Optimization of lipid nanoparticle formulation for delivery of RNA-based therapies to joint cells

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Introduction

- Osteoarthritis (OA) is a chronic joint disease characterized the degeneration of cartilage, affecting over 500 million people[1].
- The densely packed nature of the extracellular matrix (ECM) at the joint creates challenges for drug delivery to chondracytes[3]

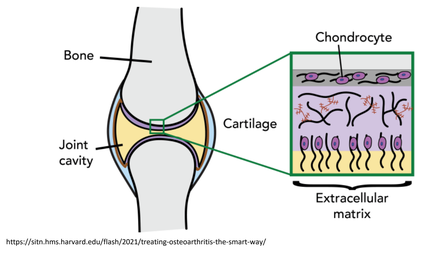


Fig 1. Diagram of knee joint and anatomy.

- RNA therapies utilize the central dogma to enable in host translation of treatment-oriented proteins
- Lipid Nanoparticles (LNPs) protect and encapsulate nucleic acids, facilitating effective cellular delivery of RNAs[2]. Optimal LNP formulation varies based on target cells and the therapy[4].
- There is little research on optimal LNP to deliver large RNAs (9kb+) to chondrocytes and synoviocytes.

Research Objective: Optimize LNP formulation for delivery of large RNAs to synoviocytes and chondrocytes isolated from immature bovine joints in 2D cultures.

Methods

(1) LNP Compositions and Synthesis

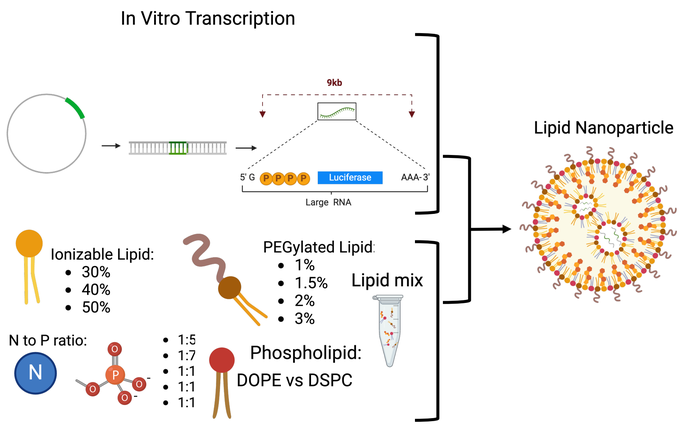


Fig 2. RNA-LNP System Synthesis. Luciferase encoding plasmid is in vitro transcribed into RNA and used to create LNPs of various lipid compositions via the vortexing method.

(2) LNP Characterization

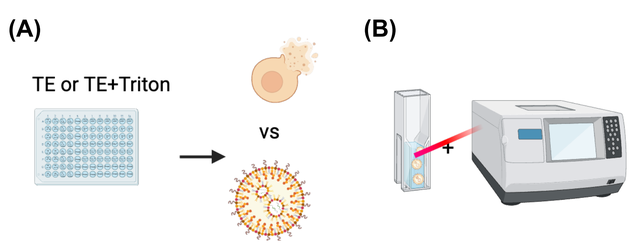


Fig 3. LNP characterization. (A) Quantiflour was used to determine encapsulation percentage of the RNA-LNP systems. **(B)** Dynamic light scattering (DLS) was used to determine the size and polydispersity (PDI) of the systems.

(3) Cell Treatment & Transfection

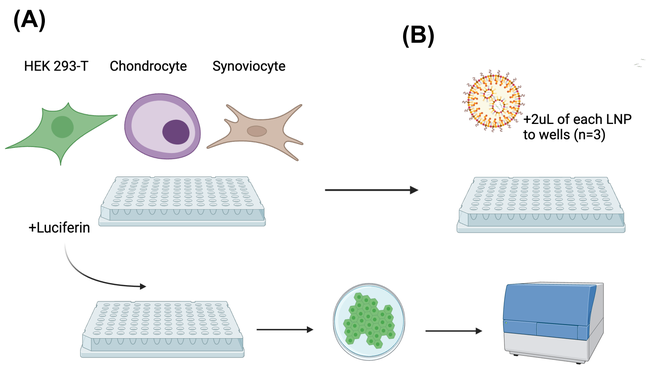


Fig 4. Cell Treatment and Transfection. (A) Cells were seeded at 10-20k cells/well and treated with 2uL of each LNP (+pos/neg controls) after 24/48 hours. **(B)** Transfected cells were incubated for 24 hours and then treated with luciferin for 10 minutes prior to taking a luminescence reading using a plate reader.

