NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2024 Project Summary

1. Student name & home university:
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2. ND faculty name & department:
Dr. Donny Hanjaya-Putra

3. Summer project title:
Enhancing Endothelial Colony Cell Targeting and Evaluating their Efficiency in Microfluidic Models for Tissue Regeneration

4. Briefly describe new skills you acquired during your summer research:
I had no research experience before starting this project this summer. Due to this, everything I did in the lab was a new skill that I acquired. I learned how to upkeep a cell culture and grow various kinds of cells and the whole process that goes along with that. I also learned how to assemble lipid nanoparticles which was very interesting. The flow experiments I performed were also new to me as well. After this summer I really have a new understanding of what all goes into a research project and I have a better understanding of what grad school will look like in the future.

5. Briefly share a practical application/end use of your research:
The research I worked with this summer centered around Human Renal Proximal Tubular Epithelial cells (HRPTEpCs), Endothelial Colony Forming cells (ECFCs), and Lipid Nanoparticles (LNPs). Using these materials, we were aiming to find the most effective method for cellular interaction between HRPTEpCs and ECFCs and cellular delivery utilizing the LNPs. This method will hopefully be used to treat acute kidney injury (AKI) or other kidney diseases through the cellular regrowth characteristics of ECFCs and the cellular transport ability of the LNPs [5].

6. 50- to 75-word abstract of your project:
Acute kidney injury is a condition that can lead to kidney failure, or in extreme cases, death. Our project aimed to utilize endothelial colony forming cells (ECFCs) to assist in the cellular growth and repair of kidney cells utilizing human renal proximal tubular epithelial cells (HRPTEpCs). These ECFCs are paired with lipid nanoparticles (LNP) along with kidney targeting peptides (KTP) and PEG in order to amplify cellular targeting of ECFCs to HRPTEpCs.

7. References for papers, posters, or presentations of your research:
[1] Bui, L., Edwards, S., Hall, E. *et al.* Engineering bioactive nanoparticles to rejuvenate vascular
[2] Stephan, M., Moon, J., Um, S. *et al.* Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nat Med* **16**, 1035–1041 (2010). https://doi.org/10.1038/nm.2198
[3] Bidwell GL 3rd, Mahdi F, Shao Q, Logue OC, Waller JP, Reese C, Chade AR. A kidney-selective biopolymer for targeted drug delivery. Am J Physiol Renal Physiol. 2017 Jan 1;312(1):F54-F64. doi: 10.1152/ajprenal.00143.2016. Epub 2016 Oct 26. PMID: 27784692; PMCID: PMC5283886. progenitor cells. *Commun Biol* **5**, 635 (2022). https://doi.org/10.1038/s42003-022-03578-4
[4] Feng, F., Zhang, R., & Long, L. (2024). LncRNA GABPB1-IT1 Is Upregulated in Ischemia-Induced

[5] Acute Kidney Injury and Downregulates miR-204-5p to Promote Hypoxia-Induced Human Renal Proximal Tubular Epithelial Cell Apoptosis. *Kidney & blood pressure research*, *49*(1), 480–489. https://doi.org/10.1159/000539342

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

This summer, the research I took part in aimed to utilize microfluidic models for tissue regeneration. Endothelial colony forming cells (ECFCs) are progenitor cells that are capable of assisting in cellular growth, repair of damaged areas, and tissue regeneration [1,2]. While these cells are helpful in the repair of damaged cells, there needs to be an effective method of transport to ensure that these ECFCs reach the cells that need repair. This is where lipid nanoparticles (LNP) come into play. LNPs are helpful in drug delivery and when modified with peptides, they can function as enhancers in cellular transport [1]. In our research, we used kidney cells in our studies. We modified LNPs with a kidney targeting peptide (KTP)[3], and then this system is attached to the surface of the ECFC to enhance its targeting. The aim of this research project is to show that LNPs with KTPs enhance the targeting ability of ECFCs to reach kidney cells to eventually be used in the treatment of acute kidney injury (AKI) or other kidney conditions. Our research aims to treat kidney diseases, such as AKI, by experimenting with and observing human renal proximal tubular epithelial cells (HRPTEpCs). AKI is a kidney condition that can lead to kidney failure and, in extreme cases, death. We utilize HRPTEpCs in our research and experiments since they are taken from proximal tubules in the nephron of kidneys, near the glomerulus. These cells play a big role in absorption in the kidney so when they are injured, the effects can be detrimental to human health [4].

