

Before arriving at the University of Washington, I considered the terms “education” and “schooling” to be synonymous. Aside from homework, my educational experiences rarely extended beyond the classroom. But the opportunity to undertake undergraduate research has shattered this misconception. Working as a member of Dr. Gerald Pollack’s laboratory and a summer intern with Syntrix Biosystems over the past year has revealed that the process of discovery provides an incredibly valuable educational opportunity. This year, I have found another such opportunity working with Dr. Tae Hee Kim in Dr. Suzie Pun’s laboratory in the Department of Bioengineering. Together, we are developing polymeric micelles to improve the delivery of a dye for near-infrared (NIR) imaging. For the first time, I am participating in research that could translate into a life-changing technology. The pure excitement of this potential drove me to take on this challenge, and already the experience has been invaluable.

Dr. Kim and I are striving to develop a polymeric micelle that can stabilize the NIR dye Indocyanine Green (ICG) for use in the body and enhance its ability to target cancerous tumors. NIR imaging has several significant advantages over other types of tissue imaging. Light at these wavelengths (700-900nm) experiences less absorption and optical scattering than visible or infrared light, and the background autofluorescence levels in this range from tissues is minimal.ⁱ Combined with the use of a contrast agent, or dye, this allows greater imaging depth and sensitivity. ICG is currently the only FDA-approved NIR dye for routine clinical use to assess liver and heart functions. However, its use is limited by several properties, including rapid aqueous, photo, and thermal instability, nonspecific binding to blood proteins, and rapid clearance from the body.ⁱⁱ

We plan to overcome these limitations by encapsulating ICG within polymeric micelle carriers. The micelles are constructed from triblock copolymers consisting of a middle

hydrophobic polymer block flanked by two hydrophilic polymers. In aqueous solution, the hydrophobic blocks cluster together, and thus the micelles self-assemble. Adding a hydrophobic salt of ICG to these solutions causes the dye to be incorporated into the core of the micelles. Previous research has already shown that this approach has great potential; Rodriguez et al. demonstrated that this approach resulted in improved aqueous and thermal ICG stability.ⁱⁱⁱ

Our research aims to build on these results by focusing on several elements of this dye-delivery system. Because we synthesize micelles ‘from scratch,’ we are examining several varieties of triblock copolymers for use in formulating micelles for ICG delivery. After the micelles form, we characterize them to evaluate their potential in our dye delivery system. To do so, we quantitatively assess the efficiency of incorporating ICG into the micelles (referred to as loading efficiency), micelle and ICG stability, and micelle size. We then assess the cytotoxicity of the micelle systems before continuing to a whole-body biodistribution study in mice to determine how the ICG-loaded micelles spread throughout the body over time. Future studies will involve administration of non-encapsulated ICG and micelle-encapsulated ICG to mice. Theoretically, micelle encapsulation should reduce nonspecific binding to blood proteins and reduce the rate of ICG clearance from the body, thereby extending the duration of ICG’s circulation in the blood.^{iv} If our data supports this prediction, we can turn our focus to assessing whether our dye delivery system also leads to increased ICG aggregation in cancerous tumors. We hope to observe this improved passive targeting ability due to the porous vasculature and increased endocytotic activity of cancerous cells. If this is the case, these dye-delivery micelles may become a valuable tool for NIR cancer imaging in the future.

Individually, my role in this research generally involves collecting and analyzing the data needed in our project, as well as synthesizing micelles. One of my major tasks to this point has

been determining the loading efficiency of ICG into micelles. In this study, I sought to determine the concentrations of triblock copolymer and ICG necessary to achieve the maximum loading efficiency of the dye. This information will be useful for synthesis of successive batches of micelles as well as a starting point for micelle formulation in the event that we begin working with different polymers. Assessing micelle stability is another one of my important research goals. This is quantified as critical micelle concentration (CMC), or the triblock copolymer concentration at which the micelles will self-assemble. This is a crucial piece of data because it determines what micelle concentration will be necessary to prevent the copolymers from spontaneously dissociating in solution. For this delivery system to be effective, the CMC of the micelles must remain well below the dosage level for imaging studies.

Throughout the course of this project, I have benefited immeasurably from Dr. Kim's support and guidance. In the whirlwind of several weeks, she introduced me to micelle formulation, fluorescence assays, UV-Vis spectrometry and dynamic light scattering. Along with such technique training, Dr. Kim has incorporated me into her own research and thus made me a part of a larger research process that includes collaboration with other UW laboratories. This has allowed me to begin developing an understanding of each of the various steps of the development of this ICG-delivery system, far beyond what would be possible without her support. She has also demonstrated an extreme willingness to help me to learn and understand anything in which I am interested. For instance, she has offered to instruct me in the culturing of cells so that I can perform micelle cytotoxicity studies in the future, an experience that few sophomores are privileged enough to have. Beyond day-to-day guidance, Dr. Kim has also expressed strong interest in my own education and has always been glad to field my endless

stream of questions, particularly about graduate school. I have been privileged to work beside her and look forward to learning even more as we continue working together.

As exciting and rewarding as day-to-day research can be, I believe that the greatest value of this research experience is the educational development and enrichment that will accompany the process. Beyond the obvious benefits, such as laboratory technique experience, I will develop a practical knowledge of many of the concepts I've had contact with only in lecture halls or relatively simple introductory laboratory sequences. And most importantly, I will continue to build upon research skills gained only through experience, such as knowing what questions to ask when planning a study, interpreting failed experiments, and learning to consider your own data objectively. Since I plan to pursue a Ph.D. in biomedical engineering following my graduation, I understand how important it is to develop these skills as an undergraduate. Perhaps the best sign of the value I place in this research project is the fact that I spend 10-15 hours per week in the lab, despite an 18-credit load of intensive math and science classes. Balancing this schedule is challenging, but my love of science and enthusiasm for this research keeps me happily committed to pursuing excellence both in the lab and the classroom.

I have always planned to try to make a valuable contribution to society through research, but I never thought I would have the opportunity to do so at age 19. If we successfully develop polymeric micelles that stabilize ICG under *in vivo* conditions and enhance the dye's ability to target tumors, we will have made considerable progress towards a valuable improvement in NIR imaging. And the practical knowledge and research skills I will develop will be of great value as I continue in my academic career. As for my mentor, I cannot thank Dr. Kim enough for her continued support and guidance both in the laboratory and for this application. I hope only that the progress that I make will be as valuable to her as her mentorship has been to me.

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- ⁱ (Zaheer et al., 2001)
ⁱⁱ (Desmettre et al., 2000)
ⁱⁱⁱ (Rodriguez et al., 2008)
^{iv} (Rodriguez et al., 2008)

References

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