NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2022 Project Summary

1. Student name & home university:
   Julia Gattozzi; University of Notre Dame

2. ND faculty name & department:
   Ryan K Roeder – Department of Aerospace and Mechanical Engineering
   Mary Ann McDowell – Department of Biological Sciences

3. Summer project title:
   Nanoparticle Carriers for Novel Anti-Leishmanial Chemotherapeutics

4. Briefly describe new skills you acquired during your summer research:
   In the Roeder lab, I learned how to synthesize gold nanoparticles, functionalize gold nanoparticles, and load functionalized nanoparticles with a chemotherapeutic. I also learned how to design a drug-loaded nanoparticle release and stability study and used ultra-sonication, UV-Vis spectroscopy, and dynamic light scattering techniques to characterize my products throughout the studies.
   In the McDowell lab, I learned how to culture mCherry *Leishmania donovani* and THP-1 line human macrophage cells. I also learned how to complete assays to test the efficacy and cytotoxicity of the chemotherapeutic alone, functionalized nanoparticle alone, and the drug-loaded nanoparticle. I also learned how to complete an *in vitro* infection of *L. donovani* in THP-1 line cells.
   In working on the entire project, the most valuable skill I developed was my ability to communicate my research with others. Throughout the summer, I had numerous opportunities to talk about the work I was doing with other researchers both informally in lab meetings and in more professional settings such as the NURF presentation and Summer Research Symposium poster session.

5. Briefly share a practical application/end use of your research:
   Drug-loaded nanoparticles have been investigated as delivery systems to combat issues in toxicity and bioavailability that often occur with traditional drug formulations. Specifically, in treating leishmaniasis, available treatments are limited by low aqueous solubility and high off target toxicities. Thus, we anticipate that using gold nanoparticles as a delivery system will enable efficient drug delivery while minimizing cytotoxic effects.

6. 50- to 75-word abstract of your project:
   Leishmaniasis is a neglected tropical disease caused by protozoan parasite *leishmania spp*. Gold nanoparticles (AuNPs) have been explored as a drug delivery system to reduce cytotoxicity and improve bioavailability of chemotherapeutics for leishmaniasis. We engineered ~13nm amphiphilic AuNPs and loaded AuNPs with a novel anti-leishmanial chemotherapeutic. Drug-loaded AuNPs were screened for potency against *L.donovani* promastigotes and cytotoxic effects on THP-1 macrophages. The 50% inhibitory concentration was ~5-10μM and drug-loaded AuNPs demonstrated minimal cytotoxicity up to 50μM.

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

[1] CDC, Leishmaniasis, Feb 2020
[2] Dakhel W, Leish in Libya, 2012
[3] Markle WH, Am Fam Physician, 2004;69(6):1455-1460
[4] Thakur L, PLOS Negl Trop Dis, 2018; 12(9):e0006659

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

Leishmaniasis is a neglected tropical disease that is endemic in ~90 countries worldwide and caused by over 20 species of protozoan parasite *leishmania.* This disease is vector-borne and transmitted by a Phlebotomine sandfly. Within the sandfly, *leishmania* exists in a life stage, known as a promastigote. When the sandfly takes a blood meal, it inserts *leishmania* promastigotes into the host where a variety of mononuclear phagocytic cells of the immune system, such as macrophages, engulf the *leishmania* promastigote. Inside the immune cell, the promastigote transitions to another life stage called the amastigote. *Leishmania* amastigotes multiply and infect additional cells to continue the infection. Symptoms of leishmaniasis range depending on the form of the disease. The deadliest form of the disease is visceral leishmaniasis which causes fever, weight loss, severe damage to the liver and spleen, and death if left untreated. *L. donovani* is one of several species of *leishmania* responsible for visceral leishmaniasis and is the species used in this study.

There are some treatments available to treat leishmaniasis, including pentavalent antimonials, liposomal amphotericin B, and miltefosine. However, these treatments are costly, time intensive, inaccessible in endemic regions, and not well tolerated by patients. Additionally, many of these compounds are limited by off target toxicities, low aqueous solubility, and the potential for disease resistance. Previous work in our lab has identified a novel compound, 1,4 diaryl-pyrazolo pyridinone (1,4 DAPP), to be a potent anti-leishmanial compound. One of the main limitations of this drug is its hydrophobic nature which could lower the drug's bioavailability. Thus, the goal of my project was to explore the feasibility in using gold nanoparticles (AuNPs) as a drug delivery system for this novel compound to improve bioavailability and decrease compound toxicity. I hypothesized that an amphiphilic AuNP could effectively encapsulate the hydrophobic drug, enable sustained delivery of the drug, and demonstrate potency against *L. donovani* without off target toxicity.

