Exploring alternatives to PEG for the formulation of lipid nanoparticles encapsulating siRNA

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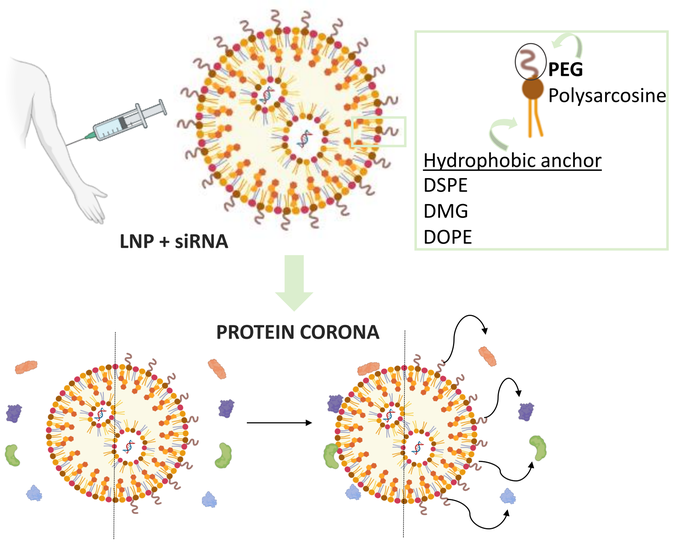
INTRODUCTION

When lipid nanovectors such as lipid nanoparticles (LNPs) are injected into systemic circulation, various processes occur and require a well comprehensive approach. Notably, the emergence of a protein corona at the LNPs' surface (formed with blood biomolecules) which significantly alters their properties and has sometimes the potential to diminish therapeutic efficacy [1]. To avoid protein corona formation, conventional LNPs are formulated by employing polyethylene glycol (PEG) to confer steric stability and extend particle half-life. However, concerns surrounding PEG-related issues pose challenges to drug efficacy, prompting exploration for alternatives to enhance both the antitumor efficacy and safety of the vector-siRNA complex [2]. In this study, polymers such as polysarcosine will be explored as an alternative to PEG. Additionally, the influence of modifying the length and number of lipid chains of the lipid-PEG on particle biodistribution will be studied. **The primary objective of this project was to assess the influence of lipid-PEG type of anchoring on particle biodistribution while exploring substitution for PEG to avoid immune adverse effects. Following thorough physicochemical characterization, the protein corona formation is evaluated for each formulation after incubation in animal serum with nanoparticle tracking analysis method (NTA) [3].**



MATERIALS AND METHODS

1. LNP FORMULATION

- SM-102 (ionizable lipid), DSPC (helper lipid), cholesterol, lipid-polymer : molar ratio of 50/10/37,5/2,5
- Lipid-polymers used *(Figure 1)* : DSPE-PEG_{2000} as control (C18:0), DMG-PEG_{2000} as control (C14:0), DOPE-PEG_{2000} (C18:1) and DOPE-$Psar_{25}$ (C18:1)
- siRNA GL3 (negative control)
- Microfluidic method for production : rapid-mixing *(Figure 1)*

