFC transfection with PREC exosomes can illustrate the impacts of preeclampsia on angiogenesis in comparison to typical angiogenesis with typical ECFCs. PE typically causes poor tube formation, lending to less angiogenesis during human development and lung defects in infants. PRECs were also transfected with ECFC exosomes in an attempt to revert tube formations to their standard state. If transfecting PRECs with ECFC exosomes results in reverting tube formations, exposing PRECs to ECFC exosomes could be used as a potential treatment for preeclampsia.

6. 50- to 75-word abstract of your project:
A fundamental question in developmental biology concerns how groups of interacting molecules and cells give rise to patterned 3D tissues with special form and function. In particular, we are interested in the process by which endothelial colony-forming cells (ECFCs) have different vascular morphogenesis patterns. The patterns of different ECFC types can be influenced by exosomes transferred between normal and disease phenotypes (e.g., preeclampsia). The impact of vascular morphogenesis by exosomes illustrates preeclampsia's potential causes and impact on angiogenesis and a potential treatment that reverts preeclampsia tube formation to original, typical tube formation.

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

Human endothelial colony forming cells (ECFCs) are a subset of endothelial progenitor cells that originate from tissue-resident vascular progenitors. ECFCs aid in the regeneration of blood vessels and potentially hold a reparative role in vascular healing.[1] ECFC-derived colonies result from a primary culture of umbilical cord monocular cells. These individual colonies are clonally isolated and grown as primary ECFC colonies.[2] Stem cell lines came from patients with both uncomplicated pregnancies and preeclampsia (PE) pregnancies—high blood pressure that reduces fetal blood supply.[3] Preeclampsia ECFCs—known as PRECs—have different vascular tube formation in comparison to ECFCs. PRECs have poor tube formation than ECFCs, causing less angiogenesis and lung defects in infants. Vascular tube formation for PRECs and ECFCs can also be influenced by exosome transfer between stem cells. By studying transfection of PRECs and ECFCs with exosomes, preeclampsia's impact on angiogenesis can be better understood while a potential treatment for that reverts poor preeclampsia tube formation to original, typical tube formation and preeclampsia's potential causes can be illuminated.

ECFC and PREC cell lines were seeded on flasks and expanded via passaging to generate an abundance of exosome-rich supernatant. Supernatant was collected at a minimum of 80% cell confluency prior to be filtered, ultracentrifuged to pellet the exosomes, and quantified using Nanosight. The exosomes were pelleted further using the ultracentrifuge before being used in Matrigel assays. Two experiments were conducted using Matrigel to study vascular tube formation following transfection. In the first experiment, ECFCs from cell line 157 were transfected with PREC exosomes from cell line 370. The ECFCs were exposed to varying quantities of PREC exosomes—10^6, 10^7, and 10^8—in sets of five Matrigel assays. Another set of ECFC and PREC exosome Matrigel assays were exposed to Dharmafect, which promotes transfection and acted as a negative control. Lastly, two sets of ECFCs acted as controls—a set of ECFCs exposed to 10^8 ECFC exosomes and a set of untreated ECFCs. In the second experiment, PRECs from cell line 370 were transfected with ECFC exosomes from cell line 150. Similar to the first experiment, the PRECs were exposed to different quantities of ECFC exosomes in sets of five Matrigel assays—also 10^6, 10^7, and 10^8. Another set of PREC and ECFC exosome Matrigel assays were exposed to Dharmafect to act as a negative control while another two sets acted as controls—a set of PRECs exposed to 10^8 PREC exosomes and a set of untreated PRECs.

After approximately 10 hours or more, images of the gels were taken in order to analyze the vascular network using Kinetic Analysis of Vasculogenesis (KAV). KAV forms a mask of the mask and then a skeleton of vascular network from the mask, as seen in Figures 1 and 2. KAV then analyzes the skeleton for total branched networks, nodes, triples, quadruples, tubes, total tube length, average tube length, tubes/nodes ratio, closed networks, and network area.

