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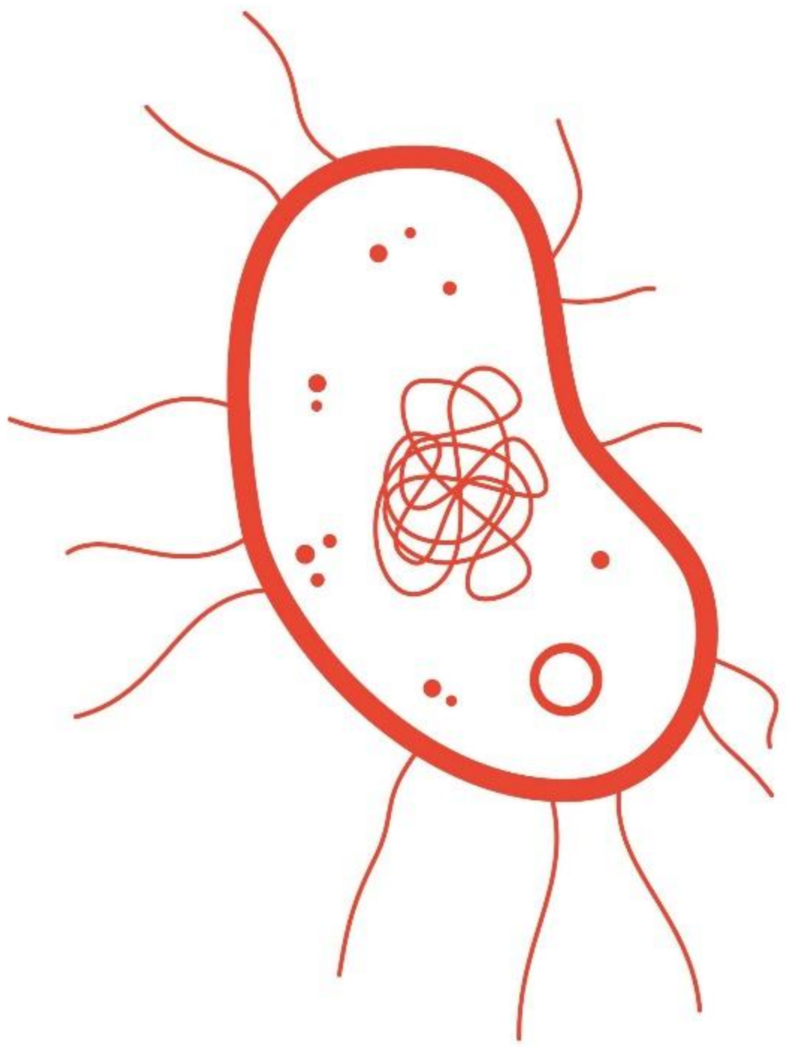

Non-Viral Gene Delivery with Lipid Polymer Hybrid Nanoparticles

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Bachelor-Thesis, Field of Study: Bioanalytic and Cell Biology

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INTRODUCTION

mRNA therapies show promise for vaccines and protein replacement but require efficient delivery systems due to their instability and membrane impermeability. Lipid nanoparticles (LNPs) are currently considered the gold standard for mRNA delivery but show limitations, particularly due to cytotoxic effects and the risk of triggering immune responses.

To overcome these drawbacks, lipid–polymer hybrid nanoparticles (LPNPs) have been developed as a next-generation delivery system. These consist of a lipid-mRNA complex as the core, encapsulated in a PLGA-based polymer shell, combining biocompatibility, mechanical stability, and enabling controlled mRNA release.

The aim of this work was to compare LNPs and LPNPs using different optimized formulations and evaluate their cytotoxicity, while also assessing mRNA retention and transfection functionality.

METHODS

LNPs/LPNPs were formulated via microfluidics with optimized ratios and analyzed by dynamic light scattering (DLS) to confirm suitable particle size and polydispersity.

Cytotoxicity was tested in HepG2 and SH-SY5Y cells using MTT and Resazurin. Due to variability, MTT was used with 12-step dilutions starting at 0.2 mg/mL. For SH-SY5Y, 0.02 mg/mL was used due to higher sensitivity.

mRNA retention was assessed via agarose gel electrophoresis and quantified using a standard curve. Cells were transfected with mCherry-mRNA NPs and analyzed by confocal microscopy. Calcein-AM and Hoechst 33342 were used to assess viability and morphology.

