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Impact of lipid-polymer type and content on Lipid Nanoparticles (LNPs) physico-chemical properties and protein corona formation

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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 (Figure 2), 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 polysarcosine and Poly(N-methyl-N-vinylacetamide) (PNMVA) have been studied and seem to be promising [4,5].**

Lipid Nanoparticle (LNP)

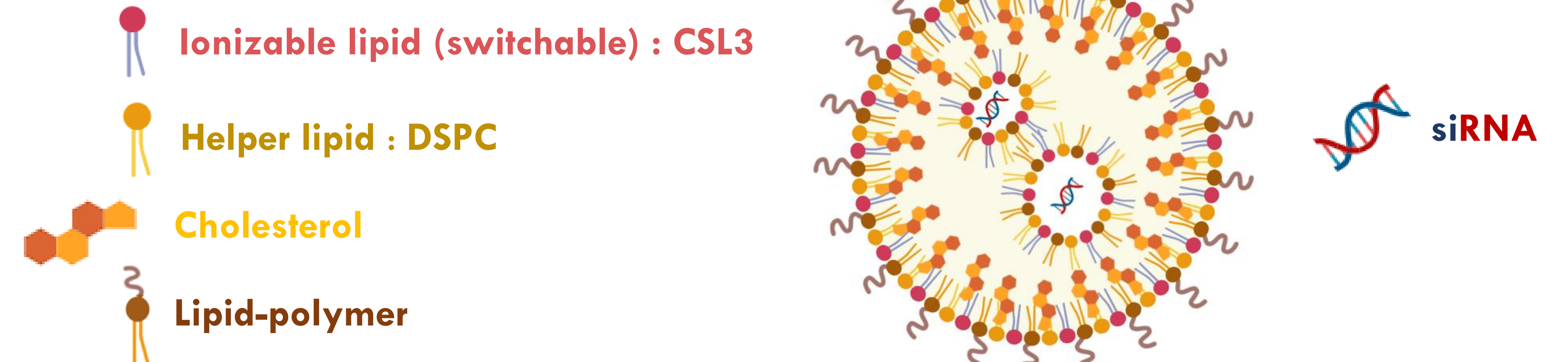

Figure 1 : Lipid Nanoparticle (LNP) structure.

