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I could not believe how fast the year went by when I realized it was time for me to move in to Irving College at Stony Brook University. It seemed like last week when I went in the lab and sat down with my mentor, Mr. Sayan Mullick Chowdhury, to discuss experiments for rest of the week. Reminiscing about the memories I made with amazing friends I had made last summer, I picked up my key and found my room. Different from last year, I had a roommate, who was also an independent researcher. Having a roommate this summer before college, I thought, was a perfect timing because I wanted to experience it. We introduced ourselves and went off to breakfast.

Starting from this summer, the dorm established a strict policy to escort students from Irving College to the Student Activities Center (SAC) for breakfast runs and back from the SAC for dinner runs. Though there were set times for escorts and students felt the loss of flexibility in lab time, I had no problem working in the lab until late at night since Sayan supervised me. When I came back from the lab on the first day, I was happy to reunite with friends who researched last summer. After playing Ultimate Frisbee, as my friends and I always have, I went in the lounge and was puzzled by one of the Residential Assistants holding a microphone and calling my name up for karaoke night. Although I discovered that my friends had signed me up, I sang and made new friends. That was how my bizarre, yet exhilarating first day in Stony Brook started and I knew I was going to have another great time this summer.

When I first walked into the lab, I was welcomed by my previous lab fellows, graduate students, and Juee, the lab manager. Perhaps because I occasionally commuted to the lab throughout the academic year, I did not feel as foreign to the environment as I did last summer. But because I missed it for a few months, I felt like I came home from a long trip back to the lab family. I caught up with the lab family and set my workspace on the same spot where I worked last summer. Subsequently, reminding myself an important lesson that I learned last summer, about how “the more I contribute to the research, the more I will gain,” I conferred with Sayan and designed experiments for the start of the summer so I could start research immediately. Throughout the research, I consulted with Sayan almost every day, whether it was about new data or about interesting journal articles that I found that often enlightened us with new perspectives to my research. Other graduate students in the lab were not only helpful like Sayan, but also cordial. Sometimes we would just joke around. One time, the high school students in the lab played basketball with Sunny, Jason, Steven, and their friends after lab. Toward the end of the summer, we had the annual bioengineering department barbeque by the building. Then our whole lab went to a bowling alley and had a fun time. At the end of the summer, the bioengineering department hosted the Annual BME Graduate Student Research Symposium for incoming graduate students. They had oral and poster sessions for graduate students to talk about their research and also the focus of their labs. Several projects were very interesting and one of them was related to my projects as well, so I learned a lot from the oral session and from simply questioning afterwards during the poster session. Some graduate students claimed that they enjoyed explaining bits and pieces to my inquisitive self, and I mutually enjoyed attending their presentations. At that moment, I reminisced about last year’s poster presentation at the Breast Cancer and the Environment Research Program Conference in Cincinnati. I remembered sharing mirth with my

audience from discussions and exchange of gratitude. With these ineffaceable memories and feelings, I was elated from thinking about attending this year's conference in San Francisco.

Compared to last summer, I took on several projects and the major ones included studying graphene oxide nanoplatelets as targeted drug delivery agents and studying single-walled carbon nanotubes as microwave-induced hyperthermia treatment agents. I consistently researched pertinent journal articles to conceptualize the overall purposes of the projects. I came upon articles about manipulating gold nanoparticles, liposomes, carbon nanotubes, and other types of nanoparticles to deliver doxorubicin, paclitaxel, and various anticancer drugs. I was inspired by those articles, which led me to classify which types of assays were essential. I also read about Kanzius Cancer Research Foundation's progress on their radiofrequency treatment, which Mr. Peter Suchmann had mentioned to me before. Their concept of using gold or other metal nanoparticles as radiofrequency induced hyperthermia is very similar to that of my study on single-walled carbon nanotubes.

I figured that the most imperative step for both of my projects is solubilizing graphene oxide nanoplatelets and single-walled nanotubes. First, since the carbon-based nanoparticles are hydrophobic (water-fearing), without any functionalization or modifications, pristine carbon-based nanoparticles will aggregate in aqueous solutions outside of our body. Once in our body, since their dimensions are in nanoscale, there is no doubt that they can move through organs and tissues and eventually bio-accumulate, which can lead to deposits that can cause inflammation and certainly intoxicate the body. Several studies show that certain nanoparticles can even cross the blood-brain barrier, which requires very specific size, electric charge, and other factors. Few years ago, a study declared that carbon nanoparticles are not efficiently cleared from airways and may accumulate after chronic exposure. Numerically, the study stated that in healthy subjects, only 25% of the nanoparticles were removed, and the remainders were not removed within 48 hours. In subjects with lung problems, the case is more adverse. Clearly, the water-insolubility of the carbon nanoparticles is a major issue. In fact, this issue is preventing many *in vivo* studies of carbon-based nanoparticles from advancing because the carbon nanoparticles are not well-dispersed and unstable during their injection into mice and rats. Therefore, for every project, the carbon nanoparticles were solubilized using several methods.

