Self-Assembling Polymer Nanoparticles in Blood Components: Towards *In Vivo* Virus Capture

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Introduction: Hepatitis B (HBV) is a condition that is estimated by the World Health Organization to affect approximately 257 million people worldwide, and is linked to the development of cirrhosis and hepatocellular carcinoma.[1,2] Although several forms of treatment are available for chronic infection, seroclearance of hepatitis B antigens occurs in 11% or fewer cases of patients receiving treatment.[2] No antiviral treatment is indicated for acute infection.[1,2] A key feature of HBV replication is the production of subviral particles, composed only of envelope proteins and containing no viral capsid. These particles are produced in concentrations 1000 to 100,000 times higher than the virus particles.[3] They are speculated to lead to T-cell anergy and downregulation of inflammatory signaling pathways, preventing an effective immune response from occurring.[2]

Although a vaccine exists, the goal of a curative treatment exists to fully eliminate HBV infection. Additionally, a small percentage of people with HBV are properly diagnosed, and an even smaller percentage actually receive treatment.[1] To that end, we are developing a theranostic tool designed to meet the ASSURED criteria, which includes sensitivity, specificity, and equipment-free devices as goals for diagnostic technologies.[4] We are aiming to develop nanoparticles that will self-assemble *in vivo* around blood-borne pathogens and clear them from the body. This will be achieved by developing polymer amphiphiles that selectively bind to proteins on the viral envelope, targeted with antibodies or antibody fragments, and recruit additional polymers to form a polymer vesicle (or polymersome). Targeting proteins on the viral envelope would capture both virons and subviral particles, which should increase the efficacy of the immune response. Upon clearance from the body, captured virus particles could also be used for diagnosis, therefore providing both therapeutic and diagnostic capabilities.

Before this is possible, it is necessary to determine the influence of blood components on the self-assembly process, as well as the presence of targeting ligands attached to the amphiphiles before polymersome formation.

Methodology: Polymersomes formed by solvent injection of PEG-b-PLA and PEG-b-PLGA have been synthesized in blood components of varying concentrations, and imaged by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Increasing amounts of red and white blood cells and plasma have been separately examined, up to physiological concentrations, to confirm the formation of nanoparticles in blood components. Ultraviolet-visible (UV-vis) absorbance measurements have been made in various solutions to determine the critical micelle concentration (CMC) of polymers being used, and experiments are being conducted to confirm that this concentration is reached in a model system mimicking blood flow. Experiments are also being conducted to confirm the directed assembly of nanoparticles around a particle, using

nanoscale latex beads as an example particle. The beads are of similar size to the infectious and subviral particles, and are coated with the same antigens that would be present on HBV particles.

Results: TEM images have shown assembly of nanoparticles for both PEG-b-PLA injected into platelet-depleted blood plasma. Imaging was successful for 5% plasma samples, where polymersome formation could be clearly seen. The average particle size in plasma was 140 nm ± 19 nm, measured by ImageJ. These particles were consistently smaller than particles formed in physiological concentrations of salt, indicating that the presence of plasma proteins has an effect on the formation of polymersomes. Samples containing higher concentrations of plasma or red blood cells could not be imaged due to excessive film thickness. Other techniques are being explored for imaging these samples. SEM imaging of plasma samples was unsuccessful for all concentrations, and imaging of red blood cell solutions is ongoing with the development of new biologically relevant sample preparation techniques. Comparisons of the CMC values in different media may help explain the discrepancy in particle size. The CMC of PEG-b-PLA was found to be approximately 3.75 μg/mL in water and 3 μg/mL in physiological concentrations of salt. Measurements were repeated for PEG-b-PLGA, with a CMC of 3 μg/mL in water and 3.5 μg/mL in physiological salt solution. Absorbance measurements could not be taken for plasma samples due to similar critical absorbance wavelengths between the plasma and the polymer, so other methods are being tested. A tubular channel was 3D-printed along with a spacer that separates the channel into discrete sections and allows local concentration to be measured over time and length. This will be used to determine the impact of flow on polymersome formation and CMC, which will provide a model more closely resembling nanoparticle formation in real blood flow.

Conclusions: Preliminary results show that blood components impact the self-assembly process, though the exact mechanisms are still unknown. To explore the effect of these components, experiments are being conducted to determine the individual contributions to nanoparticle size, morphology, and the minimum requirements for formation. The effect of flow on nanoparticle formation will also be explored, and, taken as a whole, these results form a model of blood flow that can be used to investigate the self-assembly process of polymers amphiphiles injected into the body. Additional studies will be done on amphiphiles with attached ligands, with the ultimate goal of controlled, targeted self-assembly for encapsulation of hepatitis B molecules.

References:

1. World Health Organization. “Hepatitis B”, https://www.who.int/news-room/fact-sheets/detail/hepatitis-b (accessed March 5, 2019).
2. Yuen, M.-F.; Chen, D.-S.; Dusheiko, G. M.; Janssen, H. L. A.; Lau, D. T. Y.; Locarnini, S. A.; Peters, M. G.; Lai, C.-L. Hepatitis B virus infection. *Nature Reviews Disease Primers*, **2018**, *4*, 1-20.
3. Chai, N.; Chang, H. E.; Nicolas, E.; Han, Z.; Jarnik, M.; Taylor, J. Properties of Subviral Particles of Hepatitis B Virus. *Journal of Virology,* **2008**, *82*, 7812-7817.
4. Wu, G.; Zaman, M. H. Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. *Bulletin of the World Health Organization,* **2012**, *90*, 914-920.