RESULTS

Physicochemical Characterization: DLS confirmed size and dispersity differences between LNPs and LPNPs. The 40x formulation was used for LNPs, and formulation B for LPNPs due to optimal size and low polydispersity.

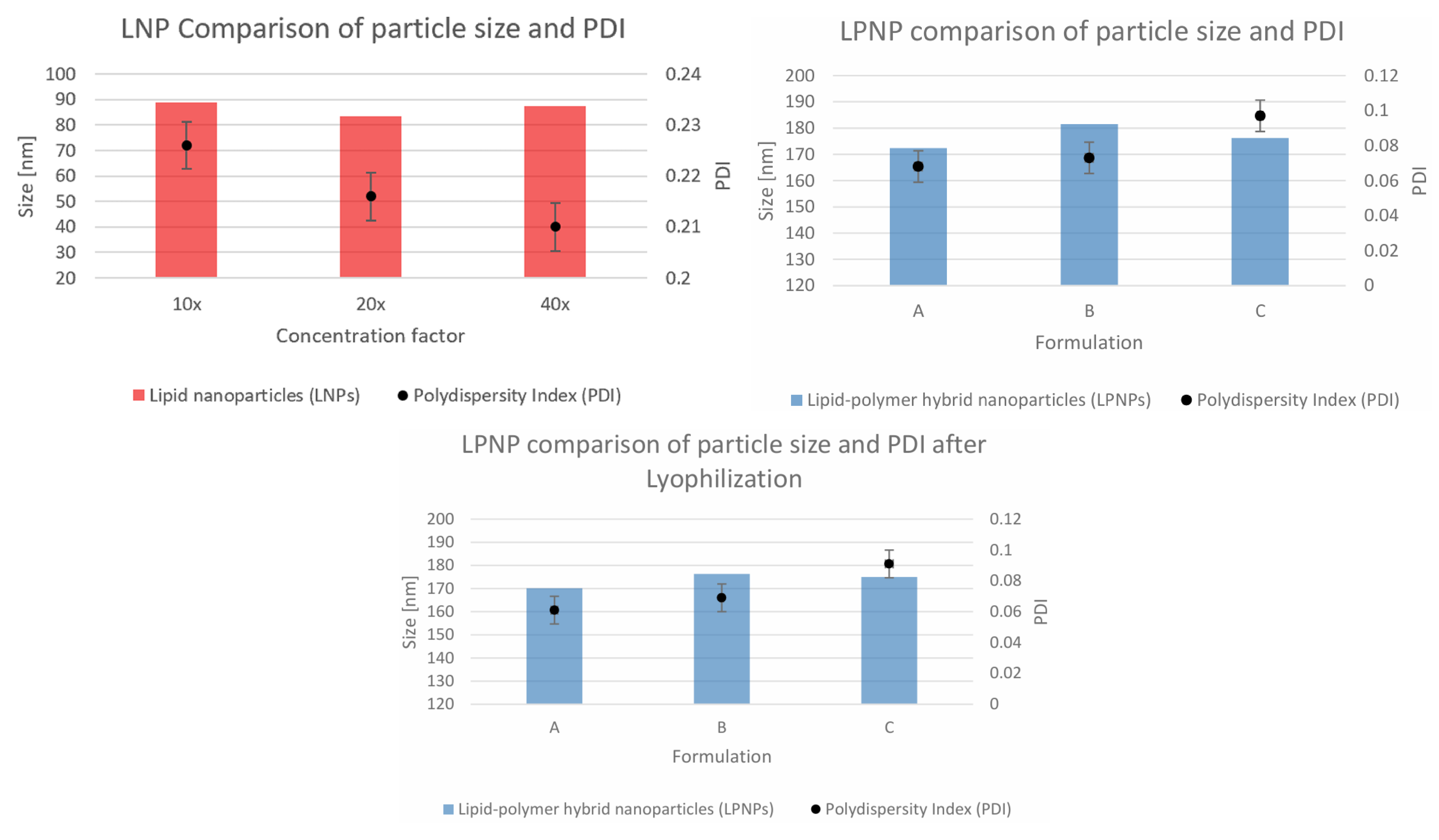

Figure 1:LNPs measured at 10x, 20x, and 40x showed sizes of 88.8, 83.4, and 87.5 nm with PDIs of 0.226, 0.216, and 0.210. LPNP formulations A–C (pre-lyophilization) had sizes of 170.4, 176.3, and 174.0 nm and low PDIs (0.065–0.095), indicating monodispersity. After lyophilization, sizes slightly increased (172.1–184.2 nm), with stable PDIs (0.069–0.100).

