Library of Gene Delivery Systems: Delivery of Genetic Material via Polyplexes Embedded in Thermosensitive Hydrogels

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Introduction

Gene therapy represents a promising strategy for the treatment of cancer, chronic and rare diseases[1]. However, the limited cellular uptake of naked genetic material necessitates the development of efficient delivery systems. In this work, cationic polymer-based polyplexes will be coated with hyaluronic acid (HA) to target CD44-overexpressing tumour cells and will be encapsulated within a dual-crosslinked, stimuli-responsive hydrogel for localised and controlled release[2].

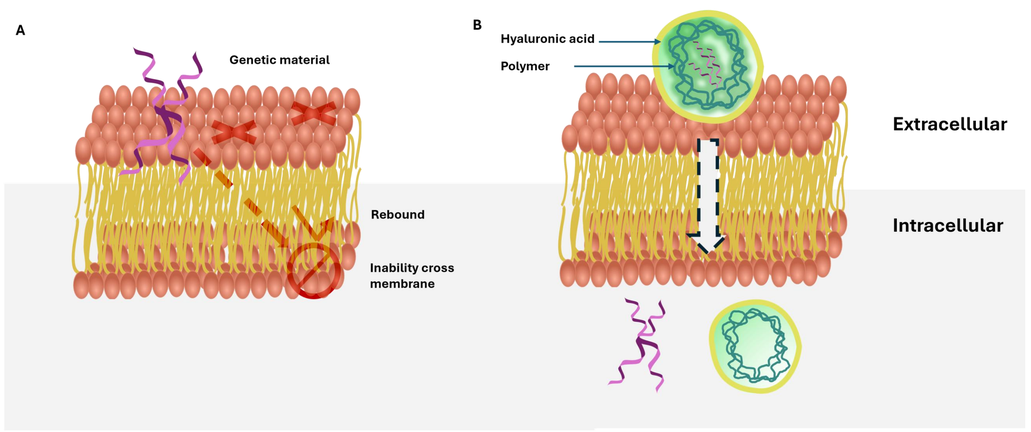

Figure 1: Gene therapy. A. Naked genetic material (purple single helices) incapable of crossing the membrane. B. Genetic material (miRNA) delivered through polyplexes, reaching the intracellular side of the membrane.

Methodology

- Formulation of polyplexes, lipo-polyplexes and lipoplexes

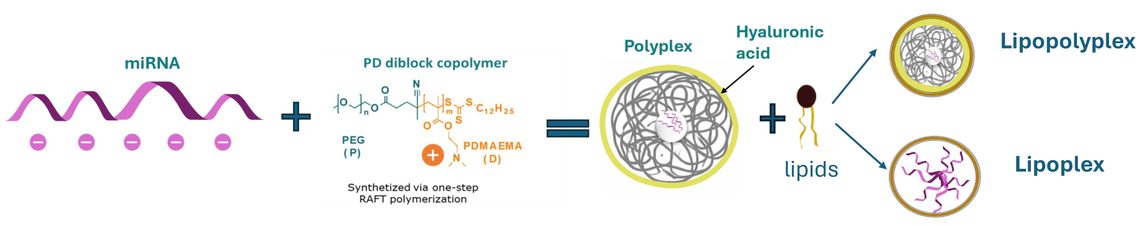

Figure 2: Negatively charged miRNA, complexed with cationic polymer (PEG-PDMAEMA) forming a polyplex, embedded in hyaluronic acid. Subsequently, hybrid complexes with lipidic shells will be formulated.

- Controlled thermosensitive release.

