Using Plasmonic Nanobubbles for mRNA Cell Transfection

Undergraduate Research Opportunities Program Final Report
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1. Project Summary

Introduction

mRNA cancer vaccines are an exciting new technology currently being explored. These vaccines involve transfecting specific codes of mRNA into immune system cells such as Natural Killer cells (NK-cells). These mRNA genes code for proteins that allow these cells to recognize and fight the cancer. However, a major challenge in the development of these vaccines is effectively transfecting the mRNA into the immune system cells. mRNA is a large, negatively charged, and unstable molecule. Thus, getting it into the immune system cells represents a significant challenge. Some of the current transfection methods include lipid nanoparticles (LPNs), viral vectors, and cationic liposomes. Each of these methods has its advantages and disadvantages. A new emerging technology that can be paired with liposomes or LPNs is the addition of metal nanoparticles that resonate with near-infrared light to create a plasmonic nanobubble. This temporarily breaks open the cell wall and allows the mRNA to be carried into the cell with high efficiency and uptake. The goal of this project was to test the feasibility of such a system with regard to cellular uptake, cellular expression, cytotoxicity, and long-term cell viability. The transfection system used in this project is a cationic liposome studded with polymer changes linked to hollow gold nanoparticles (HGNs) that resonate with NIR light to create plasmonic nanobubbles (PNBs).

Background and Outcomes

The use of plasmonic nanobubbles for cell transfection first rose to academic awareness in 2012 when researchers from Rice University (Lukianova-Hleb, Mutonga et. al.) released a paper detailing the method. Their paper outlined the potential use of PNBs. They were able to successfully transfect some cells and kill others in the same bulk (see Figure 1).[1]

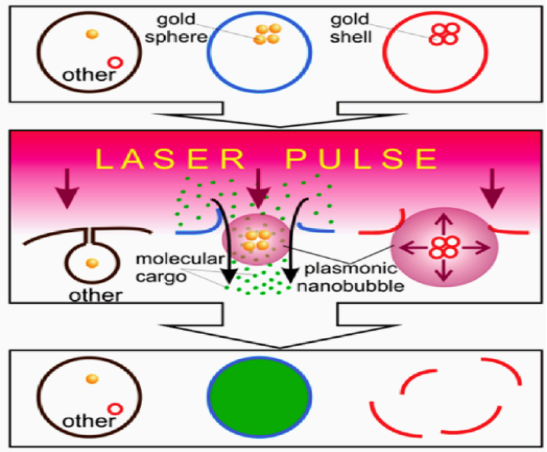

Figure 1: Multi-functional cell-specific processing of heterogeneous cell system with plasmonic nanobubbles (PNBs) that are selectively generated around the clusters of gold spheres in spheres-targeted cells (blue) and around the clusters of gold shells in shells-targeted cells (red) with a single laser pulse, resulting in the simultaneous delivery of molecular cargo into blue cells due to injection of the molecules (green dots) with small PNB and mechanical destruction of red cell with large PNB without the damage to other cells, all realized in a single pulse treatment. (Credit: Lukianova-Hleb, Mutonga et. al.)[1]

Additionally, in 2018 Ogunyankin, Shin, et al. from the University of Minnesota developed a method of synthesizing and optimizing HGNs with a resonance in the near-infrared light wavelength range; a biologically benign light frequency. In 2022, Veeran, Ogunyankin, et al. combined these ideas to create an HGN-studded liposomal transfection system to deliver biological materials to cells. They also better characterized the perforation of the lipid bilayer by the PNBs as seen in figure 2 from their paper (figure 2).[3]

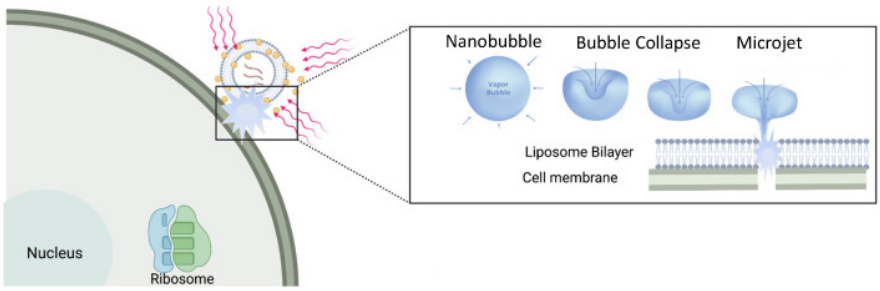

Figure 2: Plasmon-resonant gold nanoparticles tethered to liposomes can be triggered by short pulses of NIR laser light (red lines) that heat the nanoparticles. At a threshold light fluence, heat dissipating from the nanoparticles boils a minute amount of water to form unstable vapor nanobubbles that rapidly expand and contract. As the nanobubbles collapse, liquid–vapor microjets form that can perforate cell and liposome membranes. This allows the liposome contents to be rapidly released and cells to be perforated. (Credit: Veeran, Ogunyankin, et al.)[3]