Protein corona

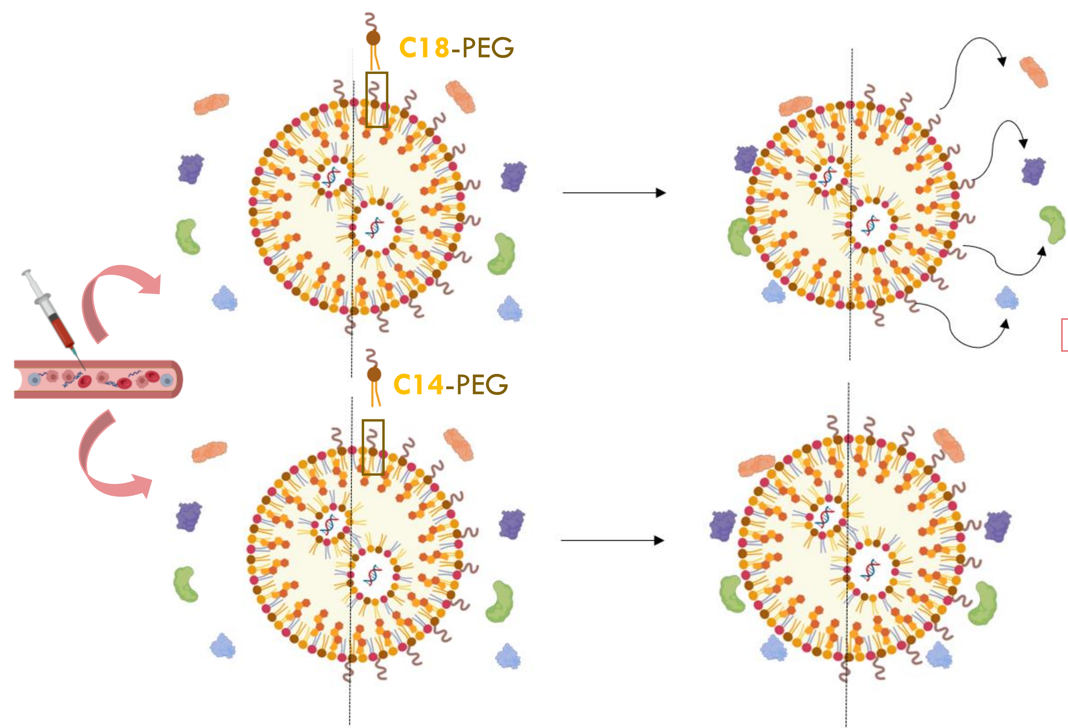

Figure 2 : Illustration of protein corona formation on LNP surface composed of long lipid-PEG (C18) and short lipid-PEG (C14).

Objectives

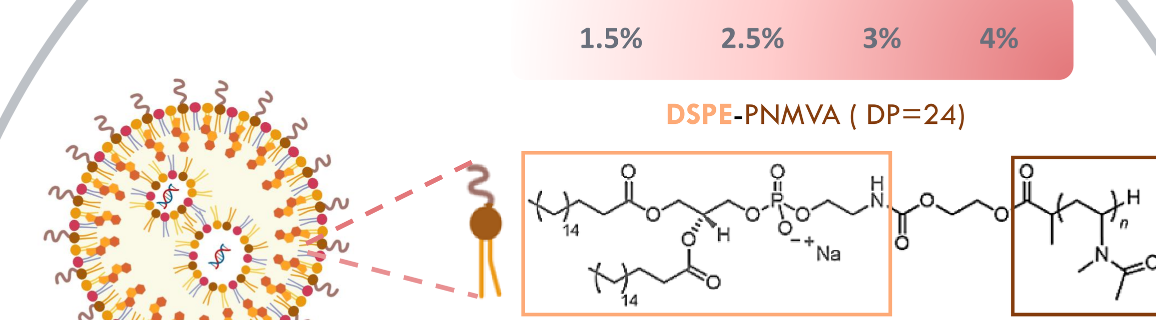

Figure 3 : Structure of **DSPE-PNMVA$_{24}$** used at different contents for LNP formulation.

- To produce LNPs by the rapid-mixing method with different types of lipid-polymers and contents (Figure 3-4)
- To analyze the impact of these modifications on physico-chemical properties and protein corona formation (Figure 2-4)
- To compare LNPs with different contents of DSPE-PNMVA$_{24}$ (recently patented) and DSPE-PEG$_{2000}$ to then compare it to another commercial polymer (polysarcosine)

Development of safer and efficient LNP formulations for an antitumoral application

Materials and methods

The LNPs, produced by rapid-mixing, are composed of CSL3, DSPC, cholesterol and different types of C18 lipid-polymers (DSPE-PEG$_{2000}$, DOPE-PEG$_{2000}$, DOPE-Psar$_{25}$, DSPE-PNMVA$_{24}$) and DMG-PEG$_{2000}$ as C14 control at a molar ratio respectively of 50:10:37.5:2.5 (Figure 4). The lipid-polymer content was decreased until 1.5% for all the formulations and increased until 3 to 4% by varying the cholesterol content only for DSPE-PEG$_{2000}$ and DSPE-PNMVA$_{24}$ formulations.

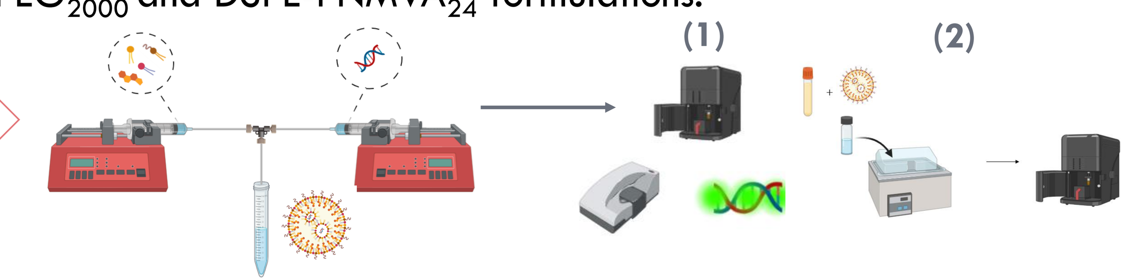

Figure 4 : Production of LNP by rapid-mixing followed by the techniques used for LNP analysis.