After learning about the paramount importance of solubilizing nanoparticles, I conceptualized the mechanisms of targeted drug delivery and of microwave-induced hyperthermia treatment. The drug delivery mechanism is fairly simple. After solubilizing the carbon nanoparticles, drug is loaded onto the nanoparticle. Then the targeting ligand or antibody for the cancer cells is attached to the loaded, water-soluble nanoparticle. After injection, these homing nanoparticles will locate and attach to the receptors on cancer cells. Once they are internalized by the cells, the acidic conditions will cause doxorubicin to be unloaded from the nanoparticles. The microwave-induced hyperthermia treatment mechanism is very similar. After the nanoparticles are solubilized, the targeting ligand or antibody is attached in a similar manner to how it was attached to drug delivery nanoparticles. After internalization of the nanoparticles, a microwave field is generated. According to recent studies, the nanoparticles that exhibit unique dielectric properties, such as single-walled carbon nanotubes, facilitate localized cytotoxic heating of malignant tissues.

With a firm grasp on each experiment, I was able to anticipate data trends and compare experimental values. Whenever there were unexpected results, I thoroughly checked for possible errors. Once one of my lab fellow and I were using the UV-Visible spectrophotometer to measure the absorbance of doxorubicin dissolved in various solubilizing agents, such as sodium dodecyl sulfate and polyethylene glycol (PEG). We noticed how the absorbance value was decreasing as the time passed. This meant that the amount of doxorubicin in the sample was decreasing over

time, which did not make sense because doxorubicin is hydrophilic, water-soluble, well-dispersed in the solution, and should not have been spontaneously degrading. We speculated that doxorubicin molecules could be settling down at the bottom of the cuvette. We tried varying the volume of the sample and discovered the minimal volume of sample needed to keep the absorbance values consistent. We modified the protocol for reading samples using the UV-Visible spectrophotometer. Afterwards from this elementary, yet fundamental discovery to the lab, my lab fellow and I high-fived and shared the excitement of genuine exploration and research.

For first few weeks I focused on graphene oxide nanoplatelets study. By using the UV-Visible spectrophotometer and the modified protocol I addressed before, I read the absorbance of doxorubicin in different types of solutions: dextran 6K, dextran 10K (6K and 10K refer to the molecular weight of dextran), pluronic, sodium dodecyl sulfate, polyethylene glycol, and water for control. I varied the concentration of doxorubicin in order to plot standard curves of each doxorubicin-solubilizer combination. Deriving from these sets of data and equations, I can calculate exactly how much of doxorubicin is loaded in graphene oxide nanoplatelets. Then I proceeded to solubilizing the graphene oxide nanoplatelets using dextran 6K, dextran 10K, and polyethylene glycol. Then I needed to check whether the physical structure and/or the chemical compositions were altered because change in structure will result in change in properties of the nanoparticles. I confirmed that the structures and properties were uninterrupted by characterizing the solubilized nanoparticles with Raman Spectroscopy, which measures the vibrational frequencies of different parts of a molecule.

Following, I determined the optimal concentration of doxorubicin that can be loaded. After varying the concentration of doxorubicin, I concluded that 200 μg of doxorubicin is loaded with the highest efficiency (over 90%). Then I determined the optimal pH (measures of the acidity, neutrality or basicity of a solution) to acidic (values below pH of 7) by observing the release of doxorubicin as a function of pH. In the neutral solution (pH = 7) and the basic or alkaline solution (pH greater than 7), the drug releases were significantly less. After that, through lactate dehydrogenase assay and presto blue assay, I assessed the efficiency of drug delivery using direct uptake instead of targeting with proteins. I experimented on two cervical cancer cells, HeLa and SiHa, which are easy to work with. Although the drug loading was very high compared to that of liposomal delivery, the efficiency was negligible. Trying to find an explanation, Sayan and I planned to take images of the cellular uptake and internalization of the nanoparticles using State-of-the-Art instrument, Transmission Electron Microscope at Stony Brook's Central Microscopy Imaging Center, and also redo the assays on three additional established breast cancer cell lines (MDA, MCF-7, and SkBr) after attaching the targeting ligand on the nanoparticles.

