Encapsulation and delivery of tumor imaging agents and chemotherapeutics via amphiphilic micelles

Historically, pharmaceutical therapies have been implemented by way of injection or ingestion of an active molecule which reaches its therapeutic target via unmediated and unprotected circulation in the vasculature. Recently, investigators in the growing field of drug delivery have begun to identify and explore the problems and limitations which arise from this treatment strategy. In the blood stream, pharmaceutics are exposed to enzymatic degradation and renal or hepatic filtration, which necessitates large doses to compensate for the drugs lost to these mechanisms. Additionally, systemic circulation of toxic drugs, such as chemotherapeutics, jeopardizes the well-being of the patient. Fortunately, recent advances in drug delivery have demonstrated excellent promise in solving or at least attenuating these problems. Drug loaded liposomal nanoparticles have been shown to decrease the systemic toxicity of chemotherapeutics[1]. Also, molecular protection via nanoparticle encapsulation has improved the circulatory half-lives of therapeutics, increasing exposure to the physiological target.[2]

In the Pun Lab at the University of Washington we are working to develop innovative drug delivery systems to maximize the clinical effectiveness of nanoparticle-mediated chemo-therapeutic treatment and tumor imaging. One specific group of drug delivery vehicles commonly used to encapsulate molecules are micelles formed from the self-assembly of amphiphilic block copolymers. Amphiphilic copolymers contain both hydrophobic and hydrophilic sections, and when these polymers are added to solution, the hydrophobic components aggregate together while the hydrophilic components extend into solution. This effectively creates a spherical micelle into which imaging agents or chemotherapeutics can be loaded. I have collaborated with Dr. Tae Hee Kim (Ph.D.) and Christopher Mount in the investigation of a novel micelle drug delivery system using mixtures of triblock copolymers. The copolymers we use consist of a hydrophobic central block flanked by two hydrophilic tails. We used mixtures of poly(ethylene oxide)-poly[(r)-3-hydroxybutyrate]-poly(ethylene oxide) **(PEO-PHB-PEO),** which I will refer to here as PHB triblock, and poly (ethylene oxide) - poly(propylene oxide) – poly (ethylene oxide) **(PEO-PPO-PEO),** which has been commercialized as Pluronic F127. The rationale behind using mixed micelles stems from the relative independent properties of these two micelle systems. Pluronic F127 does not form micelles at room temperature, but rather exhibits a reversible thermosensitive transition above 25 ˚C. At physiological conditions (37˚C) the micelles are very stable and relatively small (Diameter = 20 nm). In contrast, micelles formed from the PHB triblock are relatively unstable at physiological temperatures. However, we have demonstrated that the PHB triblock micelles have a larger diameter (60 nm) than micelles formed from Pluronic F127 and thus their maximum drug loading content is significantly higher. Therefore, we proposed that the mixture of the two polymers would yield mixed micelles which are more stable at 37˚C than the PHB triblock micelles alone, and would have higher drug loading capacity than the Pluronic F127 micelles alone. This combination of high loading content and high physiological stability would make these micelles ideal for clinical implementation. As part of this project, I had an integral role in the synthesis of the components of the micelles, as well as in the *in vitro* and *in vivo* work associated with the assessment of the therapeutic efficacy of our material. An analysis that I performed of the dynamic light scattering properties of the mixed micelles demonstrated that the micelle mixtures at various ratios of PHB triblock and Pluronic F127 did in fact occupy intermediate sizes. Then, by measuring the loading content of doxorubicin, a potent tumor suppressant, using micelles of various polymer ratios, I was able to correlate the micelle size and maximum loading content of the mixed micelles.

Moving to an *in vivo* model, we analyzed the ability of our mixed micelle system to deliver both fluorescent imaging agents and chemotherapeutics to subcutaneous xenograft tumors in mice. Our findings showed that the mixed micelle system significantly improved the contrast and brightness of solid tumor fluorescent imaging using indocyanine green. Furthermore, encapsulated indocyanine green demonstrated significantly extended circulation half-life. Also, micelle mediated delivery of doxorubicin eliminated toxicity-associated weight loss, while also providing significant multi-drug resistant (MDR) tumor suppression. These findings suggest that by using micelle encapsulated doxorubicin to treat drug resistant solid tumors, the mechanism by which drug resistant cancer cells exclude doxorubicin is circumvented.

