Stimulation of morphological changes of Lipid Nanoparticle Formulations of mRNA Leads to Improved Transfection Potency

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Abstract

Lipid nanoparticle (LNP) systems enable nucleic-acids (i.e. siRNA) to silence pathological genes and mRNA used to express therapeutic proteins [1]. Major examples include Onpattro, [2] an LNP formulation of siRNA to treat transthyretin-induced amyloidosis approved by the Food and Drug Administration in 2018, and the COVID-19 mRNA vaccines that have received regulatory approval in many jurisdictions worldwide [3] and more are under development. LNP formulations of siRNA and mRNA for in vivo applications are composed of ionizable cationic lipids, phospholipids, cholesterol, and polyethylene glycol (PEG)–lipids [4]. The transfection potency of lipid nanoparticle (LNP) mRNA systems is critically dependent on the ionizable cationic lipid component. LNP mRNA systems composed of optimized ionizable lipids often display distinctive mRNA-rich "bleb" structures. Herein, we demonstrate that such structures can also be induced for LNPs containing nominally less active ionizable lipids by formulating them in the presence of high concentrations of pH 4 buffers such as sodium citrate, leading to improved transfection potencies both in vitro and in vivo. Induction of bleb structure and improved potency is dependent on the type of pH 4 buffer employed, with LNP mRNA systems prepared using 300 mm sodium citrate buffer displaying maximum transfection. The improved transfection potencies of LNP mRNA systems displaying bleb structure can be attributed, at least in part, to enhanced integrity of the encapsulated mRNA. We conclude that enhanced transfection can be achieved by optimizing formulation parameters to improve mRNA stability and that optimization of ionizable lipids to achieve enhanced potency may well lead to improvements in mRNA integrity through formation of the bleb structure rather than enhanced intracellular delivery.

References

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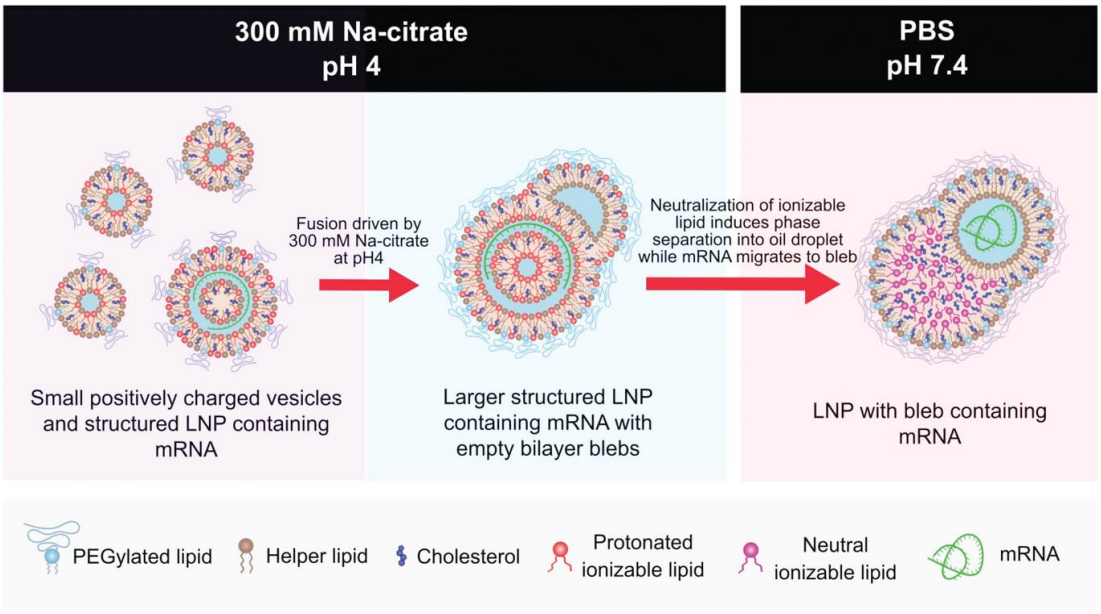

Figure 1: Proposed model of mechanism of formation of the LNP mRNA systems exhibiting bleb structures