The goal of this project was to take the systems and ideas previously researched and utilize them for mRNA transfection into human immune cells. The parameters of interest are cell viability and efficacy of transfection. Sufficiently high quantities in both these areas open the possibility of efficiently creating a CAR-NK cell bank for the treatment of different cancers.

Unfortunately, due to several issues with materials, scheduling, and various other research mishaps, no meaningful data on this project was generated over the course of my UROP on this project.

2. Discussion of Objectives

When I submitted my UROP proposal, I was extremely excited to join a research laboratory and make a meaningful contribution. In addition to the goals of the project, I had several goals that I wanted to accomplish throughout this experience. I wanted to gain a familiarity with the academic process and the day-to-day of a research scientist, see firsthand what PhD students do, and most of all learn something cool about the world. Unfortunately, I was not able to meet the objectives of my project. This was due to many factors not the least of which were a few mishaps on my end with messing up methods and getting behind schedule. Perhaps the biggest reason however was that Dr. Veeren, whom I was planning to assist in this project, got contracted to do other research shortly after I submitted my proposal.

This is wonderful for her. However, it meant that she was no longer working on the project for which I submitted my UROP. This left me trying to figure out the majority of the work and details independently. I would get occasional guidance from time to time, but Dr. Veeren was very busy with her other research. Unfortunately, I was not a decidedly competent researcher and made several simple mistakes in formulating the HGNs, liposomes, and various other parts of the project. In the lack of oversight, it took a rather long time to pinpoint exactly what I was doing wrong. Coupled this with some material supply issues that were not caught until the last minute and my project unfortunately never got properly going enough to generate meaningful results. That said, I did accomplish all my personal goals for this project. I learned so much. Not only about the practical aspects of doing research but also extremely exciting science. I met a wonderful group of people in the Zasadsinski lab and am truly glad I had this experience.

3. UROP Reflection

When I submitted my proposal, I had only known what the UROP program was for about two weeks. I just wanted to join a research lab and assist in whatever capacity I could while hopefully learning something interesting. Dr. Veeren and Professor Zasadzinski told me about the program and encouraged me to write a proposal. So, with their help, I wrote a proposal on some research that Dr. Veeren had been working on. I am so glad I did. Even though the project itself did not go as planned, I learned so much. I had the opportunity to go in a consistently work in a research lab, dive into a specific topic, and form professional relationships with researchers and professors. This experience was something that will stick with me for the rest of my life. This is not the end of my research career either. I plan on working/volunteering in the Zasadsinski lab and potentially working on this project more in the future. Regardless of exactly what I end up working on, I am looking forward to continuing to do research. A huge thank you to the Undergraduate Research Office and the University of Minnesota for funding this opportunity and making it possible.

References:

(1) Lukianova-Hleb, E. Y.; Mutonga, M. B. G.; Lapotko, D. O. Cell-Specific Multifunctional Processing of Heterogeneous Cell Systems in a Single Laser Pulse Treatment. *ACS Nano* **2012**, *6* (12), 10973–10981. https://doi.org/10.1021/nn3045243.

(2) Ogunyankin, M. O.; Shin, J. E.; Lapotko, D. O.; Ferry, V. E.; Zasadzinski, J. A. Optimizing the NIR Fluence Threshold for Nanobubble Generation by Controlled Synthesis of 10–40 Nm Hollow Gold Nanoshells. *Adv Funct Materials* **2018**, *28* (10), 1705272. https://doi.org/10.1002/adfm.201705272.

(3) Veeren, A.; Ogunyankin, M. O.; Shin, J. E.; Zasadzinski, J. A. Liposome-Tethered Gold Nanoparticles Triggered by Pulsed NIR Light for Rapid Liposome Contents Release and Endosome Escape. *Pharmaceutics* **2022**, *14* (4), 701. https://doi.org/10.3390/pharmaceutics14040701.