Cytotoxicity Evaluation: The cytotoxicity results showed that LPNPs consistently exhibited lower toxicity than LNPs across nearly all tested concentrations.

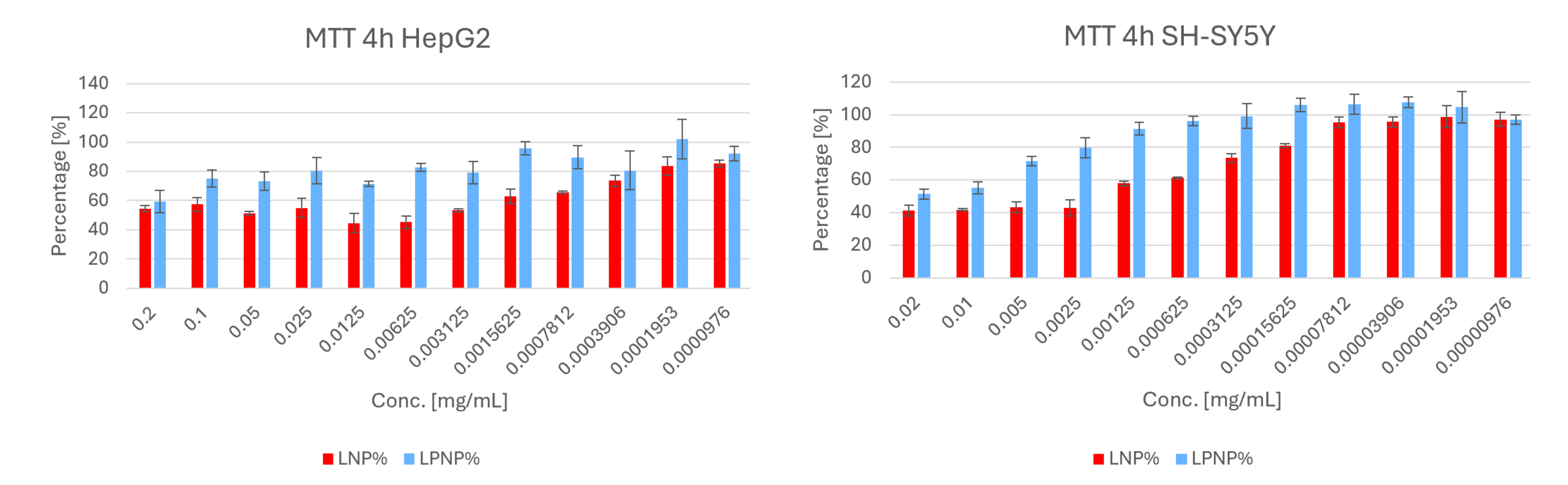

Figure 2: In HepG2 cells, the highest tested concentration (0.2 mg/mL) resulted in 54.5 % viability for LNPs and 59.1 % for LPNPs. LPNPs showed a stronger recovery, peaking at 95.8 % at 0.000153 mg/mL, while LNPs reached 85.5 % at the lowest dose. SH-SY5Y cells showed a more pronounced difference: at 0.02 mg/mL, LNPs reached 41.3 % and LPNPs 51.4 %. Across all concentrations, LPNPs remained less toxic, with both systems showing high viability at low doses.

mRNA Retention: Agarose gel electrophoresis quantified mRNA loss during nanoparticle formulation.

Table 1: Various samples were analyzed to quantify lost mRNA. The LPNP purge sample showed the highest mRNA loss (100.7 ng; 16.3 %). For LNPs, 98.0 ng and 31.1 ng were detected (4.8 % and 5.0 % loss). No signal was found in the LPNP supernatant.

