Precision delivery of siRNA using Human Transferrin Receptor-targeted lipid nanocarriers: linking nanoscale structure to in vivo performance

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RNA interference (RNAi)-based gene therapy is increasingly recognized as a promising personalized strategy for cancer treatment. At the core of this approach are small interfering RNAs (siRNAs), which enable selective gene silencing and precise inhibition of disease-associated proteins. Despite its potential, the clinical translation of siRNA therapeutics remains limited by the need for efficient delivery systems capable of crossing biological barriers—particularly the cell membrane—while protecting siRNA molecules from enzymatic degradation.

To overcome these challenges, we developed lipid nanocarriers (LNCs) engineered for enhanced delivery efficiency and biocompatibility. The formulation incorporates POPC, a naturally occurring phospholipid that improves membrane compatibility; DODMA, an ionizable lipid that promotes efficient siRNA encapsulation; cholesterol, which enhances bilayer stability; DOPE-PEG2000-maleimide, which reduces immune recognition and allows covalent conjugation of the CGSG-T7 peptide (CGSG-HAIYPRH), targeting the human Transferrin Receptor (hTfR). This strategy is expected to enhance LNCs accumulation at the disease site, thereby increasing therapeutic efficacy.

The physicochemical properties of the LNCs were comprehensively characterized. Dynamic Light Scattering (DLS) and Small-Angle Neutron Scattering (SANS) measurements indicated a hydrodynamic diameter of 91 ± 1 nm and a bilayer thickness of 0.46 ± 0.01 nm. ζ-potential revealed a positive surface charge under acidic conditions (10.6 ± 0.2 mV), reflecting the pH-responsive behavior of the LNCs, driven by protonation of the ionizable lipid DODMA at lower pH, consistent with the tumor microenvironment. In addition, Electron Paramagnetic Resonance (EPR) spectroscopy provided detailed insights into the structural organization of the lipid bilayer.

Successful incorporation of siRNA into the functionalized LNCs was confirmed by agarose gel electrophoresis and fluorescence spectroscopy (E.E. = 79% ± 4), complemented by fluorescence anisotropy to probe changes in the local microenvironment and mobility of the fluorescent probe associated with encapsulation.

In parallel, biological assays demonstrated a significant reduction in GFP fluorescence, confirming successful siRNA delivery and biological activity. Furthermore, in vivo experiments were conducted on animal models to evaluate biodistribution and uptake, with particular focus on ocular administration as a route to investigate LNCs penetration and transport across biological barriers.

These findings provide a strong foundation for the development of advanced siRNA delivery platforms and support their future translation into targeted cancer therapies.

Keywords:
siRNA Delivery, Lipid Nanocarriers (LNCs), Targeted Therapy (hTfR), Endosomal Escape, Ocular Biodistribution

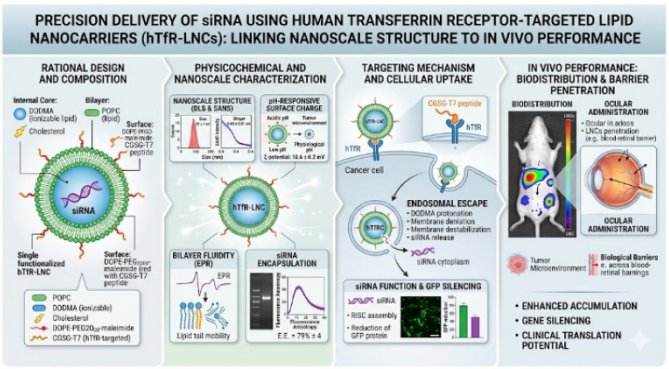

Figure 1. Structure-property relationships in hTfR-LNCs. Schematic linking nanoscale architecture to in vivo siRNA delivery. Highlights include pH-responsive targeting, endosomal escape, and ocular gene silencing. (AI-generated).