Characterization Results

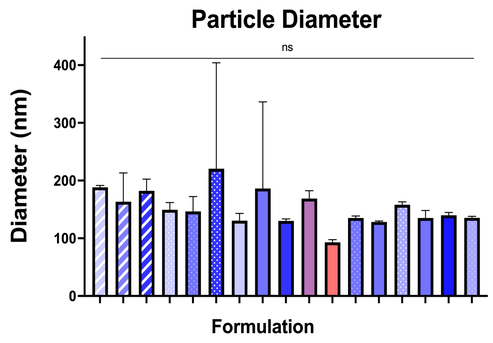


Fig 5. Graph of Diameter. No significant variation or trend across. One-way ANOVA, n=3.

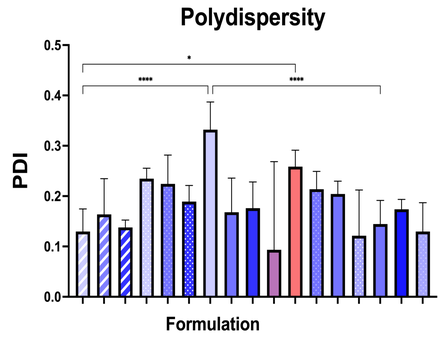


Fig 6. Graph of PDI. Significant variation across three formulations. No consistent trend between variations. One-way ANOVA, n = 3. P-values (* =<.05. **** =<.0001).

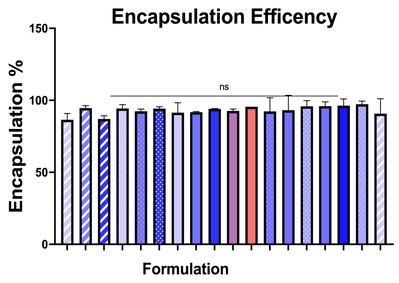
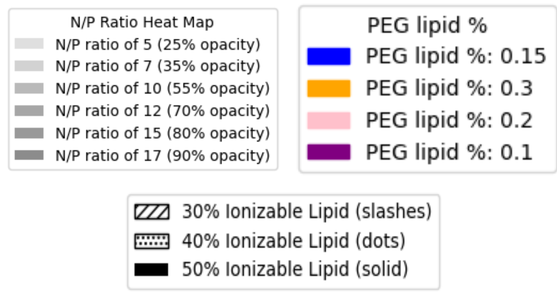


Fig 7. Graph of Diameter. No significant variation or trend across. One-way ANOVA, n = 3.



Transfection Results

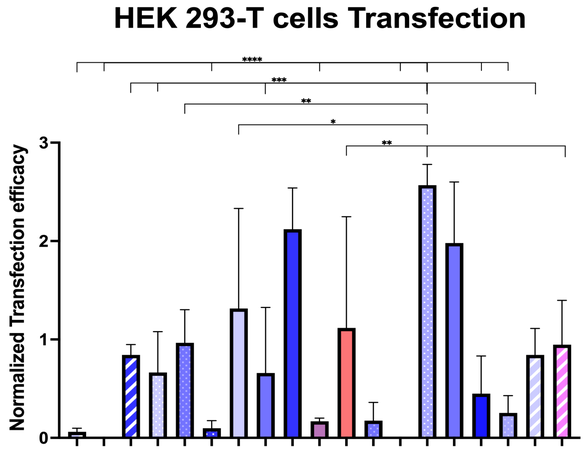


Fig 8. HEK 293-T cell transfection. The LNP with highest transfection efficacy was 10:1 n/p, 50% ionizable lipid, 1.5% PEG lipid, and used DOPE – Diameter: 135 nm, PDI: 0.0145, EE: 93%. One-way ANOVA, Tukey post-hoc test, n = 3. P-values (*=<.05, **=<.01, ***=<.001, ****=<.0001)

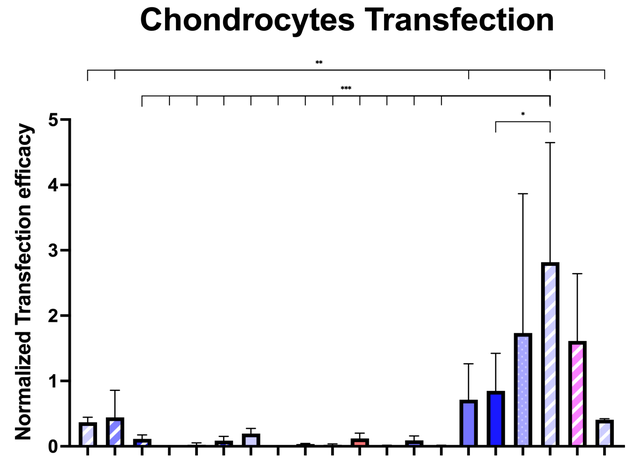


Fig 9. Graph of Chondrocyte transfection. The LNP with highest transfection efficacy was 12:1 n/p, 40% ionizable lipid, 1.5% PEG lipid, and used DOPE – EE: 96%. One-way ANOVA, Tukey post-hoc, n = 3. P-values (*=<.05, **=<.01, ***=<.001)

