Cell-Based Therapeutic System for Lowering Rates of Mucosal HIV Transmission

Despite nearly three decades of research we are still without an effective vaccine for HIV/AIDS. Current vaccine strategies that mediate systemic immunity have proven ineffective in providing mucosal immunity to prevent viral HIV transmission. Recent work in immunology and virology illuminates the key role of the mucosal immune system in HIV pathogenesis[1]. Since HIV transmission typically occurs during sexual intercourse, protecting the mucosal frontlines in rectal and vaginal mucosal membranes will a play fundamental roles in preventing HIV transmission. Eliminating HIV during the earliest stages of mucosal infection will have the greatest impact to prevent systemic dissemination of the virus[1]. Vaccines designed to elicit humoral and cellular immunity within the mucous membrane, at the site of infection, could realistically reduce rates of transmission amongst high-risk populations.

In the Woodrow Lab at the University of Washington, we are focused on engineering biomaterials that neutralize HIV virions or induce HIV-specific protective host immune response at mucosal sites of viral entry. Unfortunately, HIV employs a suite of tactics to avoid detection and elimination by the host immune system, including its high antigen mutation rate and its ability to subvert $CD4^+$ T-cell responses through immunosuppression[1,2]. Several studies indicate that effective vaccines must induce both long lasting production of neutralizing antibodies capable of eliminating free virus and T-cell responses that control infected cells[1,2]. In addition, mucosal vaccination has proven to elicit a more potent immune response in mucosal tissues, where protection against sexual HIV transmission is most needed. To date, most highly immunogenic HIV-1 therapeutics are engineered to present viral antigens in native conformations and in structures that closely mimic the live virus. Self-assembling HIV-1 virus-like particles (VLPs) are nanoparticle shells consisting of HIV-1 antigens but lack the proteins and RNA required for infectivity. HIV-1 VLPs are recognized by the host immune system and are capable of stimulating potent mucosal and systemic immune responses. However, VLPs administered mucosally are often trapped and diluted by mucosal secretions and require higher doses to be effective. **We propose to overcome obstacles to mucosal vaccination with VLPs by delivering cell based therapeutic factories housed in biocompatible alginate microcapsules that are capable of maintaining sustained levels of HIV-1 VLPs.**

My research in the Woodrow Lab has focused on engineering a biocompatible alginate hydrogel to support the viability and function of transplanted cells that express HIV-1 VLPS. Cells encapsulated in alginate microcapsules have been previously shown to remain viable for up to 6 months *in vivo*[3]. I have designed and built an electrostatic droplet generator to fabricate finely controlled alginate capsules encapsulating human cells. The method involves extrusion of an alginate solution containing engineered cells into a calcium chloride bath, which crosslinks the alginate polymer and results in a stable micron-sized hydrogel particles interspersed with encapsulated cells. By applying an external high voltage electric field, I have previously shown that altering the strength of the applied electric field can control particle size[4]. I have also demonstrated that the alginate particles can be coated with a poly-L-lysine (PLL) layer to form a semipermeable membrane that can be adjusted to control the mass transport of nutrients and waste products to and from the encapsulated cells. My current work focuses on transfecting a human embryonic kidney cell line (HEK-293T) with a plasmid that codes for the HIV *gag* structural protein conjugated to the enhanced green fluorescent protein (Gag-EGFP). Expression of the Gag-EGFP fusion has been designed to minimally impact the folding and self-assembly of the viral

[1] Belyakov, I. G. & Ahlers, J.D. What Role Does the Route of Immunization Play in the Generation of Protective Immunity against Mucosal Pathogens? *Journal of Immunology* (2009).

[2] Belyakov, I.G. & Berzofsky, J.A. Immunobiology of Mucosal HIV Infection and the Basis for Development of a New Generation of Mucosal AIDS Vaccines. *Immunity* (2004).

[3] Orive, G. *et al.* Cell encapsulation: Promise and progress. *Nature Medicine* **9**, 104–107 (2003).

