Redefining PEGylation: PNMVA-driven innovation in Lipid Nanoparticle delivery for cancer therapy

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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. 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. **Recently, alternatives to PEG such as Poly(N-methyl-N-vinylacetamide) (PNMVA) have been studied with LNPs and seem to be promising [2]. However, improvements need to be done to optimize LNP properties and protein corona formation ability.**

Materials and methods

I. Production

The LNPs, produced by rapid-mixing, 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.

Objectives

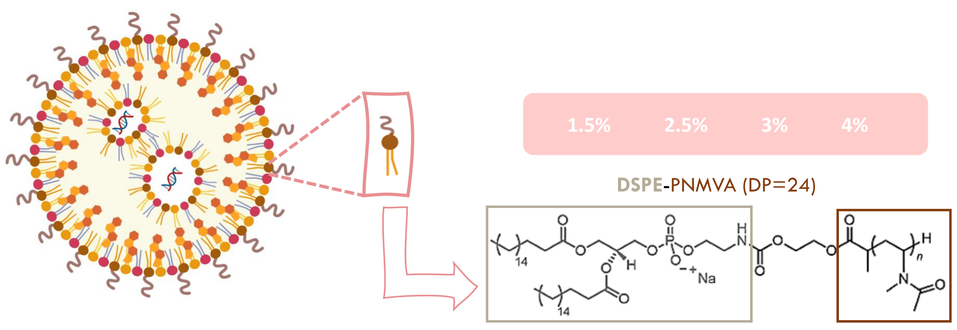


Figure 1 : DSPE-$PNMVA_{24}$ structure.

To explore the impact of different contents of a new type of lipid-polymer (**DSPE-$PNMVA_{24}$**) on LNP physicochemical properties (Figure 1) and protein corona formation to then compare it to PEG (**DSPE-PEG_{2000}**).

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

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, increasing lipid-PEG content increases PdI, while increasing lipid-PNMVA content decreases it. Encapsulation efficiency of siRNA is also changing as increasing lipid-PEG content reduces it, whereas increasing lipid-PNMVA content has no impact. So on, **a higher DSPE-$PNMVA_{24}$ content (4%) is needed to form and stabilize LNPs compared to DSPE-PEG_{2000}.** 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 <0.2, and ~80% siRNA encapsulation efficiency (Figure 2).

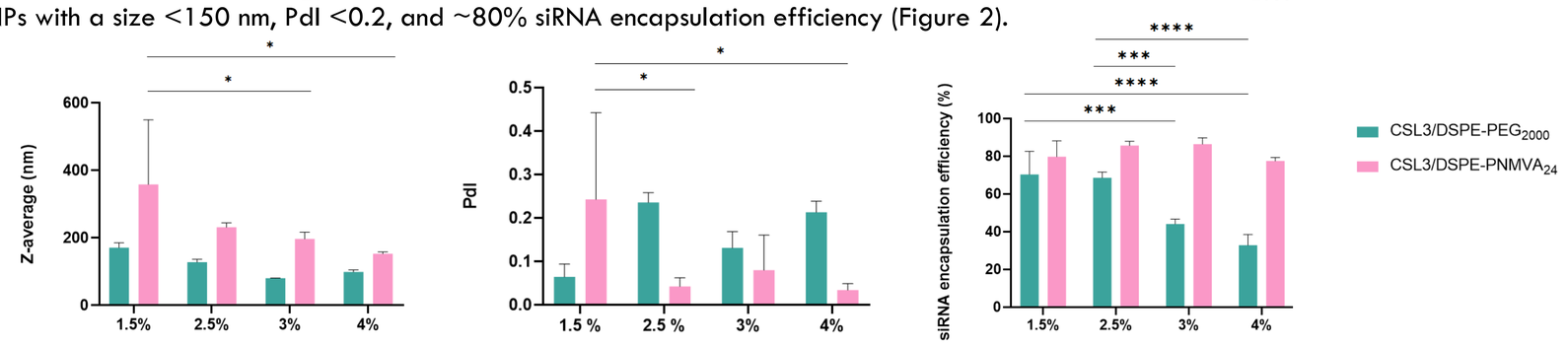


Figure 2 : Physicochemical properties of different LNP formulations.

