Controlled delivery of a cochlear therapeutic by a copolymer hydrogel complexed with α-cyclodextrin

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Past Research Experience

My research experiences at the University of Washington have been diverse. Each has provided me with unique insight and played a pivotal role in shaping the student and researcher I am today. I began conducting research during autumn quarter of my freshman year, working in the lab of Dr. Peter Ward, measuring and categorizing fossils from the Burgess Shale. That winter, to pursue my interest in biomedical research, I joined the lab of Dr. Matt Kaeberlein (UW Pathology) as a lab assistant. I was soon offered a research project measuring the genomic stability in long lived yeast strains by deleting genes known to increase lifespan and measuring the formation of extrachromosomal rDNA circles (an indicator of genomic instability). I worked on this project for about one year, and was even rewarded by the support of a Mary Gates Research Scholarship, which generously sustained me through my formative and enlightening research experience in the Kaeberlein Lab. These projects, undertaken in the early years of my academic career, have helped establish my understanding of effective and efficient research techniques. This fundamental, basal knowledge has imbued me with a voracious scientific curiosity and has motivated me take on a complex and innovative project of my own, taking the next step to becoming an independent researcher.

Current Research Experiences in the Pun Lab

During winter quarter of my sophomore year, my curiosity was piqued by an interesting opportunity. I had been doing some personal investigation into the field of drug delivery, and was simply amazed at the level of innovation that had been achieved by its investigators. The pursuit of protecting and specifically delivering molecules via nanoparticles to specific organs or cells in the body sounded to me like something out of science fiction. My fascination impelled me to apply for the summer Amgen Scholars Program in the lab of Dr. Suzie Pun (UW Bioengineering). To jumpstart my training, I began my work in the Pun lab spring quarter, doing double time with the Kaeberlein Lab.

The research experience I had already gained as a Mary Gates Scholar allowed me to rapidly come up to speed with the ongoing projects in the Pun lab. Though working in two labs tested my time management capabilities, it was indubitably worth the effort, as I learned a great deal about the synthesis and characterization of drug delivery nanoparticles. Specifically, I studied the PEO-PHB-PEO triblock, which is a copolymer consisting of a hydrophobic core polymer of polyhydroxy butrate (PHB) capped by two hydrophilic tails of polyethylene oxide (PEO). That spring, I participated in a project using drug loaded micelles formed from this material to deliver imaging agents and chemotherapeutics to murine tumor xenograft models. I became fluent in the techniques of polymer synthesis, NMR characterization, nanoparticle formulation, and xenograft tumor reduction studies. Our work showed excellent drug delivery and we are currently in the process of writing two manuscripts to be submitted for publication before the end of the year, on which I will be co-author. My newfound understanding of the PEO-PHB-PEO triblock put me in an excellent position to take on an independent project developing an innovative use of the material to achieve a novel therapeutic goal.

Independent Project

This past June, as an Amgen Scholar, I began a project developing a biomaterial to deliver protective therapeutics to the inner ear. This project is in collaboration with the Rubel lab (UW Otolaryngology). Hair cells, which are present in the cochlea of the inner ear (Figure 1), convert mechanical signals received by the ear drum into electrical signals to be sent to the brain. Strong antibiotics, including kanamycin and neomycin (the active ingredient in Neosporin), are causally linked to the loss of hair cells, and once lost, they cannot be regenerated. The Rubel lab has, through extensive drug screening, identified a molecule that protects hair cells from degradation by neomycin, called PROTO1[1]. One obstacle to clinical translation of this therapy is effective delivery of drugs to hair cells *in vivo* due to the poor vascularization of the cochlea. This limitation necessitates daily systemic doses to achieve therapeutic effects. To circumvent this clinical barrier, a biomaterial could be placed onto the round window membrane (which provides an interface between the auditory canal and the cochlea) which could gradually release the drug into the cochlea (Figure 2). Such a treatment

would eliminate the need for intravenous injections. The goals of my project are to develop a biomaterial which will provide delivery of PROTO1 to the cochlea, verify hair cell protection in an animal model, and ultimately utilize the biomaterial to deliver other promising therapeutics.