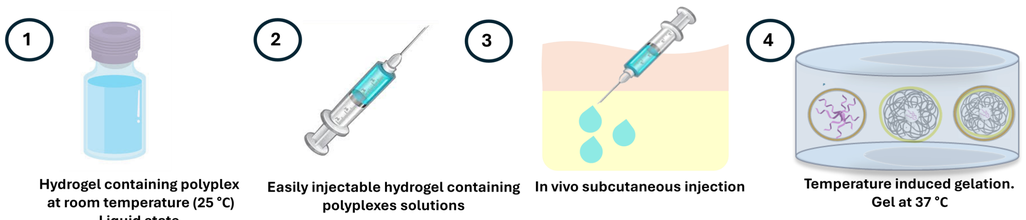

Figure 3. Encapsulation of polymeric complexes within a thermosensitive hydrogel enabling easy administration for in situ gelation.

- Formulation of lipid nanoparticles.

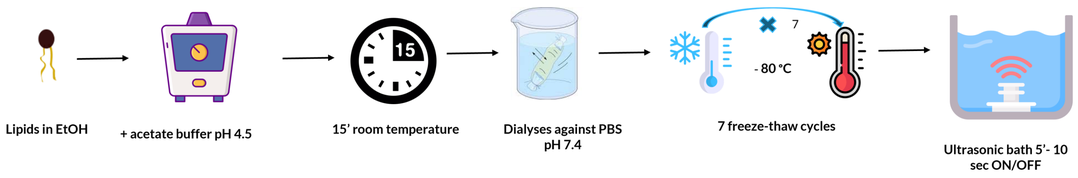

Figure 4. Schematic of the lipidic nanoparticles formulation by **solvent exchange**.
Lipidic formulation tested: SM_{-102}: DSPC: cholesterol: PEG-DMG-$_{2000}$ (50:10:38.5:1.5 mol %).

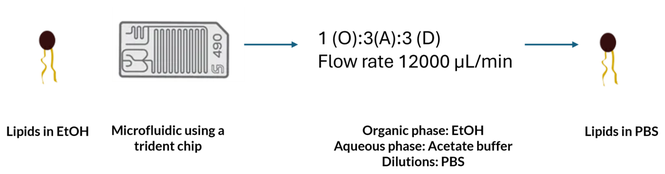

Figure 5. Schematic of the lipidic nanoparticles formulation by **microfluidic**.
Lipidic formulation tested: SM_{-102}: DSPC: cholesterol: PEG-DMG-$_{2000}$ (50:10:38.5:1.5 mol %).

- Physiochemical characterization of the copolymeric solutions:

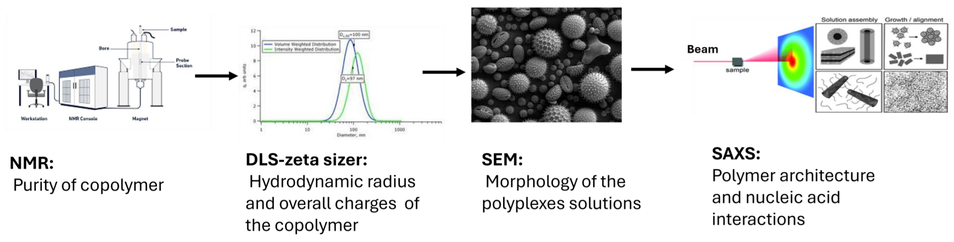

Figure 6: Polymer Characterization Workflow. Physical chemical characterization of the polyplex and lipid nanoparticles.

Results

- Size distribution and overall charges of different nuclei Polymer ratios (NP).

