ABSTRACT

Biopharmacauticals, such as insulin, monoclonal antibodies, growth hormones, and vaccines, have emerged as a major class of therapeutic molecules. Subcutaneous administration of biotherapeutics is a convenient drug delivery method that is less invasive; requires shorter clinic times; improves patient compliance; and reduces cost to the healthcare system compared to intravenous administration. The mass transport of a therapeutic injected into the subcutaneous tissue is dictated by physiochemical properties of the molecule such as size and electrostatic charge. Bioavailability and efficacy of the therapeutic formulation depend on efficient transport of the molecule from the injection site to lymphatic or blood vessels. The injected biotherapeutic needs to traverse complex structures of the subcutis and the extracellular matrix (ECM) before it arrives at the update site. In vitro transport screening platforms provide insights into the effects of tissue and therapeutic properties on macromolecular transport through biological barriers.

In this work, we develop an *in vitro* Transwell macromolecular recovery platform, an economical and high-throughput method that can be used to systematically evaluate effects of ECM components on mass transport properties of macromolecules. In the first part of the dissertation, we engineer subcutaneous tissue models based on collagen type I ((Col I), the most abundant fibrillar protein in the subcutaneous ECM), and hyaluronic acid ((HA), an anionic and highly viscous polysaccharide). First, we optimize protocols to reproducibly fabricate Col I and combined Col I and HA (ColHA) hydrogels. Collagen is a naturally sourced material; thus, inherent variabilities can occur batch to batch. In the following work, we establish a workflow to characterize collagen material from new sources (animal sources, different vendors, and between batches of identical material). We then characterize ColHA material properties, such as component retention and microstructure, across a large number of sample fabrication parameters. ColHA materials are highly tunable due to the large number of fabrication parameters (HA concentration, HA molecular weight (MW), collagen polymerization temperature) that could be altered. We developed physics-based Bayesian inference model to efficiently characterize large design spaces.

Next, we developed and optimized the high throughput Transwell platform, and we screened the transport of macromolecules. Transport of macromolecules which are representative of current therapeutics in subcutaneous injections was examined. We demonstrate that macromolecular transport within Col type I (Col I), blended collagen I and II (Col I/II), blended Col I and III (Col I/III), and combined Col I and HA hydrogels (Col(I)HA) hydrogels is inversely

related to the hydrodynamic radius of the diffusing macromolecules. Col II and III resulted in altered fibril morphologies (smaller fibril formation) of the blended gels which resulted in decreased molecular mass recovery rate. Increasing HA concentration within the Col I hydrogels decreased macromolecular recovery. This decrease is mainly a consequence of increased viscosity within the matrix. Recovery rates of large molecules such as immunoglobulin G (IgG), a molecule similar in size to therapeutic antibodies, were highly sensitive to HA concentration in Col hydrogels. Smaller molecules, such as myoglobin and lysozyme, that are similar in size to insulin experience electrostatic effects as HA concentration increases within Col I gels. Recovery of macromolecules in HA solution was a function of both electrostatic and steric interactions. The results from the studies were highly reproducible, highlighting the robustness of the optimized assay.

Our results thus demonstrate that the Transwell platform can be utilized for systematic evaluation of therapeutic transport as a function of molecular characteristics. The results presented can inform biotherapeutic physiochemical properties for efficient transport within the subcutaneous tissue.