II. Impact of DSPE-$PNMVA_{24}$ and DSPE-PEG_{2000} content on protein corona formation

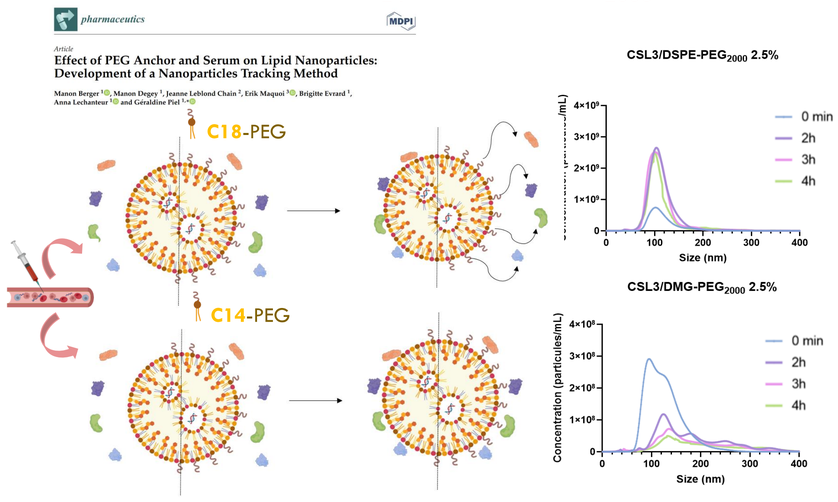


Figure 3 : Illustration of protein corona formation on LNP surface composed of long lipid-PEG (C_{18}) and short lipid-PEG (C_{14}).

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. 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 3) 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 (Figure 4).

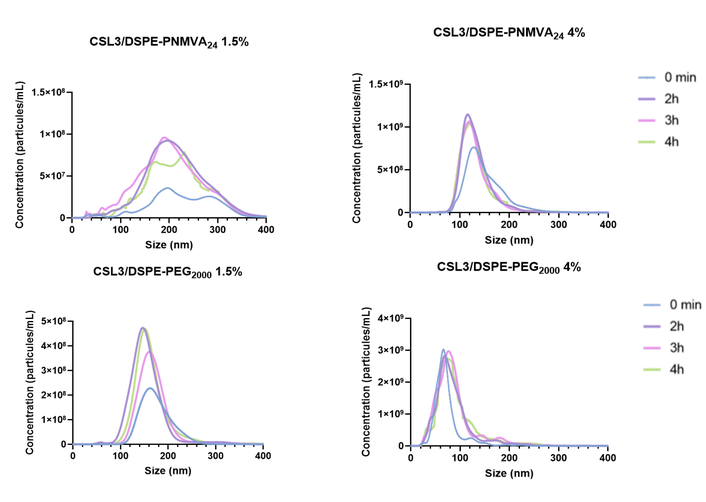
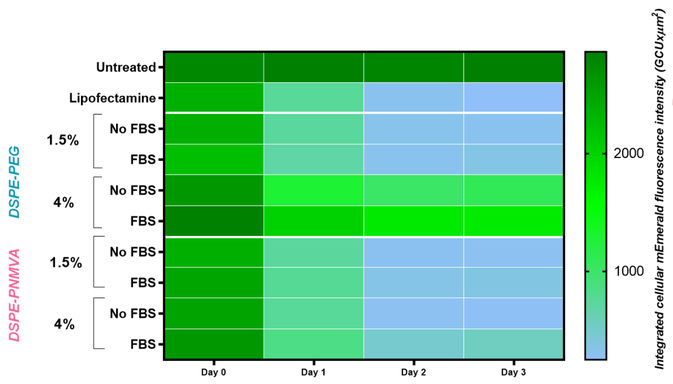


Figure 4 : NTA profiles (FBS 33.33%) of different LNP formulations.

III. Preliminary in vitro results : gene knockdownn efficiency (triple negative breast carcinoma MDA-MB-231cells expressing nuclear mEmerald)



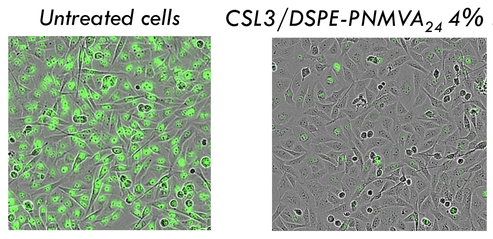


Figure 5 : Live cell imaging of MDA-MB-231 cells expressing mEmerald after 72 hours of incubation with indicated formulations of LNPs

The developed formulations were prepared with siGFP a small interfering RNA targeting GFP expression, and incubated with MDA-MB-231 cells expressing mEmerald (mutated form of GFP) for 4 hours in serum-free conditions (100 nM siGFP). Live-cell imaging was performed using the Incucyte Sx5 over 72 hours and showed a decrease of GFP expression with PNMVA formulations inserted at higher contents (Figure 5). Composite images combining phase contrast and green fluorescence are shown. After 72 hours, cells were analyzed by flow cytometry (CytoFLEX) to measure mean fluorescence intensity (MFI) of GFP.

- **Formulations with 4% DSPE-$PNMVA_{24}$** allowed the 90% of GFP fluorescence extinction

Conclusion

In conclusion, **the promising potential of DSPE-$PNMVA_{24}$ at 4% as an alternative to PEG in LNPs has been confirmed,** with findings indicating the need for a **higher concentration** than previously used.

References

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