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[1] Gabizon, A.; Shmeeda, H.; Barenholz, Y. (2003) *Clinical Pharmacokinetics*. Vol. 42, 419-436.

[2] Brigger, I.; et al. (2002) *Advanced Drug Delivery Reviews:* Vol. 54, Is. 5, 631-651.

The *in vivo* delivery efficacy that our micelles have demonstrated suggests promising clinical applicability. We believe our formulation could successfully provide a novel and functional micelle delivery system due to its high loading content, high stability at 37˚C, circumvention of multi-drug resistance, and increased fluorescent tumor imaging capabilities. We are in the process of composing two manuscripts relating to our imaging and chemotherapeutic delivery work which will be completed and submitted for publication within two months. Furthermore, as a recipient of the American Association for Cancer Research (AACR) Thomas J. Bardos Science Education Award I have been provided with funds to attend the next two AACR National Meetings to share these results and observe the approaches other investigators have used to investigate chemotherapeutic drug delivery. I intend on using this opportunity to acquire new knowledge and analytical techniques which we can use to explore novel applications of our material. One of these novel applications which we are particularly interested in is the moiety-mediated active targeting of cancer cells.

Cell specific drug delivery is a burgeoning area of investigation which has the potential to revolutionize the field of drug delivery. Currently, the clinically approved chemotherapeutic nanoparticle treatments rely on the enhanced permeability and retention effect (EPR), a property of the leaky vasculature of solid tumors, to deliver active molecules to cancerous tissue. However, this mechanism relies on passive diffusion and is only applicable to solid tumors. In contrast, cell specific delivery is accomplished by conjugating markers onto the surface of the drug loaded micelles which interact specifically with receptors or antigens present on cancer cells. Interactions of the labeled drug delivery vehicles with these receptors induce endocytosis and the release of chemotherapeutics inside of the cancer cell[3]. To actively transport the drug delivery vehicles, we must utilize a cell specific chemical interaction with the cancer cells. This can be accomplished via antibodies, peptides, or other chemical moieties for which cancer cells exhibit specific receptors. If implemented successfully, moiety directed delivery could enable the nanoparticle mediated treatment of non-solid, metastatic tumors.

The folate receptor (FR), also known as folate binding protein (FBP), is an ideal candidate for moiety mediated cancer cell targeting. It is displayed on the surfaces of numerous carcinomas, and is relatively absent from most other cells in the body. Interaction of FR with folate derived moieties has been shown to induce receptor mediated endocytosis. Thus, labeling nanoparticles with a folate derived compound could induce carcinoma specific endocytosis of the nanoparticle-encapsulated drug.[4] In the next stage of our investigation we will be modifying our material to utilize this delivery interaction. I will be developing and characterizing folate conjugated micelles using our novel mixed micelle formulations. I will then be assessing the *in vitro* cytotoxic effects of the folate conjugated micelles on a metastatic cell line. If we observe a significant increase in nanoparticle uptake and cytotoxicity relative to non-conjugated micelles, we will move into a mouse model, measuring metastatic tumor reduction using a specialized cancer cell line which produces luciferin. Luciferin expression can be visualized and quantified by luminescent imaging, enabling quantification of metastatic tumor growth. In the future, we may extend this work to utilize micelle targeting using antibodies or other chemical moieties.

Investigation of drug localization via biochemical targeting has the potential to produce innovative treatments which will improve the quality of life and prognosis for many patients suffering from cancer. The opportunities for the future implementation of drug delivery materials in clinical practice are numerous.

The time and energy I have devoted to this project has been substantial, but my involvement has proven to be an excellent experience for me to hone my research skills and investigative technique. Throughout my experience working on this project, I have learned numerous technical protocols, but more importantly, I have gained a sense of the dedication and passion that is required to be productive in scientific research. As my involvement with this project has increased, I have found myself becoming more personally attached to my work. It is a rewarding opportunity to be involved with such an interesting project which has the potential to provide meaningful clinical benefits. I am excited to extend this passion into my graduate education and beyond.

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[3] Lee, RJ; Low, PS. (1994*) Journal of Biological Chemistry.* Vol. 269, 3198-3204.

[4] Saul, Justin M; et al. (2003*) Journal of Controlled Release.* Vol 92, Is. 1-2, 49-67