Key properties such as size, PdI and surface charge were analyzed by DLS and NTA while siRNA encapsulation efficiency was evaluated by Ribogreen® assay (Figure 4 (1)).

The goal was to meet intravenous administration standards: size < 150 nm, PdI < 0.2 and maximum encapsulation efficiency. Protein corona formation was evaluated using NTA method after incubation in 33.33% of FBS at 37°C (Figure 4 (2)).

Results and discussion

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

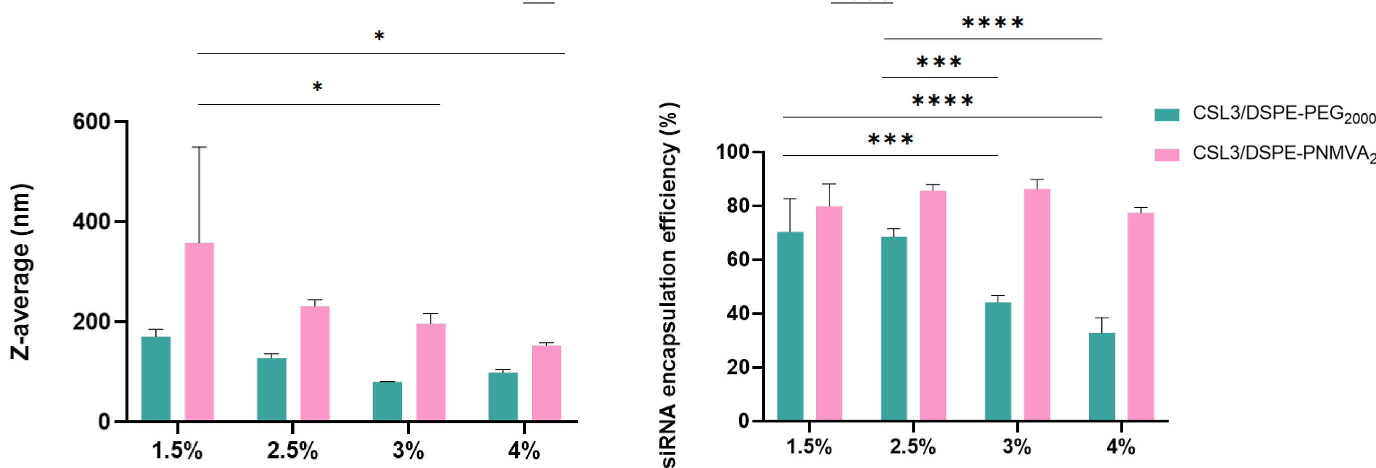

Figure 5 : Physico-chemical properties of different LNP formulations.

It has been observed that particle size and PdI were significantly decreased while the encapsulation efficiency remained constant **when increasing DSPE-PNMVA$_{24}$ content. By contrast, DSPE- PEG$_{2000}$ didn't show a decreased PdI and showed a significant decrease of siRNA encapsulation efficiency when increasing the content**, demonstrating instability (Figure 5). Moreover, the NTA test confirmed that DSPE- PEG$_{2000}$ at 2,5% hinders protein corona formation by contrast to DMG-PEG$_{2000}$ at 2,5%, demonstrated by an increased particle concentration and constant size for DSPE- PEG$_{2000}$ (due to the non-adsorption of proteins) and a decreased particle concentration and increased size for DMG-PEG$_{2000}$ (due to protein adsorption because of to the loss of the C14 anchor).

By increasing DSPE- PEG$_{2000}$ content, profiles seemed to demonstrate less protection against proteins at the opposite of DSPE-PNMVA$_{24}$ at 4% that demonstrated a similar profile to the C18 control at 2,5%, with refined peaks (Figure 6).