In addition to its ability to form of drug delivery vehicles, the PEO-PHB-PEO triblock, with which I had become quite familiar, also has the capability of forming a hydrogel when complexed with α-cyclodextrin, a cyclic oligosaccharide[2]. A "threaded necklace" is formed in which the α-cyclodextrin rings thread onto the PEO tails of the polymer[3]. Attractive forces between the polymer and α-cyclodextrin induce the formation of a supramolecular hydrogel (Figure 3). Over the summer, I worked on developing a protocol for the preparation of a stable hydrogel loaded with PROTO1. I attempted numerous formulations before I was finally able to achieve a stable, drug loaded hydrogel. Once the preparation protocol had been determined, I measured drug release from the hydrogel into PBS. To quantify drug release, I developed two novel protocols for the quantification of PROTO1 by fluorescence, and verified my results by implementing both methods. The results of the release study show a linear profile with full release occurring at around 25 days (Figure 4). Linear release is ideal for clinical application as it would maintain a constant drug concentration within the cochlea.

Proposed Work

Moving forward, there are three primary aims for this project: to ensure that hydrogel loading does not disable PROTO1 via chemical modification, to test the efficacy of the hydrogel in vivo, and to expand this therapy to accommodate other drugs. First, we will be working with the Rubel Lab to measure the hair cell protection of gel-released PROTO1 in neonatal zebra fish via fluorescent antibody hair cell labeling and quantification by fluorescent microscopy. This study will verify that hydrogel loading does not chemically disable PROTO1. Subsequently, we will move into a rat model, performing minimally invasive surgery to inject drug loaded hydrogel on to the round window membrane of our animal subjects. We will measure hair cell protection in the rats by testing their functional hearing as quantified by brainstem response to auditory signals. Finally, by modulating the parameters and preparation of the hydrogel, this therapy could be utilized to deliver other cochlear

therapeutics such as indomethacin and nordihydroguaiaretic acid[4]. If effective, this hydrogel could be administered to patients receiving neomycin doses for large wounds or serious infections and could eliminate the risk of hearing loss that accompanies such treatments. This therapy has a great potential to relieve the unnecessary suffering of thousands of patients receiving large dose antibiotic treatments.

Personal Development

My work on this project has been profoundly exciting and intellectually stimulating. I have been given the unprecedented opportunity to work on the preliminary development stages of an innovative therapy in an independent and self-directed manner. As a Mary Gates Scholar last year, I worked in coordination with a group of graduate students who were responsible for the direction and goals of our project. This year, I alone am responsible for producing results, and if something fails the responsibility of correction and redirection lies on my shoulders. Dr. Pun has shown incredible support and understanding when I have encountered difficulties, and for that I am very grateful. She has allowed me to grow and find footing in my newfound scientific independence. Nevertheless, this project is still in a nascent stage. A substantial amount of work stands between our material and a viable human therapy. However, the exciting preliminary results I collected over the summer suggest the transition into animal models will be a very exciting endeavor. Financing from the Mary Gates Research Scholarship would allow me to continue to pursue this exciting this project and demonstrate its clinical relevance. Over the course of the past two years my involvement in research has become so entangled with my academic life and so engrained in my day to day activities, that I could hardly imagine a day passing during which I did not spend time contemplating or working on my research project. I have been inspired by my work on the cutting edge of medicine and science to pursue an MD/PhD, which would allow me to continue my exploration of innovative solutions to clinical problems. The simple idea that my work could be used to brighten the prospects or quality of life of a suffering patient is a potent motivating force, one which I will embrace and cultivate with my entire intellectual capacity.