[4] Bennett, Hunter R. Encapsulated Cell Based Therapeutics for the Prevention of HIV. *University of Washington Undergraduate Research Symposium*. 2012.

Gag protein into VLPs while also allowing facile fluorescent visualization of the particles. Once I successfully engineer 293T cells to express fluorescent VLPs, I will employ microscopy-tracking methods to visualize VLPs in cell and tissue models, and to follow cell proliferation over prolonged periods of time. These experiments are designed to provide proof-of-concept that our cell-based VLP delivery system can provide sustained exposure of HIV antigens and evaluate its potential as a novel VLP vaccine delivery platform. As a recipient of the Art Levinson Emerging Scholar's Award, I have been awarded funds to attend the 2013 fall BMES conference, where I plan to present my current results and also network with other researchers to discuss potential applications of cell-based vaccine delivery systems. Funding from the Goldwater Scholarship would provide the opportunity to extend my research and examine relevant *in vivo* applications of our cell-based delivery platform.

One *in vivo* application Dr. Woodrow and I are particularly interested in is simultaneously targeting HIV-1 VLPs and alginate microcapsules to the nasopharynx associated lymphoid tissue (NALT) to enhance the immune response to vaccination. Nasal vaccination has been shown to elicit long-lasting mucosal immunity at the gastrointestinal, vaginal, and rectal mucous membranes[5]. Within the NALT, microfold (M) cells demonstrate the ability to initiate potent, antigen specific humoral and cellular immune response[5]. In order to achieve this goal, we will increase mucosal exposure to VLPs by augmenting microcapsule retention within the NALT while simultaneously using ligands to target VLPs to M cells capable of inducing an immune response. In order to increase particle adhesion, we will decorate the surface of our alginate microcapsules with a protein ligand that binds to nasal epithelial cell surface proteins. We will derive our protein ligand from the surface proteins of the hemagluttinating virus of Japan (HVJ), which effectively binds to nasal epithelial cells prior to cellular evasion. VLPs can subsequently be targeted to M cell via modification with a recombinant form of the viral protein hemagluttinin σ1, which has been shown to effectively bind M cells in the NALT tissue. These modified VLPs will be more efficiently absorbed by M Cells, enhancing the delivery of our VLP antigens. I will then assess the *in vivo* ability of the targeted microcapsules to induce humoral and cellular immune responses in intranasally immunized mice. Mucosal samples from feces, vaginal washes and nasal washes will be sampled and IgA specific to HIV-1 Gag will be detected by ELISA. Finally, immunohistochemical techniques will be used to evaluate trafficking and effective targeting of VLPs in NALT tissues. By increasing mucoadhesion and targeting M cells within the NALT, we expect to see an overall increase in induced immune response upon intranasal vaccination, a promising result that will demonstrate the flexibility and potential of this platform to provide effective vaccination for pathogens that establish primary infections within mucosal tissues, such as HIV, gonorrhea and other sexually transmitted diseases.

The careful investigation of interactions between the mucosal immune system and mucosal drug delivery systems represents a critical step in translating developments in the burgeoning field of mucosal immunology into innovative clinical tools with diverse applications. Furthermore, this research could provide key insight into the dynamics of the mucosal immune response and help develop our understanding of a complex biological system.

My work in the Woodrow Lab has allowed me to develop my interest in immunology and has given me invaluable experience with hands on research skills. Over the last two and a half years I have learned complex experimental techniques and performed diverse scientific procedures. However, the most important things I have gained from my time in the Woodrow Lab are a passion for research and an appreciation for the way in which biomedical science seeks to transform the world. The challenge of identifying some of the most intractable and important problems in drug delivery and designing dynamic solutions captivates me. I am eager to pursue this passion through research in the Woodrow Lab, graduate school and beyond.

[5]Hiroshi, K. & Fukuyama, S. NALT- versus Peyer's Patch Mediated Mucosal Immunity. *Nature Reviews Immunology* (2004)