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NDnano Summer Undergraduate Research 2019 Project Summary

1. Student name & home university:
Stephanie Wallace, University of Notre Dame

2. ND faculty name & department:
Prakash Nallathamby, Department of Aerospace and Mechanical Engineering
Paul Helquist, Department of Chemistry and Biochemistry

3. Summer project title:
Magneto-silica nanoparticles (MagSiNs) for combinatorial chemotherapeutics and gene delivery against metastatic cancers

4. Briefly describe new skills you acquired during your summer research:
This summer, I learned how to synthesize stable liposomes as well as synthesize liposomes with an additional fluorescent payload. Previously, I was familiar with running dynamic light scattering (DLS) measurements of nanoparticles size distribution. But this summer, I further expanded my knowledge of running DLS measurements to determine the size and stability of the liposomes I synthesized. Once the liposome protocol was deemed effective, I learned how to conjugate magneto-silica nanoparticles (MagSiNs) to the liposomes. I had already synthesized the MagSiNs in the spring semester of 2019, where I synthesized a cobalt ferrite core and added a silica shell and a rhodamine (RITC fluorophore).This summer I learned how to conjugate the MagSiNs onto the surface of the liposomes using 'CLICK' chemistry. Once this was accomplished, I began cell culture experiments. I learned proper sterile techniques when working in a tissue culture room as well as how to bring up different cell lines from cryo-storage and maintain them over the course of my project duration. I exposed the different cell lines with the different liposomes I synthesized and analyzed my results via a Nikon TE-2000U Epifluorescence Microscope, which I also learned how to operate.

5. Briefly share a practical application/end use of your research:
Current cancer treatments such as chemotherapy remain very toxic and dangerous on the body, reducing the patient's quality of life drastically as the systemic treatment targets all rapidly dividing cancer cells and normal cells indiscriminately. As a result, normal cells are also killed at the expense of targeting cancer cells. Therefore, there is a need for a cancer treatment that selectively targets only cancer cells. In this project, we developed a label free nanocarrier system with a fluorescent payload that mimics selective chemotherapeutics delivery to metastatic cancer cells. Recent research has showed that cancer cells have a more permeable membrane than normal cells. By fine-tuning the magnetic field at the cell membrane, we were able to show that MagSiN conjugation to the liposomes increases the efficiency of payload delivery to cancer cells in the presence of a directing magnetic field and an alternating magnetic (AC) field. This knowledge can further be applied to improve chemotherapeutics delivery by reducing the number of normal cells destroyed in the crossfire of targeting cancer cells during treatment and thus reducing the harsh side effects common chemotherapeutic treatments impose on patients today.

6. 50- to 75-word abstract of your project:
This project seeks to combine the use of magneto-silica nanoparticles (MagSiNs) conjugated liposomes with a fluorescent payload to mimic selective chemotherapeutics delivery to

metastatic cancer cells. Liposomes synthesized with a fluorescent cell-impermeant payload were conjugated with MagSiNs to create a label-free magnetic nanocarrier system. *In vitro* studies were performed to look at the difference in the efficiency and selectivity of payload delivery in different cancer cells with and without a magnetic force applied at the cell membrane.

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

Guduru, R.; Liang, P.; Runowicz, C.; Nair, M.; Atluri, V.; Khizroev, S., "Magneto-electric Nanoparticles to Enable Field-controlled High-Specificity Drug Delivery to Eradicate Ovarian Cancer Cells." Scientific Reports 2013, 3, 2953

Nallathamby, P. D.; Hopf, J.; Irimata, L. E.; McGinnity, T. L.; Roeder, R. K., "Preparation of fluorescent Au-SiO2 core-shell nanoparticles and nanorods with tunable silica shell thickness and surface modification for immunotargeting." Journal of Materials Chemistry B 2016, 4 (32), 5418-5428.

Stephan, M. T., Moon, J. J., Um, S. H., Bershteyn, A., & Irvine, D. J. (2010). Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nature medicine*, *16*(9), 1035.

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

Current systemic cancer treatment such as chemotherapy remains very toxic to the body, killing all indiscriminately rapidly dividing cancer cells as well as normal cells. This lack of selectivity towards targeting only cancer cells leads to numerous harsh side effects of treatment that reduces the quality of lives of the patients that receive it as well as increase post-treatment recovery times. Therefore, there is a need for a chemotherapeutics delivery system that can selectively target only cancer cells. Recent research has discovered that cancer cells have a much more permeable cell membrane than normal cells. This project intends to use this knowledge of cell permeability in order to target cancer cells by fine-tuning a magnetic field induced force exerted by magnetic nanocarriers to selectively permeate cancer cells and not the membranes of the stiffer, healthy cells. Thus, this project combines the use of magneto-silica nanoparticles (MagSiNs) and liposomes with a fluorescent payload to mimic selective chemotherapeutics delivery to different cancer cells.