Sample	Calculated band intensity	Calculated mRNA amount [ng]	Total volumes [µL]	Sample volumes [µL]	mRNA unencapsulated/lost	Total mRNA loss [%]
mRNA stock	61480.14	300			-	-
LPNP purge	26787.94	100.722218	110.23	15	0.740174009	16.3%
LPNP supernatant	7203.267	-11.775396	2965	15	-2.327603278	-51.3%
LNP leftover	26317.75	98.0213517	33.5	15	0.218914352	4.8%
LNP purge	14667.03	31.0976868	110.21	15	0.228485071	5.0%

Transfection: Transfection with mCherry-mRNA-loaded nanoparticles

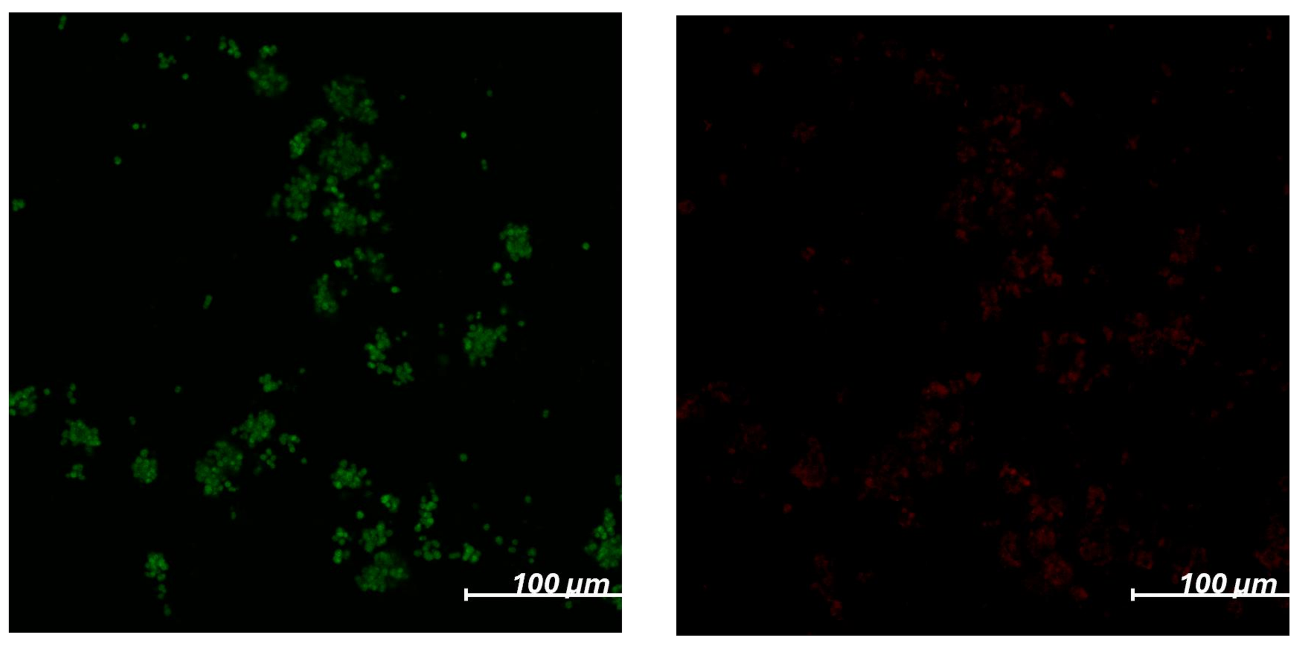

Figure 3: 4.56 ng mRNA per 0.02 mg/mL nanoparticles were used. Calcein-AM stained viable cells (green); mCherry fluorescence (red) indicated expression. A weak transfection signal was observed for LNPs before lyophilization. All other tested formulations (LNPs after lyophilization, LPNPs before/after lyophilization) showed no detectable signal under the tested conditions, indicating limited transfection efficiency.

CONCLUSION

LNPs and LPNPs were successfully formulated and characterized. As expected, LPNPs showed markedly lower cytotoxicity, likely due to their biocompatible PLGA shell, which reduces membrane interaction and cellular stress. Both systems retained mRNA to a measurable extent, but only minimal transfection was observed in LNP-treated cells. These findings highlight LPNPs as a less toxic and structurally promising alternative to conventional LNPs, while underlining the need for improved delivery conditions to enhance transfection efficiency.

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