Simultaneously, I worked with single-walled carbon nanotubes. When I learned about the mechanism of nanoparticle-facilitated hyperthermia treatments, I immediately associated it with Kanzius Radiofrequency hyperthermia treatment mechanism. Targeting strategies are similar in that both mechanisms use antibodies or ligands. I planned to use arginine-glysine-aspartic acid (RGD), a targeting ligand. This targeting tripeptide binds to the alpha v beta 3 ($\alpha v \beta 3$) integrin receptors, which are overexpressed in cancer cells. Using the RGD peptide motif, the nanotubes will target cancer cells, get uptaken by the cells, and get exposed to microwave frequency for heating.

There are only a few differences between my project and the Kanzius treatment. The Kanzius treatment uses gold colloids and metal nanoparticles instead of carbon-based nanoparticles. It uses radio frequencies instead of microwave frequencies. Due to their excellent photothermal properties and conductivity, gold nanoparticles and single-walled carbon nanotubes

can absorb waves at certain frequencies, and transduce those waves to heat waves with amplified energy.

Though studies have shown the effectiveness of single-walled carbon nanotubes to induce localized thermal cytotoxicity after exertion of microwave field, their biocompatibility has always been an issue. I tried solubilizing high concentrations of single-walled carbon nanotubes with dextran and polyethylene glycol, but the products were unstable. Without soluble nanotubes in high concentrations, we cannot experiment *in vivo*. So, Sayan and I designed a combination approach to solubilizing the nanotubes. First, I used acid treatment with sulfuric acid and highly oxidizing nitric acid. This introduced hydrophilic properties to the nanotubes, which made it water-soluble. I followed up with dextran coating. There were some problems due to highly acidic solutions, but the product was very favorable. I brought the soluble and stable concentration up to 5mg of single-walled carbon nanotubes per 1 ml of water, which was my goal. Sayan and Professor Sitharaman were impressed and asked me to increase the concentration as much as possible using the novel method and prepare samples for several concentrations. I changed some of the steps in the protocol, such as extending the time for acid treatment and also for dextran coating. The final product showed great solubility and stability at 15 mg of single-walled carbon nanotubes per 1 ml of water, which was a *huge* breakthrough. I ran out of single-walled carbon nanotubes, but once another batch is delivered, I will attempt to obtain at least 50 mg and up to 100 mg /ml. After the samples are prepared, they will be sent to Dr. Susan C. Hagness's Electrical and Computer Engineering lab at University of Wisconsin-Madison for testing the solubility of the nanotubes and also their dielectric properties, which is crucial for hyperthermia treatment. After we receive the data back from the Hagness lab, we will start RGD synthesis and attachment to the nanotubes, and conduct assays that will show change in temperature when the nanotubes are exposed to microwave. In addition, the internalization of these nanotubes will be studied using transmission electron microscope.

I believe that the emerging field of nanotechnology is promising and will extensively impact all of us the next five to ten years or so. As I have researched in the Sitharaman lab for past two years and have learned about ever-expanding spectra of potentials that nanotechnology offers, from medicine, electronics, optics, and many more, I truly believe this field will revolutionize multiple areas. As amazing as it may sound, it is also frightening. There will be more nanoparticle manufacturers, which the situation conjectures that both the workers and consumers may be prone to leaks, exposures from waste, and long term side effects. For biomedical applications, the researchers are striving to maintain nanoparticles' biocompatibility and biodegradability, because we know that pristine nanoparticles are toxic to us. Any precautionary action is worth taking. The more people are educated not only about the potential applications of nanotechnology, but also about the risks and toxicological assessments, the more they will be prepared in the future.

It is my obligation as a researcher and an advocate to research and translate results to educate others, so they could make better decisions for a better tomorrow. I thank the Great Neck Breast Cancer Coalition and Stony Brook University for allowing me to fulfill that obligation. I truly appreciate and respect the purpose of this program-- to augment the pillars for the safety of our future generations. My interest in research and my will to serve my community have burgeoned immensely. My advocacy for breast cancer prevention will not cease, but be amplified as I move on to college and the real world. Through this powerful program, I had gained more than what I hoped for. The experience of conducting experiments and running assays is not the only meaningful gain from my association with the coalition. The seemingly trivial experience of living in a dorm and commuting to lab after dorming ended; the experience of integrating individuality and ingenuity to research; the experience of communicating research in layperson

terms; the experience of discussing about research with colleagues like professionals; and lastly, the invaluable memories I made with my friends in Stony Brook and family in our coalition. All of these that I cherish, I can attest, have been consolidated into a highlight and a chapter in my life that shall never be forgotten.