

Gene Delivery Nanomedicine Platform

Gene therapy and nanoparticle (NP)-based drug delivery have revolutionized therapeutic strategies, offering new solutions for previously untreatable diseases. Despite recent advances, the challenge remains to develop scalable, non-viral delivery systems tailored to both the pathology and the specific therapeutic payload. In our laboratory, we work towards optimizing microfluidic-based methods to formulate lipid-based nanoparticles for different applications, demonstrating how fine-tuned formulations can act as platforms for multiple diseases. As a first example of our work, we designed lipid nanoparticles (LNPs) for CRISPR/Cas9 delivery to hematopoietic stem cells to treat neurometabolic disorders. The design started by using a model enzyme (β -glucosidase) that mimics the size and charge of the ribonucleoprotein (RNP) complex. Key formulation parameters such as lipid composition, flow rate ratio (FRR), and total flow rate (TFR) were screened, leading to 16 promising formulations. Following enzyme encapsulation, 9 LNPs maintained monodispersity, and the highest-performing system, named SCD5, exhibited an 87% uptake in hematopoietic stem cells at 100 $\mu\text{g}/\text{mL}$, with a good safety profile. When the RNP complex was used, we achieved an encapsulation efficiency exceeding 50%, suggesting that this formulation can be a valid tool for gene editing. As a second example, we have been working for years on cholesterol (CHOL)-based NPs for Huntington's Disease (HD) treatment, as CHOL restoration is beneficial for symptomatic HD treatment. We recently optimized a microfluidic method to maximize CHOL content in NPs while minimizing surfactants, ensuring stability and high brain-targeting efficiency while also working with a readily scalable protocol for industrial development. Furthermore, to integrate a gene therapy approach, we incorporated a cationic CHOL derivative (DC-CHOL) for RNA loading, identifying promising candidates with high siRNA and mRNA loading. These studies exemplify how microfluidic methodologies enable precise control over NP formulation, allowing customization for disease-specific applications. By optimizing systems for distinct payloads, we establish adaptable platforms that can be further expanded for various genetic and metabolic disorders, bridging the gap between laboratory research and clinical translation.