NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2021 Project Summary

1. Student name & home university:
Argerie Guevara
University of Notre Dame

2. ND faculty name & department:
Prakash Nallathamby
Department of Aerospace and Mechanical Engineering

3. Summer project title:
Optimizing PLGA derived Gel for Steady Release of Phage-Mimicking Antimicrobial Nanoparticles

4. Briefly describe new skills you acquired during your summer research:
I learned how to operate various machinery, including the ultraviolet–visible (UV-Vis) spectrophotometry and the advanced molecular imaging HT instrument that allowed me to analyze and quantify my data. I also learned how to synthesize various precursors.

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

In the future, this project aims to design a phage-mimicking antimicrobial nanoparticle (PhANPs) formulation that will release these nanoparticles from a resorbable gel at a steady rate for prolonged antimicrobial action. By incorporating the PhANPs into a poly(lactic-co-glycolic acid) (PLGA) derived gel, it will be possible to determine the kinetic release of the gel encapsulated PhANPs for a better way to stop the growth of harmful bacteria.

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

This project aims to design a PhANPs formulation that will release the PhANPs from a resorbable gel at a steady rate for prolonged antimicrobial action. By incorporating the PhANPs into a PLGA derived gel, we can determine the kinetics of release of gel encapsulated PhANPs for a better way to stop the growth of harmful bacteria. Through published literature, PLGA nanoparticles were synthesized and incorporated with fluorescein to determine the dissolution rate of PLGA.

7. References for papers, posters, or presentations of your research:
1 J. Hopf, M. Waters, V. Kalwajtys, K. E. Carothers, R. K. Roeder, J. D. Shrout, S. W. Lee and P. D. Nallathamby, Phage-mimicking antibacterial core–shell nanoparticles, Nanoscale Adv., 2019, 1, 4812–4826.
2 H. A. Machado, J. J. Abercrombie, T. You, P. P. DeLuca and K. P. Leung, Release of a Wound-Healing Agent from PLGA Microspheres in a Thermosensitive Gel, BioMed Res. Int., 2013, 2013, e387863.

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

There is a large need for a novel class of anti-infective to counter drug resistant bacterial infections. A possible solution is to mimic antimicrobial viruses (Phages) that have successfully exploited the evolutionarily constant shape and membrane composition of bacteria for millions of years. However, phages' biological origin makes them susceptible to immune reactions from the patient. To counter this problem, the Nallathamby lab has been synthesizing phage-mimicking nanoparticles (PhANPs) made from non-immunogenic components. The antibiotic-free, phage-mimicking nanoparticles demonstrated a > 99.9% bacterial kill rate against four clinically relevant multi-drug resistant infectious bacterial cultures (Staphylococcus aureus USA300, Pseudomonas aeruginosa FRD1, Enterococcus faecalis, and Corynebacterium striatum) in suspension and on implants[1]. Thus, the new class of phage-mimicking core-shell nanoparticles from the Nallathamby lab are modularly assembled with a silica core (of 65 nm or 130 nm) and a discontinuous shell composed of gold-silver nanoalloys in the range of 1.8-3.5 nm.

The goal of this project is to design a PhANPs formulation that will release the PhANPs from a resorbable gel at a steady rate for prolonged antimicrobial activity. This formulation consists of incorporating the PhANPs into a PLGA derived gel. As part of optimization we are determining the kinetics of release of gel encapsulated phage-mimicking antibacterial nanoparticles for a better way to stop the growth of harmful bacteria. By referencing published literature, poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) will be synthesized and later incorporated with fluorescein for release study purposes. This will help determine the dissolution rate of PLGA and maximize the efficiency of payload incorporation into PLGA NPs. We have also synthesized AlexaFluor 750 (AF750) incorporated PhANPs for in vivo tracking of the fluorescent PhANPs release from PLGA gel.

In order to analyze the PhANPs, the transmission electron microscope (TEM) was used. TEM allowed us to visualize the nanoparticles and determine the size of both the gold nanoalloys and the silica core. This equipment also allowed us to characterize and quantify the amount of gold present with the use of the Energy-dispersive X-ray (EDX) spectroscopy. For instance, the TEM images of one of the batches tested was AuSi-AF750 with twice the original amount of gold. This sample demonstrated that the gold nanoalloys are scattered throughout the silica nanoparticles. However, there were some difficulties in quantifying the amount of gold present using the EDX spectra. It is possible that the aggregation of the silica nanoparticles affected the signal for gold.

To analyze the composition of the various samples of PLGA microparticles, the scanning electron microscope (SEM) was used. For the first sample, several incomplete spheres were found. But, in the second and third sample, more uniform and spherical microparticles were observed. A likely reason for the error in the first sample is that the water from the sample did not completely evaporate. Thus, resulting in incomplete spheres.

As part of the release study, seven samples of fluorescein were diluted at different concentrations to obtain a calibration curve for what the required concentration of fluorescein should be. This concentration will help optimize the release study results for the PLGA microparticles that are incorporated with fluorescein. The release kinetics study should help determine the dissolution rate of PLGA. This is carried out in a five day process in which each day, multiple vials of approximately 1.5 mL of 1% w/v of all three samples of PLGA-fluorescein are taken at different time points and stored in an incubator. The absorbance of the contents of these vials are measured using UV-Vis spectroscopy at the maximum wavelength of the fluorescein concentration. The first few time points indicate relatively good absorbance values of PLGA-fluorescein, but as the time increases, there are poor absorbance values from all three samples. To rectify this issue, we may either need to redo the release study analysis or change the concentration used for fluorescein.