Optimization of Lipid Nanoparticle Formulation for Delivery of Large RNA-Based Therapies to Cartilaginous Cells

Rozenberg, Mia (School: Pine Crest School)

Osteoarthritis (OA) is a chronic inflammatory joint disease affecting over 500 million people, leading to cartilage degeneration and severe pain. Current treatments are invasive, costly, and fail to address the underlying disease. Large self-amplifying mRNA (9Kb+) therapies present a viable alternative, enabling sustained therapeutic protein production with smaller, less frequent doses. However, no RNA-based treatments for OA exist, and effective delivery to synoviocytes and chondrocytes remains a major challenge due to densely packed cartilage and the extracellular matrix, which limit transfection. This research is the first to optimize lipid nanoparticles (LNPs) for large RNA delivery to cartilaginous cells, systematically varying ionizable lipid percentage, nitrogen-to-phosphate (N:P) ratio, PEGylation, and phospholipid type. LNPs were synthesized using a modified vortex-based method and a luciferase encoding large mRNA, with dynamic light scattering confirming particle sizes of 90-220 nm, polydispersity of 0.09-0.3, and a quantifluor assay determining RNA encapsulation efficiency of 87-97 percent. Transfection efficiency was assessed via luminescence assay 24 hours post-treatment. Results identified distinct optimal formulations for joint cells, demonstrating that 30-40% ionizable lipid, 1.5% PEGylated lipid, DOPE, and N:P ratio of 17:1 maximized transfection. Notably, reducing ionizable lipids from 50 percent not only improved transfection to joint cells but also lowered costs by 45%, enhancing the potential for clinical translation. These findings provide the first systematic optimization of LNPs for joint cell transfection, addressing a critical barrier to OA therapies and offering a scalable, cost-effective alternative to current treatments.

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