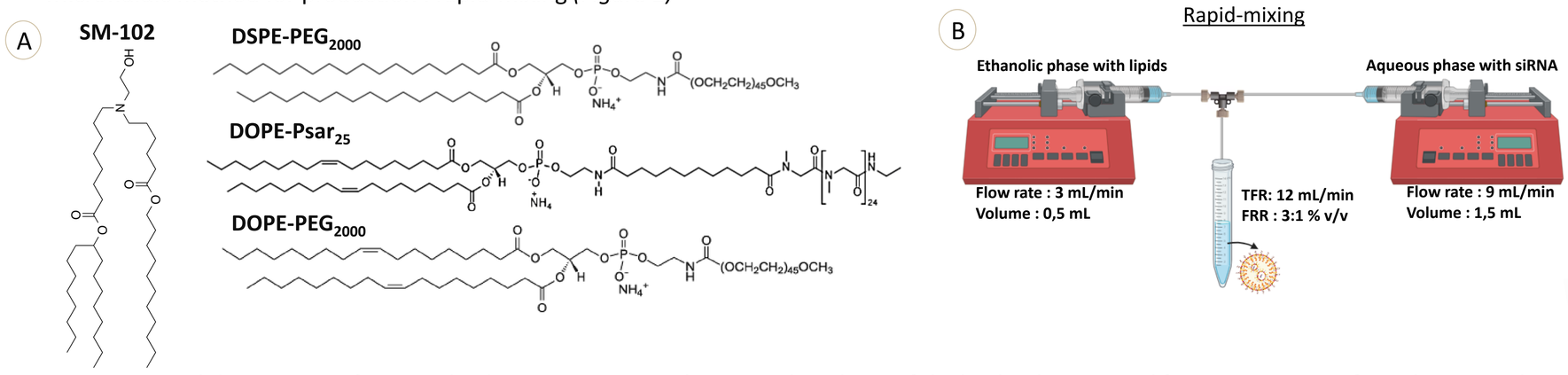


Figure 1 : (A) Structures of SM-102 lipid, DSPE-PEG_{2000} used as control, and two of the lipid-polymers used for innovative LNP formulations and (B) experimental conditions for the production of LNPs by rapid-mixing (created with Biorender).

2. PHYSICOCHEMICAL CHARACTERIZATION

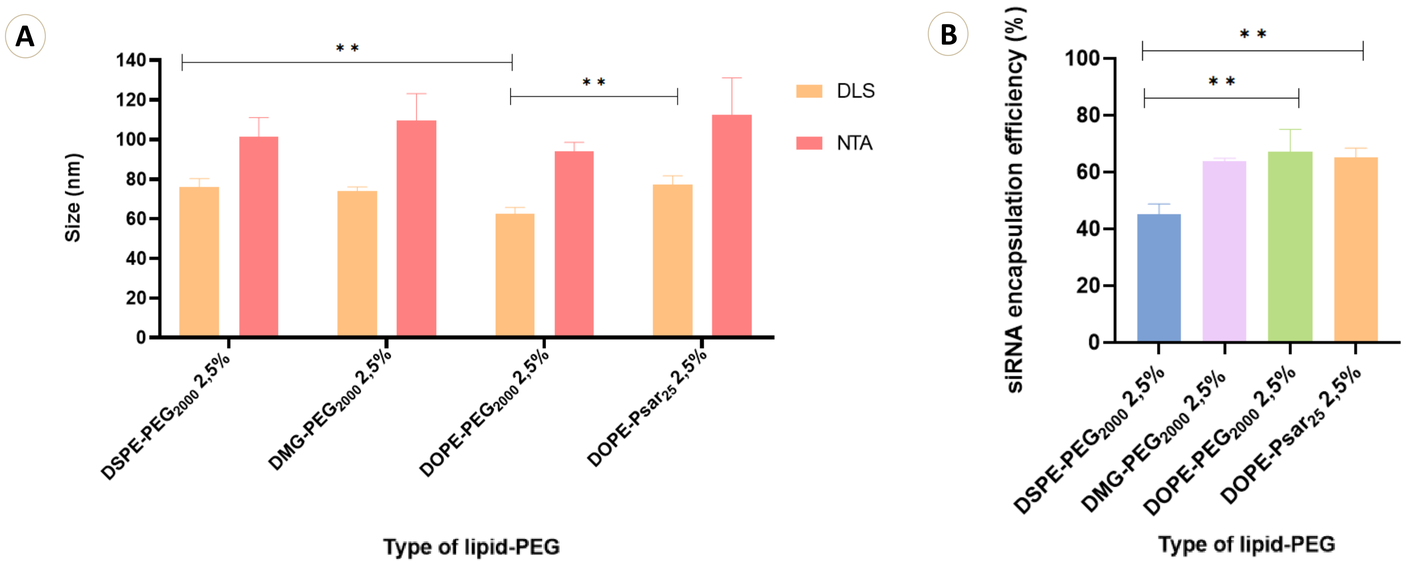
Characterization included DLS measurements (Z-average, PDI, zeta potential with Malvern Zetasizer Ultra Red).

The formation of the protein corona post two-hours incubation in 33,33% complete fetal bovine serum (FBS) was evaluated with NTA analysis, assessing particles mean size and mean total particles concentration.

Characterization included Ribogreen assay® (siRNA encapsulation efficiency).