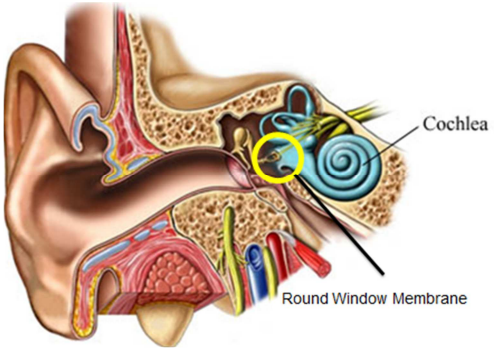

Figure 1 *– Shows the location of the cochlea in the inner ear and the round window membrane onto which the hydrogel will be injected.*

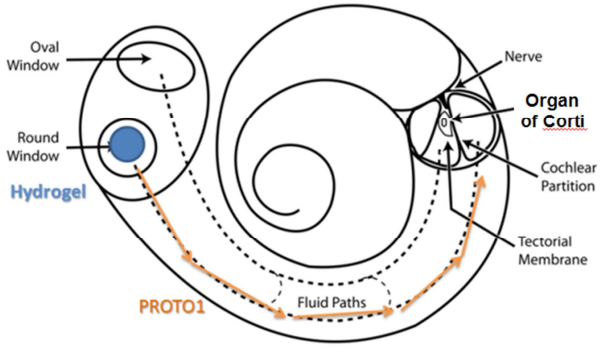

Figure 2 *– Intended therapeutic approach: PROTO1 loaded hydrogel will be injected onto the round window membrane and release PROTO1 through the membrane over a period of 2-3 weeks into the fluid paths of the cochlea.*

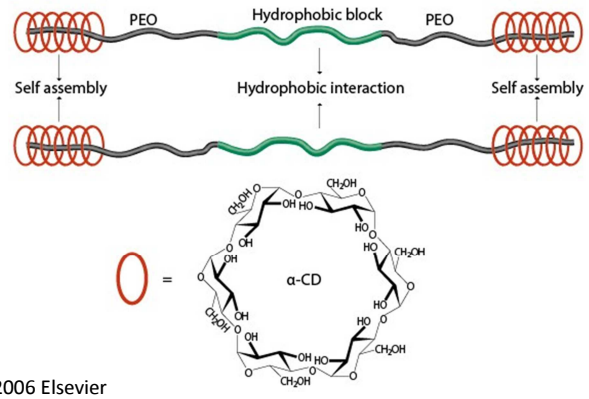

Figure 3 *– α-cyclodextrin rings are included onto the ends of PEO-PHB-PEO triblock copolymer. Interactions between the hydrophobic portions of the polymer and self-assembly of the α-cyclodextrin rings induce the formation of a supramolecular hydrogel.*

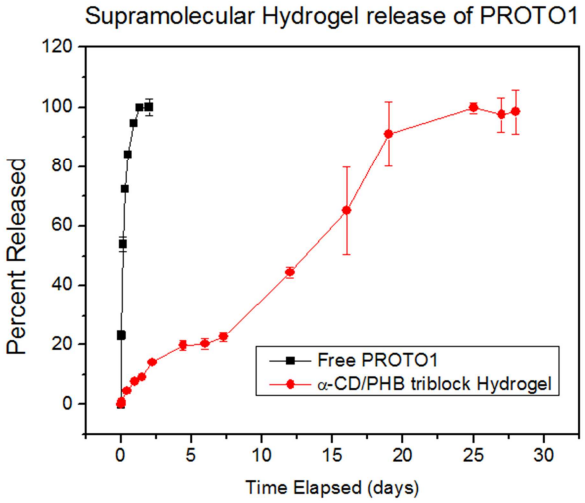

Figure 4 *– Release of PROTO1 loaded α-cyclodextrin / PHB triblock hydrogel through a 10kDa dialysis membrane is compared with the release of free PROTO1 in an aqueous solution over the period of two weeks. The hydrogel gives controlled linear release for over 3 weeks.*

References

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(3) Li, Jun, et al (2001). Formation of Supramolecular Hydrogels Induced by Inclusion Complexation. *Macromolecules* 34: 7236-7237.

(4) Hushino, T. (2008) Non-steroidal anti-inflammatory drugs protect mouse cochlea against acoustic injury. *Tohoku J Exp Med.* 216(1): 53-59.