ADVANCING siRNA DELIVERY: DSPE-PNMVA$_{24}$ AS A SOLUTION TO THE PEG DILEMMA IN LNP FOR TUMOR TARGETING

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1 Introduction

Since COVID-19 pandemic, Lipid Nanoparticles (LNPs) encapsulating mRNA have emerged as a new class of therapeutic agent as well as Onpattro®, the first FDA-approved drug based on LNPs and siRNA for a hepatic disease [1]. Indeed, siRNA and mRNA need vectors to reach the target undamaged. This is the reason why LNPs have garnered attention in this context (Figure 1). To target beyond the liver, notably for cancer treatment, LNPs need to be protected from blood biomolecules because of the formation of a protein corona, affecting their efficacy. In this context, polyethylene glycol (PEG) is used to prevent this phenomenon, but its issues initiate the search for alternatives [2,3]. **Recently, alternatives to PEG such as Poly(N-methyl-N-vinylacetamide) (PNMVA) have been studied with LNPs and seem to be promising [4,5]. However, improvements need to be done to optimize LNP properties and protein corona formation ability.**

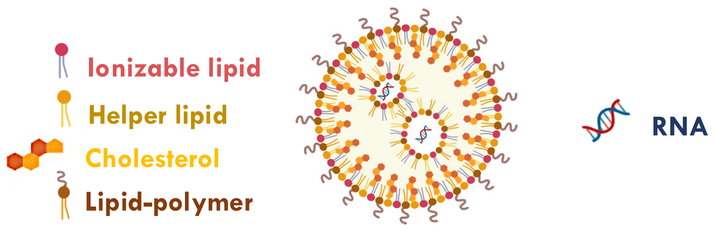

Figure 1 : Lipid Nanoparticle (LNP) structure.

2 Objectives

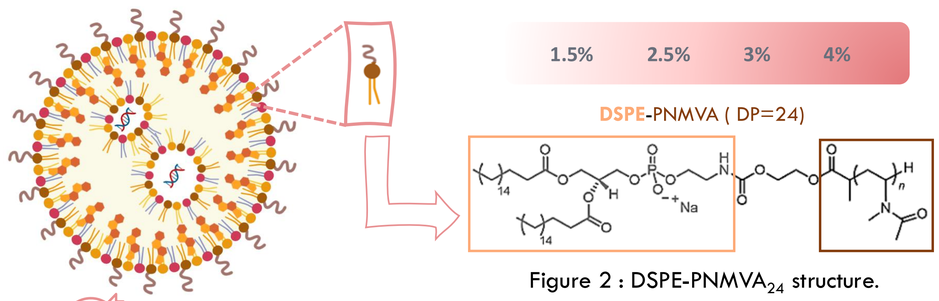

Figure 2 : DSPE-PNMVA$_{24}$ structure.

To explore the impact of different contents of a new type of lipid-polymer (**DSPE-PNMVA$_{24}$**) on LNP physicochemical properties (Figure 2) and protein corona formation to then compare this polymer to PEG (**DSPE-PEG$_{2000}$**).

Development of safer and efficient LNP formulations for a future antitumoral application.

3 Materials and methods

I. Production

The LNPs, produced by rapid-mixing (Figure 3), are composed of CSL3 (switchable lipid), DSPC, cholesterol and different types of C_{18} lipid-polymers (DSPE-PEG$_{2000}$ and DSPE-PNMVA$_{24}$) and DMG-PEG$_{2000}$ as C_{14} control at a molar ratio respectively of 50:10:37.5:2.5. The lipid-polymer content was decreased to 1.5% for all the formulations and increased to 3 to 4% by varying the cholesterol content only for DSPE-PEG$_{2000}$ and DSPE-PNMVA$_{24}$ formulations.

II. Physicochemical characterization

Key properties such as size, PdI and surface charge were analyzed by DLS and NTA while siRNA encapsulation efficiency was evaluated by Ribogreen® assay. The goal was to meet intravenous administration standards: size < 150 nm, PdI < 0.2 and maximum encapsulation efficiency.