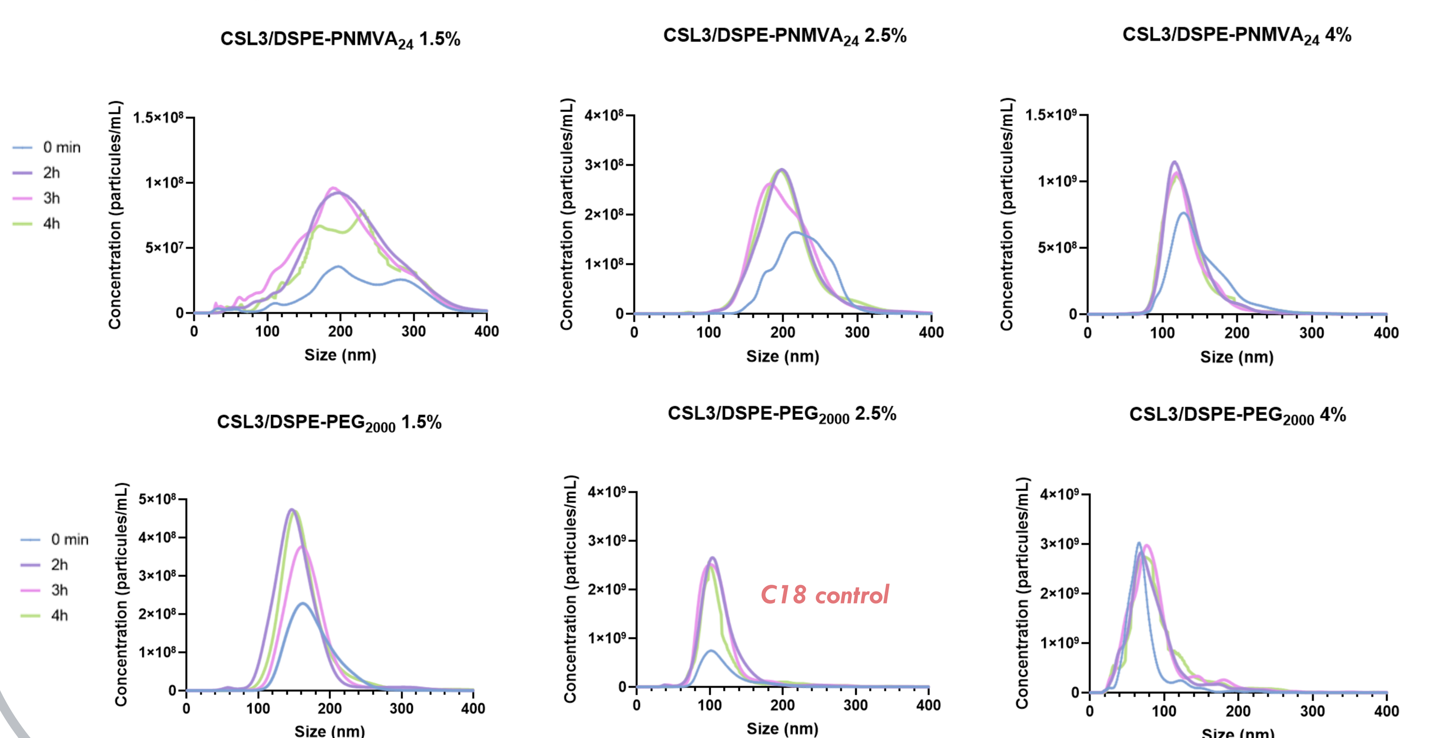

Figure 6 : NTA profiles (FBS 33.33%) of different LNP formulations.

