**Impact of surface chemistry on lipid nanoparticle targeting and transfection**

by

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Gene therapies offer vast therapeutic potential to treat and cure diseases that impact the quality of life of millions of people worldwide and have evaded treatment by other modalities. Synthetic nanoparticles, such as lipid nanoparticles (LNPs), can be engineered to facilitate the encapsulation, protection, and delivery of nucleic acid cargos to cells and tissues. However, targeted gene delivery with high specificity to certain cell types remains a critical challenge within the landscape of nanoparticle design.

Among the panoply of diseases that stand to benefit from the targeted delivery of gene therapies, hemoglobinopathies such as sickle cell disease (SCD) can be treated permanently through genetic editing of particular cells. SCD arises due to mutations in the gene encoding hemoglobin, a protein in red blood cells responsible for facilitating gas exchange throughout the body. Mutated hemoglobin creates “sickled” or half-moon shaped red blood cells, which cause a broad range of acute and systemic complications in SCD patients.

Hematopoietic stem and progenitor cells (HSPCs), a rare and quiescent population of cells resident in the bone marrow, are responsible for differentiating into all blood lineages, including impacted red blood cells. Therefore, HSPCs are a highly valuable therapeutic target for long-term treatment of these diseases. Nonetheless, the relative inaccessibility and quiescence of these cells make them a challenging target.

Nanoparticle surface chemistry is a powerful parameter that can be engineered to drive targeted and efficacious transfection, as well as stealth from off-target interactions. In particular, layer-by-layer (LbL) electrostatic self-assembly enables the modular design of polymer-based surface chemistries with affinities to particular tissues and cells. **The over-arching objective of this thesis is to investigate the impact of surface chemistry variation on the capacity of nano-systems, such as LNPs and polyplexes, to facilitate delivery of nucleic acid cargos to target cells, including HSPCs.**

In this body of work, we design and delineate the layered lipid nanoparticle (LLNP), or an LNP construct with an electrostatically-bound outer polyanion coating deposited via LbL techniques. Libraries of LLNPs with outer chemistries, including polysaccharides, homopolypeptides, and acrylic polymers, demonstrate significant variations in association, trafficking, and transfection of immune cells *in vitro* and critical tissue types *in vivo*.

We next apply the LLNP platform for the targeted transfection and gene editing of hematopoietic stem and progenitor cells (HSPCs) for the curative treatment of sickle cell disease. To accomplish specific editing, targeting antibodies (Abs) are attached to LLNP acrylic surfaces, chosen for their stealth properties in intravenous contexts. Targeted

Ab-LLNPs achieve high transfection specifically of less differentiated cell subpopulations in the bone marrow, and gene editing *in vitro* and *in vivo* in humanized mice. Furthermore, LNP cores are systematically optimized using design-of-experiment methodologies to achieve meaningful HSPC transfection. To improve Ab-LLNP access to HSPCs, bone marrow cryogels that recruit HSPCs from the bone marrow are evaluated in parallel, ultimately achieving similar therapeutic gene editing *in vivo* in humanized mice.

LLNPs are additionally evaluated for targeted delivery to ovarian cancer, a fatal gynecological cancer with poor survival outcomes. LLNP surface chemistries of polysaccharides or homopolypeptides are screened for efficient and specific transfection of ovarian tumor cells and tissues, in mouse models of metastatic ovarian cancer. LLNPs with polypeptide outer layers drive two-fold increases in tumor accumulation and transfection, preferential transfection of tumor cells over immune cells, and significant CRISPR-Cas9 gene editing of oncogene *PLK1*.

Beyond therapeutic applications, we illustrate the utility of the outer layer as an alternative to PEGylation, or inclusion of poly(ethylene glycol) (PEG) in LNPs. While PEGylation confers stability and circulation benefits to many therapeutics, it can hinder gene delivery by reducing cellular association and cargo release and inducing production of anti-PEG antibodies that drive rapid clearance of subsequent doses. We demonstrate that addition of an outer polyanion coating stabilizes non-PEG LNP formulations against biological stresses. Furthermore, non-PEGylated LLNPs exhibit improved transfection to PEGylated LNPs *in vitro*, and marked extrahepatic transfection upon systemic administration.

Ultimately, this thesis illustrates the many ways LNP surface chemistry can be engineered for advantageous nucleic acid delivery. Here, we develop a layered LNP platform that enables the modular design of various surface chemistries. We use this approach to deliver gene editing cargos to stem cells and ovarian tumors, as well as stabilize non-PEGylated LNP systems.

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