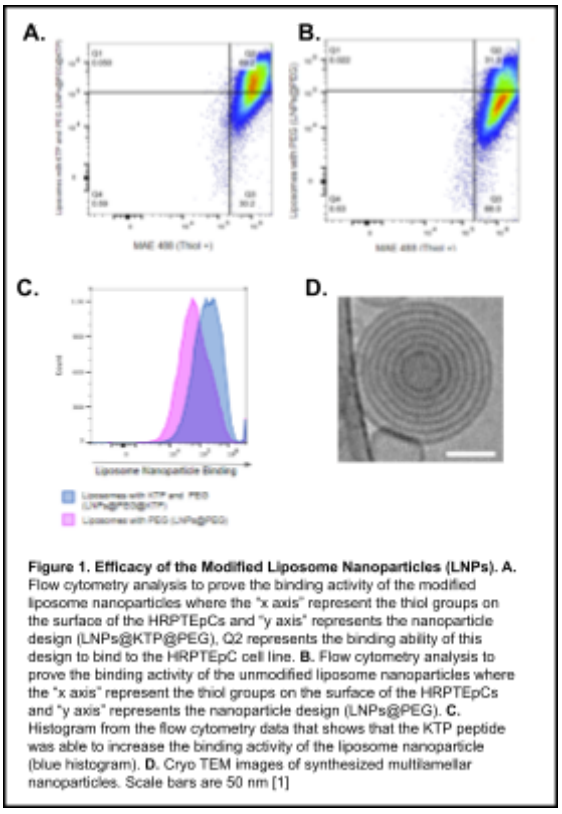

Figure 1. Efficacy of the Modified Liposome Nanoparticles (LNPs). A. Flow cytometry analysis to prove the binding activity of the modified liposome nanoparticles where the "x axis" represent the thiol groups on the surface of the HRPTEpCs and "y axis" represents the nanoparticle design (LNPs@KTP@PEG), Q2 represents the binding ability of this design to bind to the HRPTEpC cell line. **B.** Flow cytometry analysis to prove the binding activity of the unmodified liposome nanoparticles where the "x axis" represent the thiol groups on the surface of the HRPTEpCs and "y axis" represents the nanoparticle design (LNPs@PEG). **C.** Histogram from the flow cytometry data that shows that the KTP peptide was able to increase the binding activity of the liposome nanoparticle (blue histogram). **D.** Cryo TEM images of synthesized multilamellar nanoparticles. Scale bars are 50 nm [1]

To test and analyze the LNPs, we performed flow cytometry analysis where we blocked the thiol groups on the surface of the HRPTEpCs and tested two different LNPs. We used LNPs with PEG as negative control and analyzed the LNPs with KTP and PEG. **Figure 1 C** is a histogram that compares these two LNPs. In quadrant 2 in **figure 1 B** on the graph with LNP@KTP@PEG, we are found an increment on the binding of the liposome nanoparticle that has the KTP (blue histogram in **figure 1 C**). This shows that LNP with KTP have an increase in binding ability when compared to LNP without KTP. This supports our hypothesis that KTP will increase the targeting and binding ability of ECFCs to the HRPTEpCs.

To test this, we ran several flow studies at different conditions to simulate a kidney environment to observe the interactions between ECFCs and HRPTEpCs. We had three different pumps set up which would flow ECFCs in growth media across a chip that had HRPTEpCs seeded onto it. The kidney cells were dyed green and the ECFCs were dyed blue in order to differentiate between the two at the end of the experiment. In each of the different pumps there were ECFCs in different conditions. This varied from experiment to experiment, but we typically had one pump that was flowing plain ECFCs, one that contained ECFCs with blank LNPs and one that had ECFC with LNP@KTP@PEG. What we hoped to see was an increase in cellular interaction between ECFCs and HRPTEpCs across the pumps. Our

hypothesis was that pump 3 would have the highest interaction due to the kidney cell targeting properties of the KTP on the LNPs.

The flow experiments we ran throughout the summer were set at various time lengths and shear stresses. Some ran for one day while others ran for multiple days. While doing our literature review for the flow experiments, we found that there is not one universally agreed upon value of shear stress that is used across literature in the scientific community. Shear stress is a force that is applied to cells, caused by the flow of fluid in the kidneys. During our literature review, we found that the values used ranged from 0.1 to 10 dynes. Due to this, later in the summer in our experiments we tried to vary the shear stress we used to see how this affected cellular interaction. This research is ongoing, and more experiments and analysis are yet to be done. Although our results don't show statistically significant ECFCs sequestration when using the peptide, we do see some interesting interactions between the ECFCs and HRPTEpCs when the KTP is present on the LNPs. Going forward, this experiment can be optimized by changing the ratio of the KTP into the surface of the LNPs, and we are also working on quantifying these results by the proximity of the different cell lines, which will give us a better idea about the interaction between the cells.