II. Impact of DOPE-Psar$_{25}$ and DOPE-PEG$_{2000}$ content on LNP properties

Compared to its lipid-PEG analogous, DOPE-Psar$_{25}$ followed the same tendances for size and PdI when increasing the content : a decrease of the size and an increase of the PdI. In contrast, the encapsulation efficiency remained constant **when increasing DOPE-Psar$_{25}$ content while DOPE-PEG$_{2000}$ showed a decrease in encapsulation efficiency (not significant) as well as DSPE-PEG$_{2000}$**. As well as DSPE-PNMVA$_{24}$, **DOPE-Psar$_{25}$ showed disturbed profiles at 1.5% and 2.5% (broad peaks) demonstrating the potential need of a higher content. In contrast, DOPE-PEG$_{2000}$ already showed protein corona formation at 2,5% demonstrated by flattening of the curves (as a C14 lipid-PEG) starting to 3h of incubation (Figure 7).**

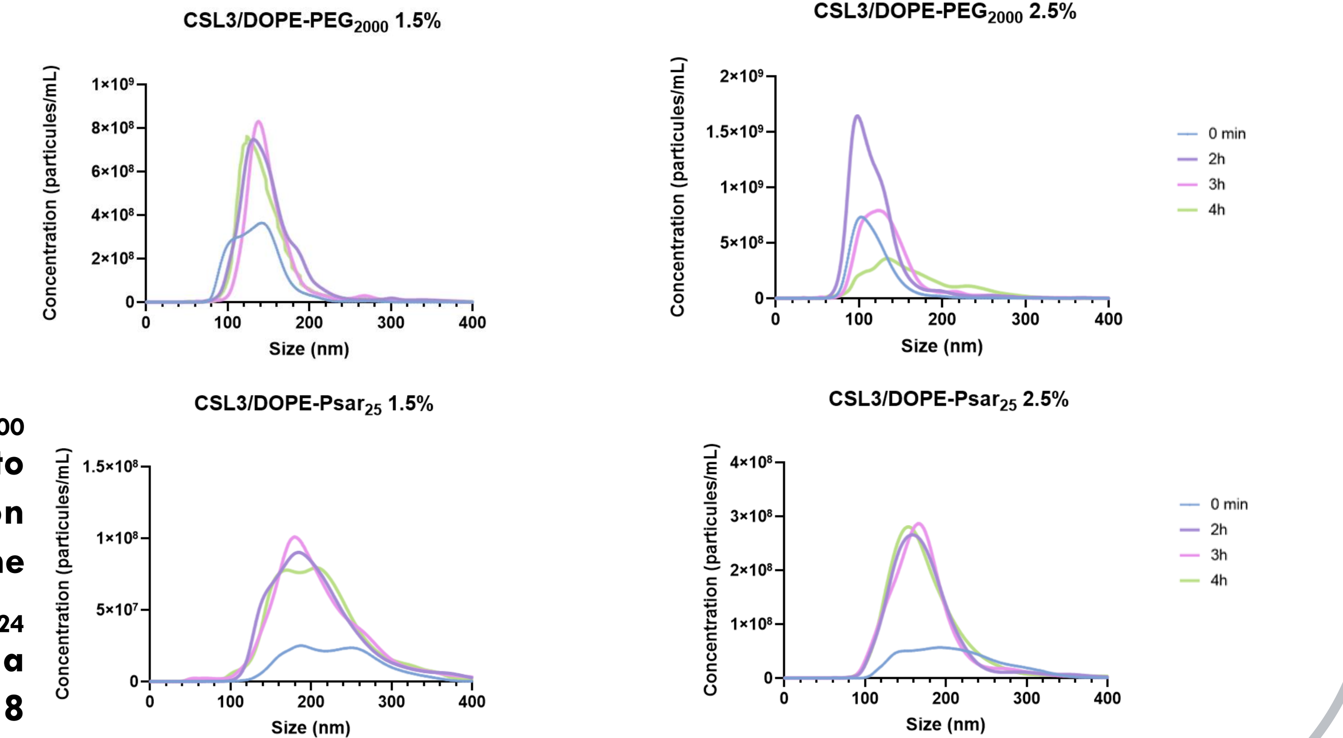

Figure 7 : NTA profiles (FBS 33.33%) of different LNP formulations.

Conclusion and perspectives

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. These conclusions have been transferred to another commercial polymer for further optimization. Moreover, *in vitro* tests will be realized to confirm that a higher content of PNMVA allows siRNA delivery and that it is not cytotoxic.

References

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