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Optimizing lipid nanoparticle (lnp) delivery systems for mRNA-based therapeutics: A translational approach

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Statement of the Problem: The success of mRNA-based vaccines has highlighted the critical role of lipid nanoparticles (LNPs) as delivery vectors. However, challenges such as cellular uptake, endosomal escape, and biodegradability limit their broader therapeutic use. This research aims to optimize LNP formulations for improved delivery efficiency, stability, and target-specific mRNA expression in vivo.

Methodology: LNPs were formulated using microfluidic mixing, incorporating ionizable lipids, cholesterol, DSPC, and PEG-lipids. Several formulations varying in lipid composition and molar ratios were evaluated. Encapsulation efficiency was quantified using RiboGreen assays, and particle size was measured using dynamic light scattering (DLS).In vitro studies were conducted in HEK293 and HeLa cell lines to assess mRNA transfection efficiency via luciferase reporter expression. Cytotoxicity was evaluated using MTT assays. In vivo biodistribution studies were carried out in murine models using fluorescently labeled LNPs. Tissue-specific mRNA expression was measured using qRT-PCR and in vivo imaging.

Results: Among the tested formulations, LNP-4 (with an ionizable lipid:cholesterol:DSPC:PEG-lipid ratio of 50:38:10:2) exhibited the highest encapsulation efficiency (95%) and optimal particle size (~80 nm). LNP-4 demonstrated superior mRNA expression in both in vitro cell lines and in vivo liver tissues with minimal cytotoxicity. Biodistribution studies confirmed targeted delivery to hepatic and splenic tissues, with reduced off-target accumulation compared to other formulations.

Conclusion: This study successfully identified and optimized a lipid nanoparticle formulation that enhances mRNA delivery and expression while maintaining safety. The findings support the translational potential of LNPs in the development of next-generation mRNA therapeutics beyond vaccines, including in oncology and rare genetic disorders.