RESULTS AND DISCUSSION

1. PHYSICOCHEMICAL CHARACTERIZATION



For DLS analysis, formulations containing 2,5% **DOPE-PEG_{2000}** showed a significantly smaller mean size than formulations with 2,5% DSPE-PEG_{2000}. Regarding the formulations containing **DOPE-$Psar_{25}$**, there was no significant increase or decrease for the mean size compared to DSPE-PEG_{2000}.

For NTA results, the same tendency than DLS is observed for **DOPE-$Psar_{25}$** but no significant decrease for the mean size is observed for **DOPE-PEG_{2000}** .

These innovative formulations have also shown that **DOPE-PEG_{2000}** and **DOPE-$Psar_{25}$** seem to provide the higher siRNA encapsulation efficiency compared to DSPE-PEG_{2000}.

LNPs formulations based on DOPE-PEG_{2000} and DOPE-$Psar_{25}$ seem to show interesting physicochemical properties.

Figure 2 : (A) LNPs Z-average and mean size using respectively DLS and NTA methods (B) siRNA encapsulation efficiency using Ribogreen assay ®.

2. PROTEIN CORONA FORMATION KINETICS

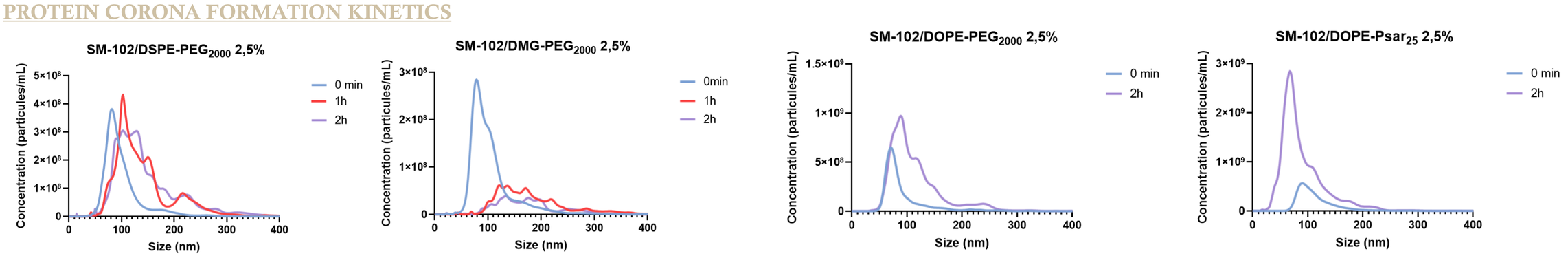


Figure 3 : Particles distribution profiles given by NTA.

The formation of the protein corona is limited when a long-chain lipid is used (DSPE-PEG_{2000}). In contrast, short-chain lipid rapidly lose its anchoring in LNPs and are incapable of adequately protecting LNPs from the formation of protein corona. **Regarding formulations with DOPE-PEG_{2000} and DOPE-$Psar_{25}$ (long-chain lipids), the protein corona seems to be formed much less easily than with formulations with DSPE-PEG_{2000} and certainly DMG-PEG_{2000} after a two-hours incubation in 33,33% FBS.**

CONCLUSION

Further investigation is warranted **for DOPE-PEG_{2000} and DOPE-$Psar_{25}$** formulations, as they seem to exhibit a reduced formation of the protein corona, along with interesting physicochemical characteristics. Other alternative compounds to PEG will be explored and the most promising formulations will undergo cytotoxicity evaluations through *in vitro* and *in vivo* tests.

REFERENCES

[1] V. Francia, R. M. Schiffelers, P. R. Cullis, et D. Witzigmann, « The Biomolecular Corona of Lipid Nanoparticles for Gene Therapy », Bioconjug. Chem., vol. 31, no 9, p. 2046-2059, sept. 2020, doi: 10.1021/acs.bioconjchem.0c00366.
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[3] **M. Berger et al.**, « Effect of PEG Anchor and Serum on Lipid Nanoparticles: Development of a Nanoparticles Tracking Method », Pharmaceutics, vol. 15, no 2, p. 597, févr. 2023, doi: 10.3390/pharmaceutics15020597.