

**NDnano Summer Undergraduate Research
2025 Project Summary**

1. Student name & home university: Kaitlin Ryan, University of Notre Dame

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

3. Summer project title: Hypoxic Stress Induces Angiogenic Dysfunction in Endothelial Colony-Forming Cells

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

This summer, I was excited to be able to work more independently, doing assays like tube formation, sEV extraction, and more. I also developed skills with staining/ImageJ and using R for analyzing/plotting data files, as well as running statistical testing.

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

The main goal of the summer segment of this research project was to isolate the impact of hypoxia on the angiogenic function of endothelial colony-forming cells (ECFCs), and my further extension of this throughout the academic year will explore genetic content, as well as investigate the therapeutic potential of small extracellular vesicles (sEVs). The downstream use of this research would be to have better, more targeted treatments for preeclampsia. By isolating the root cause of preeclampsia (or at least narrowing down the list), I hope this can be achieved by researchers in this field in the coming years.

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

Preeclampsia, a hypertensive disorder, is the most common cause of maternal and fetal mortality out of all pregnancy-related complications.¹ The cause of preeclampsia is not well-understood, and the only cure is delivery.^{7,8} This study isolates the impact of hypoxia on the angiogenic function of endothelial colony-forming cells (ECFCs). Hypoxic stress resulted in poorer tube formation compared to the ECFC control, mimicking what is observed in preeclamptic ECFCs (PRECs). Such results are consistent with our proposed mechanism in which hypoxia is a strong inducer of angiogenic dysfunction.

7. References for papers, posters, or presentations of your research:

- (1) Karrar, S.; Hong, P. L.; Martingano, D. J. *Preeclampsia*. PubMed. <https://www.ncbi.nlm.nih.gov/books/NBK570611/>.
- (2) Matsubara, K. Hypoxia in the Pathogenesis of Preeclampsia. *Hypertension Research in Pregnancy* **2017**, 5 (2), 46–51. <https://doi.org/10.14390/jsshp.hrp2017-014>.
- (3) Chaiworapongsa, T.; Chaemsathong, P.; Yeo, L.; Romero, R. Pre-Eclampsia Part 1: Current Understanding of Its Pathophysiology. *Nature Reviews Nephrology* **2014**, 10 (8), 466–480. <https://doi.org/10.1038/nrneph.2014.102>.
- (4) Tal, R. The Role of Hypoxia and Hypoxia-Inducible Factor-1Alpha in Preeclampsia Pathogenesis. *Biology of Reproduction* **2012**, 87 (6). <https://doi.org/10.1095/biolreprod.112.102723>.
- (5) Varberg, K. M.; Winfree, S.; Dunn, K. W.; Haneline, L. S. Kinetic Analysis of Vasculogenesis Quantifies Dynamics of Vasculogenesis and Angiogenesis in Vitro. *Journal of Visualized Experiments* **2018**, No. 131. <https://doi.org/10.3791/57044>.
- (6) Parashar, D.; Mukherjee, T.; Gupta, S.; Kumar, U.; Das, K. MicroRNAs in Extracellular Vesicles: A Potential Role in Cancer Progression. *Cellular Signalling* **2024**, 121, 111263–111263. <https://doi.org/10.1016/j.cellsig.2024.111263>.
- (7) Mayo Clinic. *Preeclampsia - Diagnosis and Treatment*. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/preeclampsia/diagnosis-treatment/drc-20355751>.
- (8) Schlembach, D. Pre-Eclampsia--Still a Disease of Theories. *Fukushima Journal of Medical Science* **2003**, 49 (2), 69–115. <https://doi.org/10.5387/fms.49.69>.

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

Preeclampsia, a hypertensive disorder that can occur with pregnancy, is the most common cause of maternal and fetal mortality out of all pregnancy-related complications, impacting around 5 % of pregnancies and claiming the lives of over 50,000 mothers and 500,000 babies around the world each year.¹ The issue is that the causes of preeclampsia are complex and not fully understood, but one known cause of worsening preeclampsia is hypoxia (insufficient oxygen).²

This project, which will remain ongoing into the academic year, focuses on endothelial colony-forming cells (ECFCs), as well as their diseased counterparts, the preeclamptic endothelial colony-forming cells (PRECs). ECFCs are critical for vessel formation and placental development but known to be functionally compromised in preeclampsia.³ The goal of this project is to isolate the impact of hypoxia on the angiogenic function of ECFCs.

First, the extent in which hypoxic stress can induce angiogenic dysfunction (which is observed in PRECs) in ECFCs was investigated. There were 3 groups of cells cultured and tested: ECFC (control), PREC, and hypoxic ECFC. The hypoxic ECFCs were placed in a hypoxic incubator for 24 hours, while the other 2 groups were placed in a normoxic incubator before a tube formation assay was conducted as a measure of angiogenic (vessel forming) potential. The images were processed with Labkit and quantified with Kinetic Analysis of Vasculogenesis in ImageJ.⁵

Based on the results, hypoxic stress resulted in poorer tube formation compared to the ECFC control group, mimicking what is observed in PRECs, and such results are consistent with our proposed mechanism in which hypoxia is a strong inducer of angiogenic dysfunction.

The next steps, which are in progress, include an interrogation of genetic content. First, small extracellular vesicles (sEVs) were extracted from cell conditioned media of each of the samples and RNA will be isolated in the coming weeks. The specific point of interest is the miRNA, which plays a large role in controlling gene expression. Specifically, miRNA contained within sEVs is crucial, as sEVs play a role in communication between cells, make miRNA degradation slower (providing a longer period of time from which the sample was taken to when it needs to be analyzed), and can be easily extracted from biological fluids for future applications within the medical field.⁶ This leads to the next goal of determining if miRNA content in ECFC sEVs becomes altered when put in hypoxic stress, going beyond just a functional analysis of the impacts of hypoxia on ECFCs.

Next, an investigation into the therapeutic potential of sEVs comes into play, as there is possibility of healthy ECFC sEVs being able to partially restore functionality in the diseased cells. Unleashing the potential of sEVs as a mode of therapy for conditions like preeclampsia could be magnitudinous in a field where treatments are presently to reduce symptoms rather than the disease itself.⁷ While clinical applications of this are likely still a long ways away, progress in this field is critical, as the stresses and potential heartbreak families impacted by preeclampsia must go through should be of the utmost importance in research.