In this research project, I first synthesized stable liposomes. To prepare for this synthesis, I learned the proper technique of working in a dry box in order to aliquot maleimide (MBP-PE) in an anhydrous environment due to its reactivity with water. MBP-PE is an essential component to the lipid, so it was necessary for this aliquoting to take place prior to liposome synthesis. Once this was completed, I was able to combine all the lipid components, which included DOPC, DOPG, MBP-PE, and Dil, into a small vial before placing it in the transfer chamber of the dry box to vacuum dry the chloroform solvent. After dehydration, the dry lipid component was rehydrated in a water-soluble buffer before being vortexed and extruded. Following extrusion, the liposome sample was split and stored in 4˚C and 37˚C for characterization tests. Stability tests were conducted via DLS and results showed a stable liposome for more than two weeks. Once this protocol was finalized, liposomes with an additional fluorescent payload were synthesized following a similar procedure, the only difference being the dry lipid was rehydrated in a water-soluble buffer with 1μL of green or blue dye added. DLS measurements confirmed that the addition of the fluorescent payload did not destabilize the liposome.

After the liposomes with a fluorescent payload were synthesized, I 'CLICK'ed MagSiNs tagged with a Rhodamine Isothiocyanate (RITC) fluorophore to the surface of the liposomes. I had already synthesized the MagSiNs in the spring semester of 2019. In this process, I synthesized a cobalt ferrite core and added a silica shell to the surface as well as the RITC fluorophore. To check that proper conjugation took place, I added MagSiNs with a Fluorescein isothiocyanate (FITC) fluorophore to the liposome and checked fluorescence under a microscope. MagSiNs with a FITC fluorophore were used in this step in order to distinguish the MagSiNs from the liposomes as the liposomes possess Dil, also a red fluorophore similar to RITC. Fluorescence images showed strong red fluorescence from the liposomes as well as strong green fluorescence from the MagSiNs, thus showing an effective conjugation took place. Stability tests via DLS were run to show a stable liposome conjugation for more than a week.

Now, once all of the liposomes were synthesized, I began cell culture experiments. Using proper cell culture techniques, I brought up three different cancer cell lines: MDAMb231(breast cancer), A2780 (ovarian cancer), and PC3 (prostate cancer). HUVEC vein endothelial cells were also brought up to serve as a non-cancer cell control. I applied five different liposome treatments to the different cell lines with and without a magnetic field applied at the cell membrane. I then imaged the cells under a fluorescent microscope in order to determine the efficiency of fluorescent payload delivery. The cells with a more intense fluorescence suggested that the liposomes with the fluorescent payload were able to pass through the cell membrane and enter into the cell. Therefore, when equal brightness intensity was applied to the different cell lines under the different liposome and magnetic field treatments, the images with a greater sum of fluorescence intensity suggested a more efficient payload delivery to the cells. Results showed

that the conjugation of MagSiNs to the surface of the liposomes increased the efficiency of payload delivery to cancer cells in the presence of a magnetic field.

Fluorescence imaging and data analysis are still being conducted on the PC3, MDAMb231, and HUVEC cell lines. This continued work will then show whether payload delivery is selective to only cancer cells. Future experiments will also be conducted with the addition of PEG-maleimide to the liposome and MagSiN conjugation to see if further selectivity of payload delivery to cancer cells can be obtained.

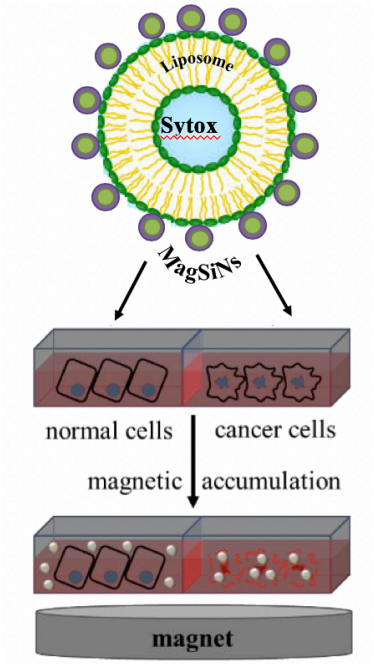

Figure 1. Schematic illustration of the workflow for the addition of liposomes conjugated to MagSiNs (Lipo-MagSiNs), to normal control cells and to cancer cells. Magnetic fields were used to permeabilize Lipo-MagSiNs into cancer cells. An alternating magnetic field was then used to trigger payload release from the liposomes into the cancer cells. Illustration is not to scale.