III. Protein corona formation

Protein corona formation was evaluated using NTA method after incubation in 33.33% of FBS at 37°C.

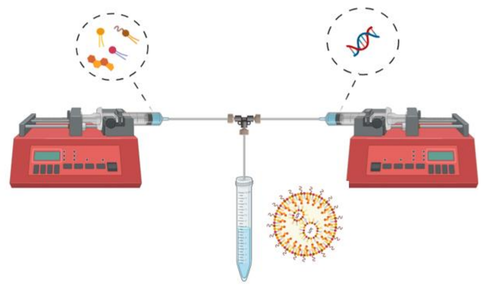

Figure 3 : Illustration of the rapid-mixing method for LNP production.

4 Results and discussion

I. Impact of DSPE-PNMVA$_{24}$ and DSPE-PEG$_{2000}$ content on LNP properties

The impact of DSPE-PNMVA$_{24}$ and DSPE-PEG$_{2000}$ content (1.5–4%) on LNP physicochemical properties was studied. Increasing the lipid-polymer content reduces Z-average for both LNP types. However, lipid-PEG increases PdI, while lipid-PNMVA decreases it. Encapsulation efficiency of siRNA also changes: lipid-PEG reduces it, whereas lipid-PNMVA has no impact. So on, **a higher DSPE-PNMVA$_{24}$ content (4%) is needed to form and stabilize LNPs compared to DSPE-PEG$_{2000}$ (2.5%).** While 1.5% lipid-polymer is used in the COVID-19 vaccines, 4% of DSPE-PNMVA$_{24}$ ensures LNPs with a size <150 nm, PdI <0.1, and ~85% siRNA encapsulation efficiency (Figure 4).

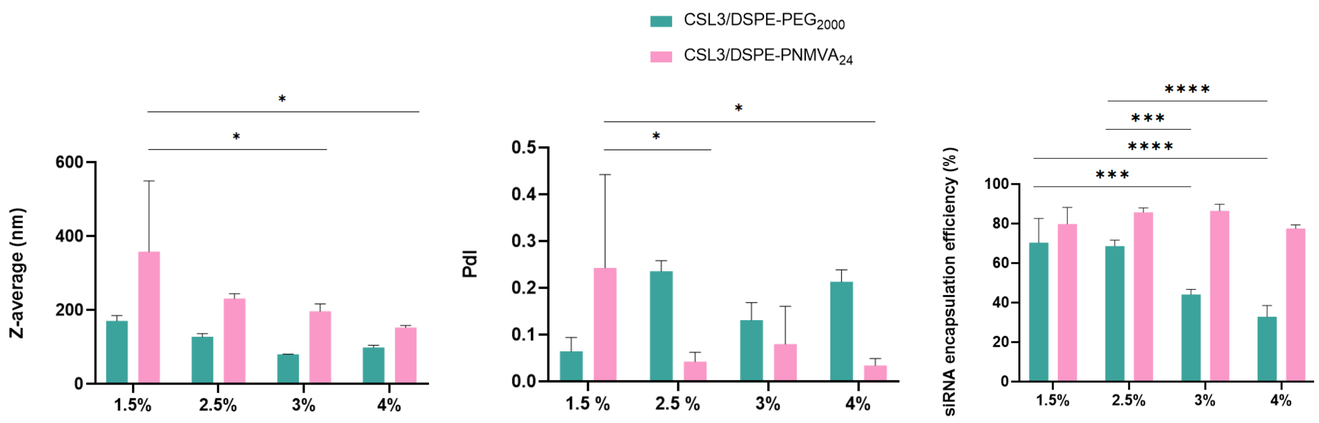

Figure 4 : Physicochemical properties of different LNP formulations.

