Despite decades of research, an individual diagnosed with cancer today still has a limited set of treatment options available. One of the most common treatments is the use of chemotherapeutic agents to destroy cancerous tissues. To be effective in treating solid tumors, chemotherapeutics must overcome a series of delivery obstacles including delivery of the drug to the tumor site, penetration of the chemotherapeutic into the tumor, accumulation and retention of the agent, and intracellular uptake of the drug. The effectiveness of many chemotherapeutic agents is limited by these barriers. Indeed, several studies suggest that limited tissue penetration can be linked to a cancer's resistance to chemotherapeutic treatment.[1] Therefore, to enhance the effectiveness of chemotherapy drugs, my research goal is to improve the drug's ability to penetrate solid tumors through the development of advanced drug delivery formulations.

As an undergraduate researcher collaborating with Dr. Tae Hee Kim, a senior fellow in Dr. Suzie Pun's lab in the Department of Bioengineering at the University of Washington, I sought to achieve this goal by applying polymers that self-assemble into spherical shells called micelles and encapsulate doxorubicin (DOX), a chemotherapy drug. DOX often cannot effectively diffuse into the interior of a tumor because it can bind non-specifically to proteins and also because it is sequestered by cells surrounding the tumor; this limits its effectiveness as a chemotherapeutic agent.[2] By loading DOX into the core of the micelles, I hope to show that these DOX-loaded micelles effectively permeate tumors after being injected into the bloodstream, and after collecting in the tumor site, that they release the chemotherapy drug to destroy the cancerous tissue. Therefore, Dr. Kim and I needed to prepare biocompatible and biodegradable micelles that could efficiently encapsulate doxorubicin, penetrate tumors and slowly release active drug.

After considering each of these design goals, we decided to encapsulate DOX within particles assembled from a biodegradable copolymer. The term 'copolymer' indicates the presence of multiple units per polymer molecule: each consists of three distinct units, or 'blocks': a middle hydrophobic (water-avoiding) polymer block flanked by two hydrophilic (water-seeking) polymers. In aqueous solution, the hydrophobic blocks of nearby copolymer units cluster together, and thus self-assemble into small (<100nm) spherical micelles. Since hydrophobic compounds tend to cluster together in aqueous environments, adding a hydrophobic complex of doxorubicin to an aqueous solution of the triblock copolymer causes the drug to incorporate into the micelle core. In addition to our work with doxorubicin, this approach is easily translatable to other deliverables because most hydrophobic compounds can be incorporated into the micelles. For instance, Dr. Kim and I have authored a paper recently submitted to *Biomaterials* describing the use of polymeric micelles to improve the stability of an imaging dye, and other researchers may follow-up on our work with additional compounds. If these micelles improve the delivery characteristics of a wide range of drugs, there would be major commercialization potential for the material as a broadly-applicable delivery vehicle.

Many researchers investigating methods for improving chemotherapeutic delivery are particularly interested in targeting the delivery of their agent to the tumor site. I aim to demonstrate that the micelles we are developing can achieve this targeted delivery by taking advantage of the unusually large pores in the blood vessels that permeate solid tumors. If the DOX-loaded micelles are smaller than these pores, typically less than 200 nm in size, there is literature evidence to suggest that drug concentrations in the tumor can reach levels as much as 10-50 fold higher than in the non-cancerous surrounding tissue within several days.[3] This phenomenon is commonly referred to as the Enhanced Permeation and Retention (EPR) effect. In addition, the Pun laboratory recently showed that nanoparticles that are about 40 nm in diameter or smaller can more

[1] Trédan et al. "Drug Resistance and the Solid Tumor Microenvironment." *J Natl Cancer Inst* 2007;99: 1441 – 54.

[2] Minchinton et al. "Drug penetration in solid tumors." *Nature Reviews: Cancer*. 2006; 5:583-592.

[3] Iyer et al. "Exploiting the enhanced permeation and retention effect for tumor targeting." *Drug Discovery Today.* 2006; 11: 17-18.

efficiently penetrate tumor tissue.[4] In a recent study, I have been able to show that the doxorubicin-loaded micelles have an average size of approximately 37 nm, well within the range of particle sizes that can exploit the EPR effect and small enough to potentially penetrate tumor tissue.

When evaluating any compound that is being developed for use as a pharmaceutical, it is common to investigate the effects of that drug in cell lines before pursuing animal studies. These assays are often conducted using single layers of cells. However, this 2D layout is a poor model of an actual tumor, and cannot be used to assess how a chemotherapy drug permeates a 3D tissue environment. Therefore, we cultured spherical cancer cell masses as tumor mimics, hereafter referred to as spheroids. These spheroids consisted of SiHa cells, which are derived from human cervical cancer cells, and were approximately 400 microns in diameter when they were used for studies. They exhibit a number of properties that make them useful for drug penetration assays, including reproducible, relatively consistent shape, the presence of extracellular matrix – a collection of proteins and other molecules that presents a major barrier to drug delivery – an oxygen-deprived interior often present in tumor tissue, and a necrotic core that is also common in tumors. In these tumor mimics, fluorescent microscopy studies indicate that DOX-loaded micelles penetrate the spheroids more quickly than DOX itself, demonstrating improved penetration efficiency in the micelle formulations. This suggests that Dr. Kim and I have made major progress towards the completion of my major research aim: to enhance the penetration efficiency of doxorubicin by encapsulating the drug within polymeric micelles. I presented this result at the Biomedical Engineering Society's Annual Fall Meeting in October 2009, where I discovered that I had truly made a meaningful contribution to the field of drug delivery, and that I could share this knowledge with seasoned professionals in this area of research.

That experience has motivated me to continue to seek methods to improve our studies, and I have identified several potential areas for future improvements and research. One of my major goals is to develop a method for quantifying the results of our spheroid penetration assays. As an engineering student, this challenge is particularly relevant to me because I understand that it is more prudent to draw conclusions from reproducible, quantitative data than from sets of qualitative data, particularly when it is impossible to represent all of the qualitative data as is the case with these fluorescence microscopy images. I therefore am devising an algorithm that will calculate the distribution of DOX within the spheroids as a function of time, thereby allowing a quantitative comparison of the rate at which different drug formulations penetrate the tumor mimic. Another challenge of working with the spheroids is inconsistent spheroid size. It is impossible to select spheroids with a narrow size distribution by hand, and during the course of the penetration studies I surmised that this variation could have a significant impact upon the results of the penetration assay. Therefore, I developed a design for a microfluidic device that would sort a mixture of these tumor mimics by size and retain them for use in subsequent assays. I am currently collaborating with Dr. Barry Lutz, also of the Department of Bioengineering, to develop this technology, and if successful it should simplify future research conducted with this tumor model and make the results more accurate and reliable.

However, no amount of data from cellular studies can categorically predict the performance of our micellar delivery system in live tissues, and therefore Dr. Kim and I are in the process of conducting studies on mice to determine whether the encapsulation of DOX within polymeric micelles impedes or enhances the drug's ability to destroy cancerous tissue. If DOX retains its ability to inhibit tumor growth in this animal model, these DOX-loaded micelles would not only be a notable advance within the field of drug delivery but could also find their way into clinical use.

Signed:

Date:

[4] Goodman et. al. "3-D tissue culture systems for the evaluation and optimization of nanoparticle-based drug carriers" *Bioconjug. Chemistry*. 2008; 19(10): 1951–1959.