

Lipid-poly(N-methyl-N-vinylacetamide) conjugates for surface modification of lipid membranes and nanoparticles

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Introduction

Efficient gene delivery is crucial for many therapeutic strategies, especially those involving nucleic acids, which are unstable in biological environments and therefore require nanocarriers such as lipid nanoparticles (LNPs). To improve their stability and prolong circulation time in the bloodstream, LNPs are often PEGylated; however, PEG has several limitations, including accelerated blood clearance (ABC) caused by anti-PEG antibodies, immunogenicity, and reduced cellular uptake and endosomal escape. Preliminary studies suggest that PNMVA could be a safer and more effective alternative to PEG, showing reduced ABC effects and improved performance in RNA/DNA delivery¹⁻³. This work aims to expand the range of lipid-polymer conjugates used in LNP formulation and to investigate how their structure influences interactions with lipid bilayers.

Experimental Methods

Polymer Synthesis: The synthesis and characterization of the PNMVA derivatives (DSPE-PNMVA, DPPE-PNMVA and DMPE-PNMVA) were inspired from a previous work¹.

Membrane Interaction and protection from BSA: QCM-D analyses of the polymer/membrane interactions and deposition of the DOTAP/Chol/DOPE membrane onto the gold coated sensors (Q-Sense AT-cut quartz crystal sensors, fundamental frequency of 5 MHz) and protection from BSA were carried out following a procedure described elsewhere^{1,2}.

Statistics: Values were expressed as means $\pm$ standard deviations (SD) for at least $n = 3$. PRISM (GraphPad Prism 9) was used for statistical analysis.

Results and Discussion

Lipid-PNMVA conjugates with different PNMVA molar mass and lipid anchor were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization, giving well-defined lipid-PNMVA conjugates (Figure 1).

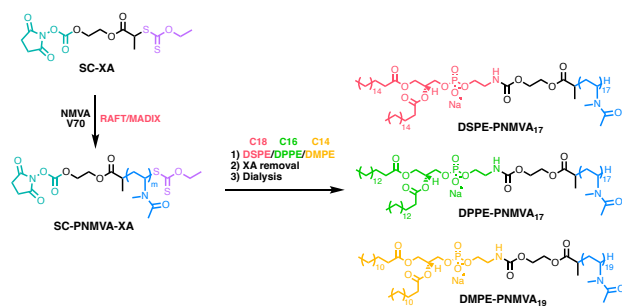


Figure 1. Synthetic strategy for DSPE-PNMVA, DPPE-PNMVA, and DMPE-PNMVA derivatives.

Particularly, a RAFT agent bearing a succinimidyl carbonate group (SC-XA) was synthesized, enabling the controlled radical polymerization of NMVA to yield polymers with tunable molar masses (1600-3000 g/mol) and low dispersity. The resulting SC-PNMVA-XA were efficiently coupled to various lipids namely DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine, C18:0), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, C16:0), and DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine, C14:0). Finally, removal of the xanthate end group and the purification via dialysis ensured the stability and biocompatibility of the lipid-polymer conjugates. This approach provided a versatile platform of different lipid-PNMVA systems for lipid nanovectors design in gene therapy.

Subsequently, the interaction of selected lipid-PNMVA conjugates with model lipid bilayers (mimicking the surface of a lipid nanocarrier) was investigated by QCM-D, in order to assess both adsorption and anchoring stability (Figure 2).

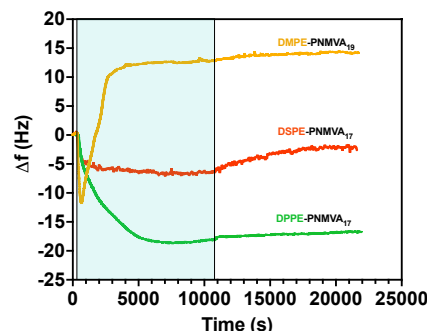


Figure 2. Overlay of Δf traces from QCM-D monitoring of the deposition of DSPE-PNMVA₁₇, DPPE-PNMVA₁₇ and DMPE-PNMVA₁₉ on lipidic membranes. Blue regions correspond to polymer solution addition while white regions correspond to buffer elution periods.

Supported bilayers on the quartz crystal were formed from DOTAP/Chol/DOPE liposomes, and mass deposition was quantified using the Sauerbrey equation. Representative conjugates from the DSPE-, DPPE-, and DMPE-PNMVA series were evaluated by exposing the bilayer to polymer solutions, followed by PBS rinsing to evaluate desorption behaviour. DPPE-based conjugates exhibited rapid and stable interactions with minimal desorption, whereas DSPE derivatives showed partial loss upon rinsing. In contrast, conjugates with the shortest lipid chain, i.e. DMPE, displayed significant desorption occurring already during the adsorption phase. The same technique also demonstrated the protein repellency of the modified lipid bilayer using BSA.

Conclusions and future insights

A library of lipid-PNMVA conjugates was successfully developed by varying both the lipid anchor and the PNMVA chain length, yielding promising candidates for lipid nanocarriers surface modification. Preliminary QCM-D studies demonstrated that lipid structure plays a key role in governing membrane affinity and surface stability of lipid-polymer systems. These findings suggest that the choice of the lipid is expected to influence the characteristics of PNMVA-modified LNPs. Future work will focus on the formulation of LNPs, followed by physicochemical characterization, evaluation of siRNA delivery, hemocompatibility and immunogenicity studies via in vitro and in vivo testing.

References

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- ² Berger M. et al. Advanced Healthcare Materials 2024, 13(8), 2302712.
- ³ Debuigne A. et al. Patent 2024, WO2024227713A1 & EP4458870A1.

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