The first aim of this study was to design an amphiphilic AuNP that can carry a hydrophobic drug such as 1,4 DAPP. First, a 13 nm gold core was synthesized through a sodium citrate reduction and surface functionalized with amphiphilic molecule 11-mercaptoundecanoic acid (11-MUA). The resulting AuNP has a hydrophilic surface with hydrophobic pockets (Figure 1a). To determine the ability to load the amphiphilic AuNP with a hydrophobic compound, a variety of drug loading experiments were completed using 1,4 DAPP and varying concentrations of AuNP, including [mM AuNP]:[mM drug] ratios of 8:1, 4:1, 2:1 and 1:1. UV-visual spectrometry and inductively coupled mass spectrometry were used to determine loading efficiency. Drug loading was efficient with 86% loading efficiency for ratios 8:1 and 4:1 and 79% and 77% for 2:1 and 1:1, respectively.

The second aim of this study was to examine release of the drug from the AuNP delivery system. Drug-loaded AuNPs were suspended in both deionized water and phosphate buffered saline (PBS) adjusted to a pH of 5.6 to mimic conditions within the parasitophorous vacuole of a leishmania-infected phagocytic cell. Drug release in each solvent was measured by UV-visual absorbance of each solvent at wavelength 262 nm at several time points over ~8 days. Drug release was slower in acidic conditions as cumulative drug release reached 50% in acidic solution and 70% in neutral solution.

The next aim was to begin initial screening of the drug-loaded gold nanoparticle system in its potency against *leishmania* promastigotes. mCherry *L. donovani* promastigotes were cultured with decreasing concentrations of 1,4 DAPP alone and drug-loaded AuNPs, starting at 100µM. After 48 hours, a CellTiter-Blue cell viability assay was completed, and the 50% inhibitory concentration (IC50) was determined. 1,4 DAPP loaded onto AuNPs was found to have similar potency to that of 1,4 DAPP alone with both treatments having IC50 values ranging ~5-10 µM (Figure 1b).

The final aim was to screen the drug-loaded nanoparticles for off target toxicity. THP-1 line macrophages were cultured with decreasing concentration of drug-loaded AuNP starting at 100 µM drug.

Cell viability was determined after 48 hours using a CellTiter-Blue cell viability assay. Roughly 80% cell viability was observed with 68 µM drug-loaded AuNP, suggesting minimal AuNP toxicity (Figure 1c).

Going forward, the next phases of this study will be to screen the drug-loaded nanoparticle for potency against *L. donovani* axenic amastigotes and complete an *in vitro* infection with and without drug-loaded nanoparticle treatments. However, based on this preliminary data, drug-loaded nanoparticles demonstrate great promise in treating leishmaniasis.

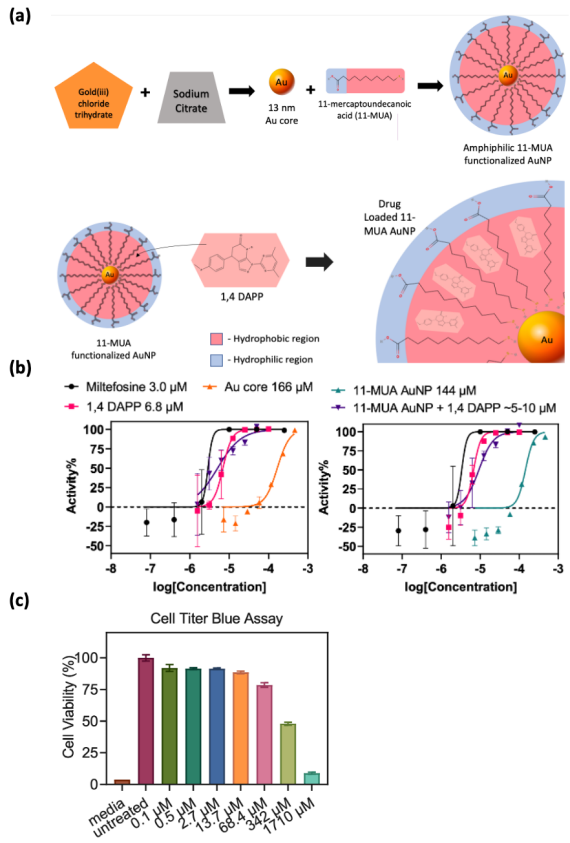

Figure 1: (a) AuNP Synthesis, Functionalization and Drug Loading Scheme (b) 50% Inhibitory Concentration of Drug-Loaded Nanoparticles with *L. donovani* Promastigotes (c) Cytotoxicity of AuNPs Against THP-1 Line Macrophages