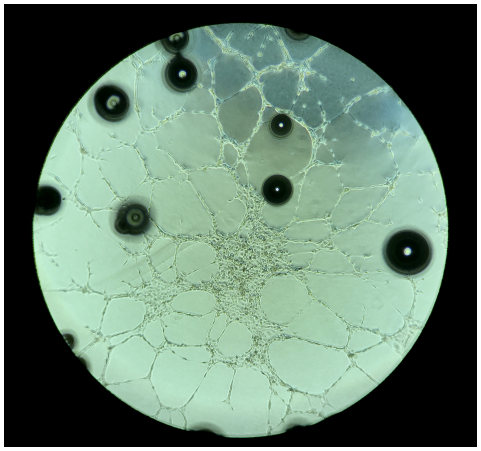

Figure 1. PRECs transfected with ECFC exosomes and exposed to Dharmafect.

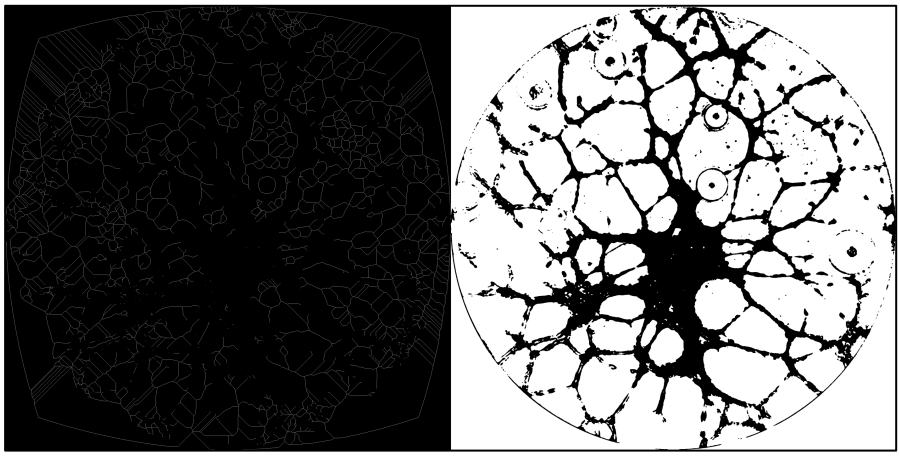

Figure 2. Mask (left image) and skeleton (right image) of Figure 1.

Unfortunately, KAV occasionally has issues in mask formation and thus, in turn, skeleton formation and analysis, which can lend to outliers. To lessen the impact of these outliers, the median rather than the mean of the number of tubes formed in each vascular network. However, the results were still fairly inconclusive and point to the need for more transfection experiments to verify the impact of exosome transfection on vascular tube formation. For the first experiment, the exposure of the ECFC 157 cells to 10^8 PREC 370 exosomes resulted in a decrease in the average number of tubes formed compared to the exposure of the ECFC 157 cells to 10^8 ECFC 157 exosomes, going from 3809 tubes to 3471 tubes. The negative Dharmafect control closely reflected this trend with an average number of 3604 tubes. However, when exposing the ECFCs to 10^6 and 10^7, the results were vastly different and contributed heavily to inconsistencies in the data. The average number of tubes formed decreased from 10^6 PREC exosomes to 10^7 PREC exosomes, going from 2677 to 2279 tubes, respectively. However, the average number of tubes formed increased tremendously to 3471 upon exposure to 10^8 PREC exosomes. Moreover, the negative Dharmafect control had more average tubes than the untreated control, with 3604 compared to 2389.5 respectively, despite PE's negative effect on angiogenesis and tube formation. KAV inaccuracies may have contributed to this data, but errors in the Matrigel assay may have also contributed. There may also other unknown causes to these inconsistencies involving the cells and the exosomes themselves.

For the second experiment, the exposure of the PREC 370 to 10^8 ECFC 150 exosomes resulted in an increase in the average number of tubes formed in the network compared to the 10^8 PREC exosome control exposure. The exposure of 10^8 ECFC 150 exosomes resulted in the formation of an average of 2458.5 tubes, whereas the exposure of 10^8 PREC 370 exosomes resulted in the formation of an average of 2049 tubes. The negative Dharmfect control matched this trend with an average formation of 2554 tubes. Similar to the first experiment, exposure of the PREC 370 cells to 10^6 and 10^7 ECFC 150 exosomes resulted in data inconsistencies. Exposing the PREC 370 cells to 10^6 ECFC 150 exosomes resulted in an average formation of 2553 tubes. Exposing the PREC 370 cells to 10^7 ECFC 150 exosomes resulted in a decrease in the average number of tubes at 2120 tubes. The average number of tubes increased at an exposure of 10^8 ECFC exosomes to 2458.5 tubes. Moreover, despite PE's negative impact on angiogenesis and tube formation, the untreated control has the largest average number of tubes formed at 2785. The inaccuracies in this experiment were most likely very similar to the first experiment. To clarify,

a number of issues KAV has in analysis includes forming a mask and skeleton of the background, which then leads to inaccurate values as KAV is recognizing nonexistent vascular networks or vascular networks not in main focus. KAV is also impacted by the presence of bubbles, as the bubbles are present in the mask and in turn acknowledged in the skeleton as networks, leading to inaccurate values. This is best illustrated in Figure 3.