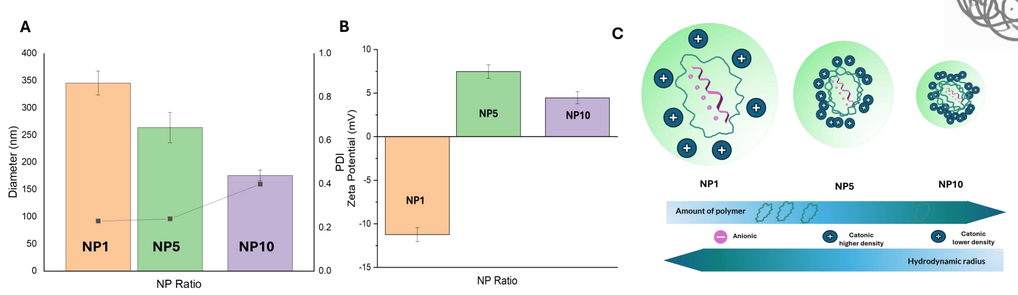

Figure 7: A) Diameter and **PDI** (grey squares) of NP1, NP5, and NP10 versus NP ratios. B) Zeta potential of the NPs using the same colour codes. C) Schematic trend showing that the size distribution decreases and the overall charge shifts from negative to positive with increasing NP ratio

- SEM and STEM of the polyplex NP10

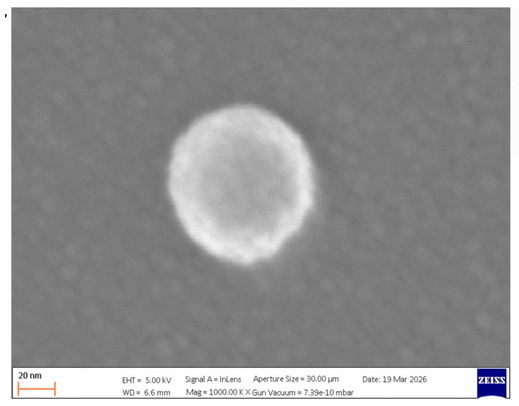

Figure 8: SEM of the polyplex NP10.

- SAXS of the polyplex

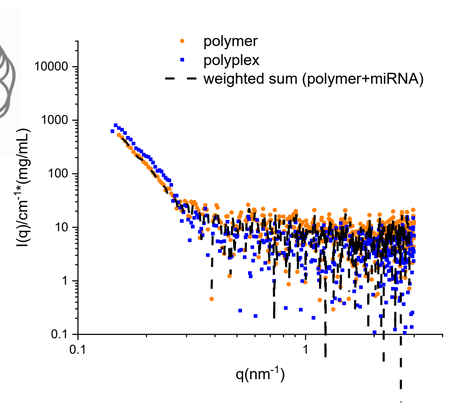

Figure 9: SAXS: The Interaction of the genetic material with the polymer is confirmed.

- Analyses of the lipidic nanoparticles

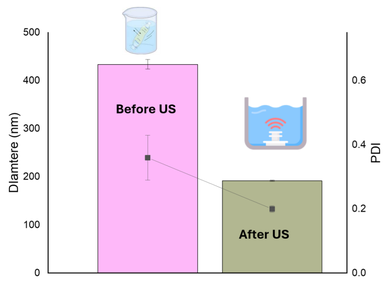

Figure 10: DLS of the lipidic nanoparticle formed with solvent exchange, before and after ultrasonication. The diameter dramatically reduced, as well as the PDI of the nanoparticles

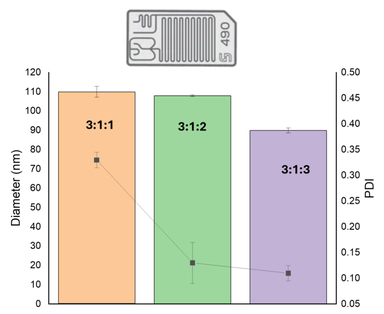

Figure 11: DLS of the lipidic nanoparticle formed with microfluidics, employing 3:1 (aqueous to water phase) at different to dilution rate (from 1 to 3).

Conclusion & Future perspectives

- NP10 ratio demonstrated to be the best composition, showing the lowest diameter and polydispersity.
- Association between polymer and nucleic acid were demonstrated
- SAXS and SANS analyses of the polymeric solutions will be performed.
- SANS rheology will be done to study the hydrogel viscoelastic properties.

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References

[1]Bulaklak, Nature communication, 2020
[2] Casadidio, Journal of Controlled Release, 2025