II. Impact of DSPE-PNMVA$_{24}$ and DSPE-PEG$_{2000}$ content on protein corona formation

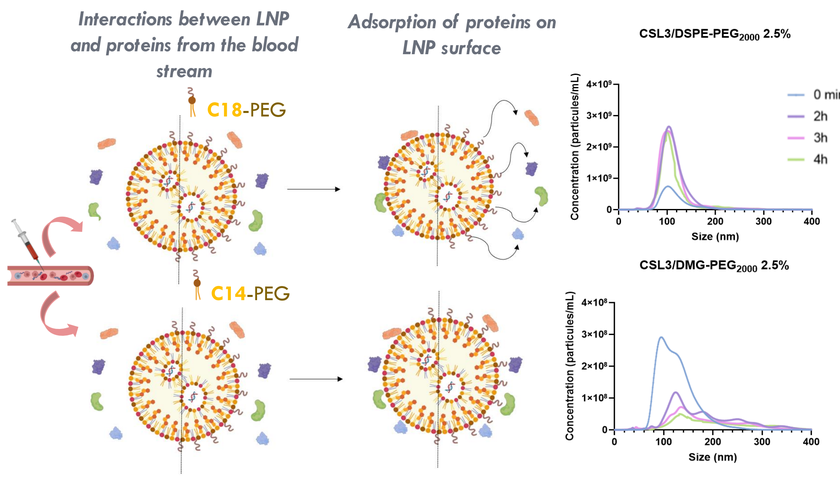

Figure 5 : Illustration of protein corona formation on LNP surface composed of long lipid-PEG (C_{18}) and short lipid-PEG (C_{14}).

A previously developed NTA method was used to assess the impact of the nature and lipid-polymer ratio on their ability to protect LNPs from protein corona formation [3]. According to this study, long lipid chains as C_{18} lipid-PEG protect against protein corona. Indeed, in the presence of 33% of FBS, LNPs covered with 2.5% of this type of lipid-PEG showed no significant variation in size and concentration (Figure 5) at the opposite of LNPs composed of C_{14} lipid-PEG.

A concentration of 1.5% of DSPE-PEG$_{2000}$ is sufficient to stabilize LNPs in FBS, maintaining consistent size and concentration over time. In contrast, DSPE-PNMVA$_{24}$ requires 3–4% to ensure stability, as lower concentrations (<3%) result in irregular size and concentration profiles over time. This indicates that 4% DSPE-PNMVA$_{24}$ is necessary to enhance shielding capacities, while DSPE-PEG$_{2000}$ achieves similar stabilization at a lower content (Figure 6).

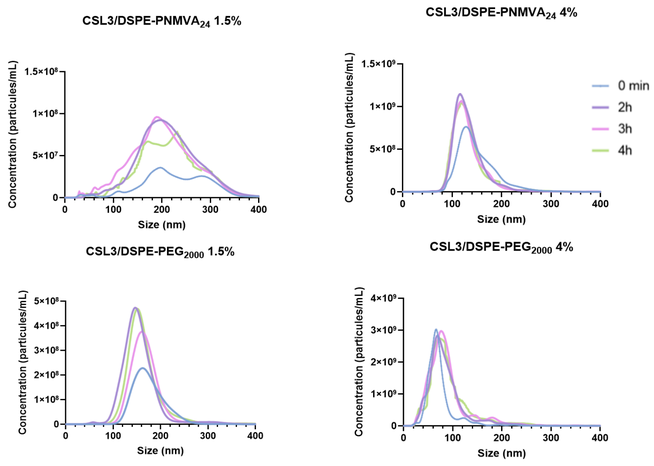

Figure 6 : NTA profiles (FBS 33.33%) of different LNP formulations.

5 Conclusion

In conclusion, **the promising potential of PNMVA as an alternative to PEG in LNPs has been confirmed,** with findings indicating the need for a **slightly higher concentration** than previously used. The comprehensive set of tests clearly demonstrates its effectiveness. This higher required amount of DSPE-PNMVA$_{24}$ allows the formation of LNPs with IV standards and a limited protein corona formation.

6 References

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