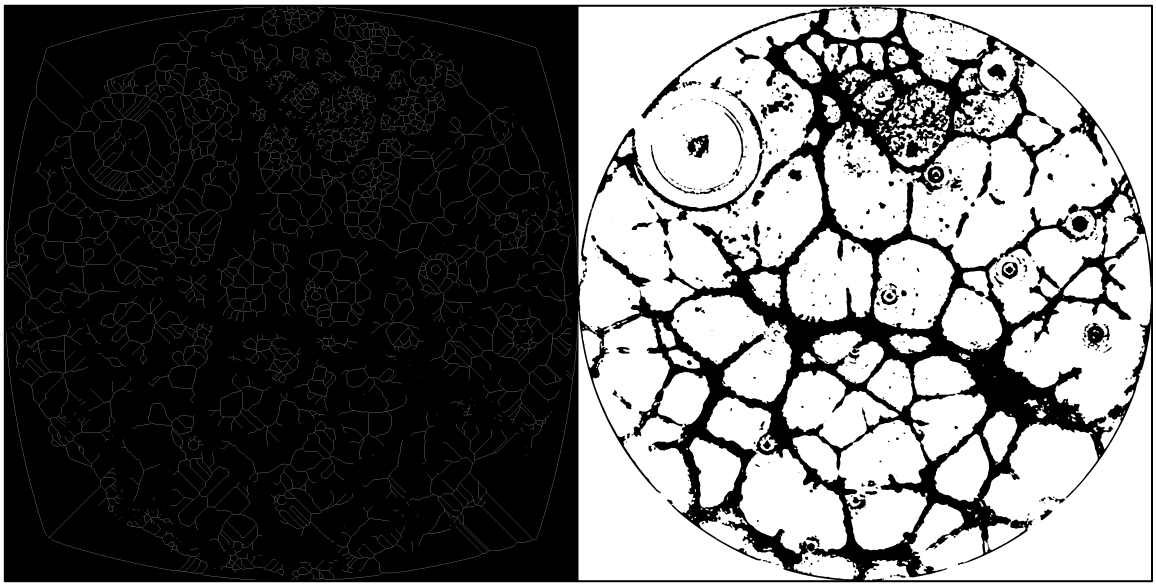

Figure 3. PREC 370 transfection with ECFC 150 exosomes and exposure to Dharmafect. The large bubble in the top left of the mask is registered as a part of the vascular network in the skeleton in the top left of the mask.

Although firm conclusions cannot be drawn from the results currently, the techniques and outcomes of this project can be used to more fully develop the study of vascular morphogenesis of endothelial colony forming cells and the impact of exosome transfection of vascular tube formation in both ECFCs and PRECs. With further development of the project and more experiments, it is possible that more conclusive results will emerge regarding PE's impact on angiogenesis and tube formation and the potential impact ECFC exosomes have on PE tube formation.

References

[1] O'Neill, C.L., McLoughlin, K.J., Chambers, S.E.J., Guduric-Fuchs, J., Stitt, A.W., & Medina, R.J. (2018). The Vasoreparative Potential of Endothelial Colony Forming Cells: A Journey Through Pre-clinical Studies. *Frontiers in Medicine, 5*(273). https://doi.org/10.3389/fmed.2018.00273.

[2] Mead, L.E., Prater, D., Yoder, M.C., & Ingram, D.A. (2008). Isolation and Characterization of Endothelial Progenitor Cells from Human Blood. *Current Protocols in Stem Cell Biology, 6*(1), 2C1.1-2C.1.27. https://doi.org/10.1002/9780470151808.sc02c01s6.

[3] Eunice Kennedy Shriver National Institute of Child Health and Human Development (2017, January 31). *Preeclampsia and Eclampsia.* National Institute of Health. https://www.nichd.nih.gov/health/topics/preeclampsia.