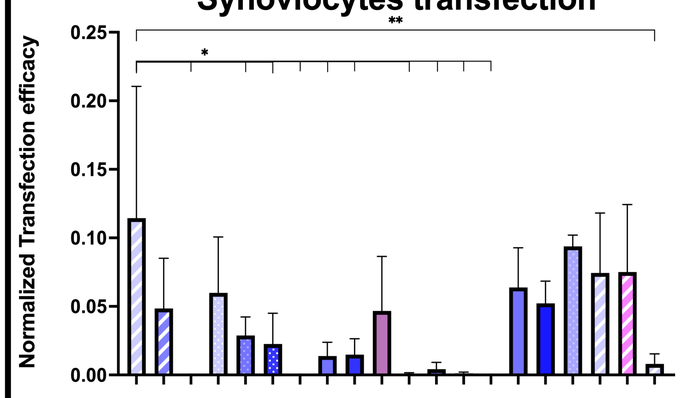


Fig 10. Graph of Synoviocyte transfection. The LNP with highest transfection efficacy was 5:1 n/p, 30% ionizable lipid, 1.5% PEG lipid, and used DOPE – Diameter: 188 nm, PDI: 0.013, EE: 86%. One-way ANOVA, Tukey post-hoc, n = 3. P-values (*=<.05, **=<.01)

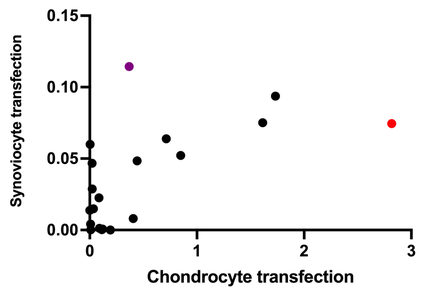


Fig 11. Graph of Synoviocyte (Y-axis) and chondrocyte(X-axis) transfection efficacy by formulation. Greatest Magnitude (Δ=1.94 – marked red) = optimal formulation: 12:1 n/p, 40% ionizable lipid, 1.5% PEG lipid, and used DOPE – EE: 96%. Optimal for synoviocyte in purple. N=3 for values.

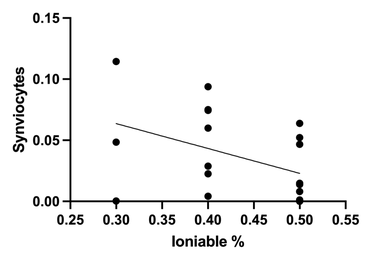


Fig 12. Linear regression with Ionizable lipid percent. Significant inverse relationship between lipid percentage and transfection efficacy. P-value = 0.0073 (***), n=3 for transfection values.

Discussion

Results provide insight into LNPs for enhanced delivery of large RNAs to chondrocytes and synoviocytes which seeks to support future OA research and treatment.

- The LNP with the greatest efficacy for both chondrocytes and synviocytes was statistically comparable to the individual optimal formulations:

Combined: **Chondrocyte/synviocyte:**

- 40% Ionizable Lipid → 40%/30% ionizable lipid
- N/P ratio 12:1 → N/P ratio 12:1/5:1
- PEG lipid 1.5% for all
- DOPE for all

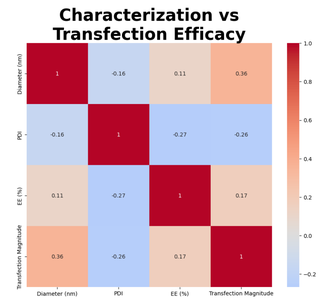


Fig 13. Correlation matrix between synoviocyte and chondrocyte transfection characterization data. Illustrates no strong relationships.

- Data analysis suggest for optimal delivery to synoviocytes and chondrocytes:
  - 30%-40% ionizable lipid is preferable to the standard 50%
  - Optimal PEG lipid content is 1.5%
  - DOPE was preferable to DSPC
  - N to p ratios for seems to have little effect on transfection
- Differences between cell transfection efficacies may be due to cell size, shape, and cellular metabolic activity, underscoring the need to optimize LNPs for specific cell types.

Limitations

- The volume of variables led to multidimensional results which are difficult to perform simple analysis on
- Cell culture was often unpredictable and timing confluency before treatment was not possible for all cell lines.

Future Work

- Multidimensional analysis on current data is still needed to fully understand complete effects of each variable
- Further data analysis will be conducted to better understand variables and components of optimal formulations
- 3D culture and in vivo treatment will be needed in the future to assess transfection with the added challenge of the ECM
- Further LNP compositions could be tested: analyzing the effect of different ionizable lipids or synthetic strategies

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Acknowledgements

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