NDnano
Center for Nano Science and Technology

NDnano Summer Undergraduate Research 2019 Project Summary

1. Student name & home university: Katherine La Costa, University of Notre Dame

2. ND faculty name & department: Dr. Matthew Webber, Department of Chemical and Biomolecular Engineering

3. Summer project title: Dynamic Peptide-Based Nanomaterials for Enzyme Targeted Drug Delivery

4. Briefly describe new skills you acquired during your summer research:

This summer, I learned chemical synthesis and characterization techniques including solid phase peptide synthesis, the use of high performance liquid chromatography and reverse phase flash chromatography to perform analytical and preparative separations, and electrospray-ionization mass spectrometry to identify compounds in solution and assess purity of material. Additionally, I gained experience on standard laboratory machinery such as the centrifuge, lyophilizer, and rotary evaporator.

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

This research has direct applications as a new way to deliver anti-inflammatory therapeutics to sites of inflammation in the body. It is a component of a larger project design in which the fiber-forming peptide and drug conjugate will be attached to a short enzyme-specific peptide sequence, which in turn is attached to a large, bulky polyethylene glycol (PEG). The PEG promotes the molecules to form micellar nanoparticles in aqueous solution, a nanostructure which is maintained until the particles come into contact with the enzyme, which will remove the second peptide sequence and PEG group, freeing the fiber-forming peptide, and allowing it to create a nanostructure around the diseased site. At this point, the drug will be slowly hydrolyzed from the peptide-linker and delivered directly to the diseased cells for treatment. We hope that this technology can be used to make treatment more effective and minimize side effects associated with traditional corticosteroids.

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

Drug delivery systems control the spatiotemporal characteristics of drugs in the body, increasing drug efficacy while decreasing adverse effects on healthy cells. This project focuses on the synthesis and characterization of anti-inflammatory drug corticosteroid Dexamethasone conjugated via a hydrolysable hydrazone linker to short amphiphilic peptides, designed to form nanofibers by supramolecular self-assembly and provide drug release over time by the slow hydrolysis of the linker. Future work includes modifications to the peptide sequence to incorporate enzyme sensitivity to target inflammation.

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

1. Webber, M. J., et.al. *Nature Materials*, 15, 13 (2015).
2. Cui, H., et.al. *Biopolymers*, 94: 1-18 (2010).
3. Lin Lock, L., et.al. *ACS Nano*, 11(1), 797–805 (2017).
4. Ortony, J. H., et.al. *Nature Materials*, 13, 812 (2014).
5. Kalafatovic, D., et.al. *Biomaterials*, 98, 192–202 (2016).
6. Kramer, et.al., *TIPS,* Vol. 38, No. 10 (2017).

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

This project aims to create a new supramolecular self-assembling nanomaterial which may have significant applications as a component of a targeted drug delivery system. Drug-conjugated materials which self-assemble into nanostructures are desirable components of a drug delivery system because they accumulate at the diseased site, allowing for high drug concentrations as well as long-term drug release. Additionally, nanostructures which macroscopically form hydrogels have significant therapeutic benefits such as site-injectability and controllable release kinetics. Peptides, especially the peptide-drug conjugate which is used in this project, are useful building blocks for these nanomaterials due to their innate biological composition, highly defined chemical structure, and ability to form tunable, self-assembled nanostructures. A hydrazone linker was used to connect the drug, dexamethasone (DEX), to the peptide because of its established controlled hydrolysis, which accelerates at s acidic conditions (pH 5).

Preliminarily, we expected our peptide drug conjugates would form its self-assembled nanostructure in the presence of diseased cells. The peptide sequence mimics a well-known nanofiber forming material due to the hydrophobic and hydrophilic driving forces, as well as beta-sheet-like hydrogen bonding along the axis of the fiber. The addition of the drug will further contribute to the self-assembly driving forces due to its hydrophobicity and ability to pi stack. Once the fibrous nanostructure has been formed around the diseased site, slow hydrolysis of the hydrazone linker will release the drug directly to diseased cells.

The goals for the summer research project focused on synthesizing, characterizing, and purifying the peptide drug conjugates, as well as initial studies into the ability of the conjugates to form nanostructured hydrogels. We synthesized the monomer species by creating the peptide sequence using solid phase peptide synthesis, then performed an additional amide coupling reaction to add the hydrazone linker, which had been previously synthesized in the Webber Lab. Once the product was purified by reverse phase flash chromatography, dexamethasone was then conjugated to the peptide-linker, in a reaction that took about a week. The progress of this reaction was monitored by H1-NMR at various points in the reaction, and by comparing the integration of proton peaks in the free and conjugated DEX, we determined that the progress of the reaction began to level out around five days. The purity of the compound was verified by high performance liquid chromatography and mass spectrometry, which validated that there were no significant contaminants remaining in our material. From this evidence, we concluded that our purification methods were sufficient to achieve a clean product, and we will continue to use these conditions as we make more batches of our material.

Once the material was synthesized, we created samples at concentrations of 1%, 2%, and 3% by weight of the biomaterial in 0.5X PBS, and placed them in a 60°C water bath for one hour. At 1%, the sample remains a solution, however at 2% and 3%, samples form opaque hydrogels. This gelation is macroscopic evidence of self-assembly on the nanoscale. As a baseline study to verify that self-assembly will occur with our peptide-drug conjugate, this test was very successful, and has given us information about how we can conduct further studies of the fiber-forming kinetics and structural properties of the material. Additionally, a drug release study has been started, in which we keep the material in deuterated water, and analyze the NMR at various points over time to observe the shift of the spectrum of DEX-conjugated peptide to the spectrum of an unconjugated peptide and DEX in solution. There is no conclusive data from this test thus far. Kinetics of self-assembly and drug release studies will be continued as a way to characterize our material and evaluate its therapeutic value, as well as other methods of material characterization, such as TEM.

As a long-term goal for this project, we would like to incorporate the fiber-forming biomaterial into a larger drug delivery system. To do this, we would attach an enzyme specific peptide to the amino groups on the lysine amino acids, which would then be PEG-ylated. The polyethylene glycol group (PEG) is

hydrophilic and bulky, which will drive the assembly of micelles in aqueous solution. An enzyme present at the diseased site will cleave the targeting enzyme from the fiber-forming peptide-drug conjugate, leaving only the fiber-forming material to undergo self-assembly at the disease site and release the drug over time directly to the diseased cells. The full micelle-to-fiber transformation is shown below.