Long Term Storage of Lyophilized mRNA Lipid Nanoparticles via Biomimetic Minerals

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Statement of Purpose: The use of mRNA as a therapeutic has significantly expanded, as demonstrated by the mass production of mRNA lipid nanoparticle (LNP) vaccines during the COVID-19 pandemic. However, global distribution of mRNA therapeutics has been limited due to the need for cold chain storage. Improving the stability of mRNA LNPs to maintain activity after storage at ambient temperatures could lead to 'off the shelf' vaccines that are able to be distributed at a global scale.

Paleontology suggests that bone related minerals, such as calcium phosphate minerals, may play a critical role in the stabilization of nucleic acids (1). Previously, we have described how mineral coated microparticles (MCMs) can promote the stabilization of mRNA complexes after lyophilization and long-term storage at room temperature (2). Here, we have prepared a library of calcium-phosphate containing MCMs to interact with and stabilize mRNA LNPs during lyophilization and long-term storage at ambient temperature.

Methods: 4-component LNPs were prepared at a molar ratio of 50:10:38.5:1.5 of ionizable lipid:1,2-DSPC:Cholesterol:PEG lipid. For additional 4-component LNPs, 1,2- DSPC was fully replaced with cationic lipid DOTAP or anionic lipid 18-PA. 5-component LNPs were prepared as previously mentioned, with the addition of DOTAP or 18PA (0-40%). Additional components are compensated by a reduction of cholesterol.

Mineral coated microparticles were prepared in modified simulated body fluid (mSBF) as previously described for 2 days (2). MCMs are then incubated in experimental mSBF containing fluoride and citrate dopants for an additional 3 days before being washed with PBS twice, undergoing filtration, and lyophilization. mRNA LNPs were mixed with MCMs to promote binding before centrifugation to isolate the mRNA LNPs only bound to MCMs ("bound"). mRNA LNPs were also co-delivered with MCMs ("adjuvant"). Samples were mixed with trehalose, maltose, and/or sucrose before freezing at -80C and/or lyophilization with long term storage at 4C, 25C, or 37C.

Human mesenchymal stem cells were transfected with fresh, frozen, or lyophilized firefly luciferase mRNA LNPs. After 24 hours, the cells were washed, lysed, and luciferase substrate was added before measuring cellular luminescence with a plate reader.

Mice were intradermally immunized twice with fresh, frozen, or lyophilized and stored LNPs carrying ovalbumin mRNA and firefly luciferase mRNA, with or without MCMs. IVIS was used to measure luciferase expression after 24 hours. Splenocytes were harvested and stained for ovalbumin expression.

Results: 4-component mRNA LNPs containing 10% DOTAP enhanced transfection efficacy when bound to MCMs (Fig. 1A). The addition of lyoprotectants (trehalose, sucrose, and/or maltose) to LNPs +/- MCMs before lyophilization increased luciferase transgene expression compared to the same conditions without lyoprotectants (Fig. 1B). Following lyophilization and long-term storage at various temperatures, mRNA LNPs bound with MCMs produced enhanced transfection when compared to those without MCMs (Fig. 1C). After an initial reduction in mRNA expression, lyophilized mRNA LNPs with MCMs maintained transfection efficacy for 3 months, outperforming mRNA LNPs without MCMs (Fig. 1D).

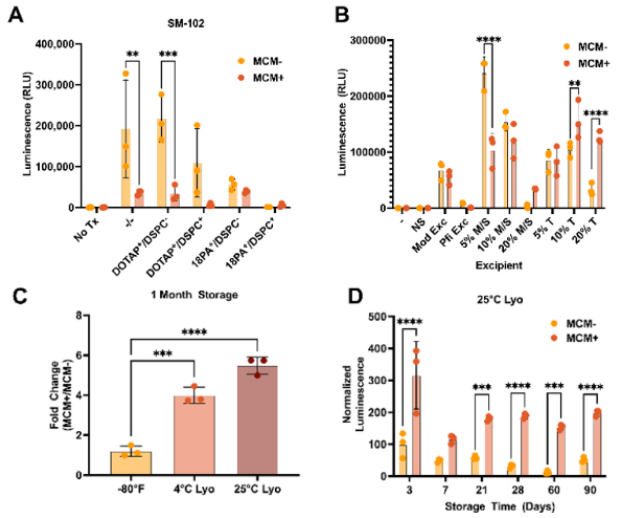

Figure 1. LNPs with MCMs show extended stabilization after long-term storage. (a) Firefly luminescence of 4 or 5 component LNP formulations with or without MCMs. (b) Screening of excipients as protectants during lyophilization. (c) Fold change in firefly luminescence of samples with/without MCMs after storage for 4 weeks frozen at -80C, or lyophilized and stored at 4C or 25C. (d) Luminescence normalized to cell viability of mRNA LNPs with (co-delivery) or without MCMs after lyophilization and storage at 25C.

Conclusions: mRNA LNPs delivered with mineral coated microparticles were able to maintain their ability to transfect target cells, even after lyophilization and storage at ambient temperatures. Further understanding of the mechanisms behind mineral stabilization of mRNA is needed. This work furthers the possibility of the production of "off the shelf" mRNA therapeutics that can be distributed at a global scale.

References:

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