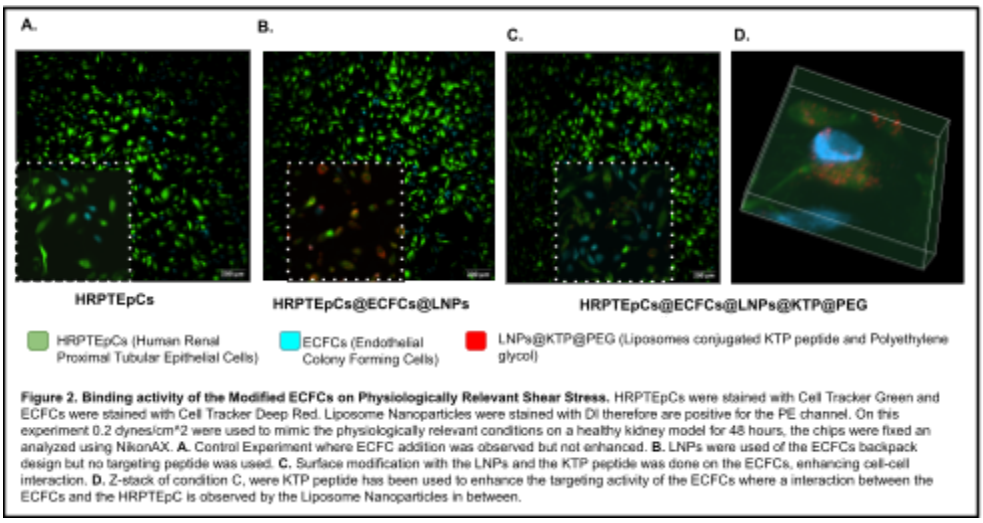

Figure 2. Binding activity of the Modified ECFCs on Physiologically Relevant Shear Stress. HRPTEpCs were stained with Cell Tracker Green and ECFCs were stained with Cell Tracker Deep Red. Liposome Nanoparticles were stained with DI therefore are positive for the PE channel. On this experiment 0.2 dynes/cm^2 were used to mimic the physiologically relevant conditions on a healthy kidney model for 48 hours, the chips were fixed an analyzed using NikonAX. **A.** Control Experiment where ECFC addition was observed but not enhanced. **B.** LNPs were used of the ECFCs backpack design but no targeting peptide was used. **C.** Surface modification with the LNPs and the KTP peptide was done on the ECFCs, enhancing cell-cell interaction. **D.** Z-stack of condition C, were KTP peptide has been used to enhance the targeting activity of the ECFCs where a interaction between the ECFCs and the HRPTEpC is observed by the Liposome Nanoparticles in between.

To quantify our results, we imaged the three chips under a confocal microscope and compared what we saw. We ran into an issue with this step due to the fact that there is not an effective way to count the cells present on the chips. If there was, we could count the number of HRPTEpCs and ECFCs that are on each of the three chips and compare to see if the ratio increased across the various ECFC environments. However, removing the cells from the chips and using the cell counter proved to be difficult as the cells would not detach. We also tried using various computer programs, such as Fiji, where we would upload an image of the chip and let the program quantify the data. However, unfortunately this also proved to be difficult, as there are numerous ways to have the program count the cells, and we got different values for the same chip every time. Nevertheless, we were able to see increased interactions between ECFCs and HRPTEpCs across the chips. **Figure 2** compares the three different systems based on confocal microscopy. **Figure A** is ECFCs with no LNPs, **figure B** is ECFC@LNP, and **figure C** is ECFCs@LNP@KTP@PEG. The 3D rendering in **figure 2 D** showcases up close imaging of the increased cell interaction when KTP is present on the LNPs. As the figure shows, an ECFC is attached to a HRPTEpC which helps demonstrate the potential cellular regeneration characteristics of the ECFCs, which increases when KTP is present.

Moving forward, the next steps for this project are to continue flow studies with various ECFC environments, while working to find a way to effectively count the cells present on the chips. Also, the shear stress studies can also be further pursued to try and identify the ideal shear stress value to use in the flow experiments. Finally, our research this summer focused on in vitro studies. In vivo studies with live animal subjects seem to be the next step to see if this method of treatment is effective and if it could possibly be used to treat human patients one day in the future.