Molecular Therapy
Commentary

Overcoming the challenge of long-term storage of mRNA-lipid nanoparticle vaccines

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Lipid nanoparticles (LNPs) have rapidly gained public attention as the delivery platform for messenger RNA (mRNA) vaccines against the virus that causes coronavirus disease 2019 (COVID-19). LNPs have been developed and evaluated for years in preclinical studies and in humans for a variety of diseases with high potency and safety, but a major challenge to their worldwide dissemination is the required storage conditions.[1–4] Unlike most other vaccines that are stored at ambient temperature or under refrigeration, mRNA-LNPs are stored frozen at −20°C or −80°C, which poses a significant challenge for transportation and long-term storage. In this issue of *Molecular Therapy*, Muramatsu, et al. demonstrate that mRNA-LNPs can be lyophilized (freeze-dried) and stored at ambient temperature for 12 weeks and at 4°C for at least 24 weeks without substantial changes to their physical properties or mRNA delivery efficiency.[5] Their findings on the long-term storage and stability of lyophilized mRNA-LNPs are critical to the widespread development and implementation of LNPs for COVID-19 and other diseases.

LNPs are comprised of an ionizable lipid, phospholipid, cholesterol, and lipid-conjugated poly(ethylene) glycol (PEG) with encapsulated nucleic acids, such as mRNA.[6] Each of the lipid components in LNPs plays a critical role toward successful mRNA delivery. The PEG-lipid conjugates enhance LNP stability and avoid immune recognition in circulation. The cholesterol are important for lipid membrane integrity and structure, and the phospholipids resemble lipids present in cell membranes.[7] Ionizable lipids utilize charge-reversal properties to perform two key functions: (1) high mRNA encapsulation due to electrostatic interactions of mRNA in acidic buffer and (2) efficient endosomal escape to increase cytoplasmic mRNA delivery.[3] Despite these advantages contributing to the success of LNPs as delivery vehicles in the Pfizer-BioNTech (Comirnaty) and Moderna (SpikeVax) COVID-19 mRNA vaccines, a huge challenge remains to increase the storage and stability of these platforms, as studied in this article.

Prior research has evaluated the stability and delivery potency of lyophilized LNPs with encapsulated small interfering RNA (siRNA) or mRNA toward the goal of enabling long-term storage.[8,9] Lyophilization of siRNA-LNPs in the original formulation buffer followed by reconstitution in deionized water resulted in a substantial decrease in gene silencing efficiency.[8] However, lyophilization in sucrose or trehalose buffers yields comparable siRNA or mRNA activity compared to freshly prepared LNPs due to the cryoprotection effects of the sugar.[8–10] This demonstrates the importance of the lyophilization buffer for the long-term storage and stability of LNPs. More recently, Ai et al. demonstrated that lyophilization and reconstitution of mRNA-LNP vaccines yield similar protection against COVID-19 variants in mice compared to freshly prepared LNPs, although this study evaluated LNPs stored for only 20 days.[11] While these prior studies have demonstrated that lyophilization can retain nucleic acid efficacy, a thorough assessment of the impact of lyophilization on the long-term stability and immunogenicity of mRNA-LNP vaccines was necessary.

In this article, Muramatsu, et al. thoroughly examine the stability and mRNA delivery efficiency of lyophilized LNPs stored under various storage conditions (−80°C, −20°C, 4°C, 25°C, or 42°C) at multiple timepoints over the course of 24 weeks.[5] Results from each storage condition were compared to LNPs stored in their frozen liquid form (not lyophilized) at −80°C to assess the impact of lyophilization, temperature, and storage time on LNP performance. They studied two nucleoside-modified mRNA cargos, luciferase mRNA or influenza virus hemagglutinin (PR8 HA) mRNA, to evaluate delivery by imaging or immunogenicity, respectively. First, the authors present a thorough assessment of LNP stability following lyophilization in 10% sucrose and 10% maltose and reconstitution in nuclease-free water after storage. The hydrodynamic diameter of both LNPs increased after storage at ambient temperature or 42°C, but it did not change after storage at or below 4°C for the entirety of the 24-week study. Similarly, mRNA integrity decreased ∼10%–15% and ∼30% following storage of the lyophilized LNPs at 4°C or ambient temperature, respectively, for 24 weeks. Of note, storage of lyophilized LNPs at all temperatures tested did not result in changes to LNP polydispersity or encapsulation efficiency. These results demonstrate that the stability and integrity of lyophilized LNPs, particularly LNP size and mRNA integrity, is dependent on the storage temperature and length of storage time.

The authors evaluated mRNA delivery efficiency in terms of luminescence (for the luciferase mRNA-LNPs) or immunogenicity (for the PR8 HA mRNA-LNPs) in mice following intradermal or intramuscular injection.[5] Luciferase delivery was determined by imaging mice using an *in vivo* imaging system, which revealed that lyophilized LNPs stored at ambient temperature for 4 weeks retain high protein production that then modestly decreases after storage for 24 weeks. Further, storing LNPs at 4°C does not alter luciferase protein production for at least 24 weeks compared to LNPs stored at −80°C. The authors found similar results in terms of immunogenicity following treatment with PR8 HA mRNA-LNPs. HA inhibition titers revealed

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that storage of lyophilized LNPs at ambient temperature or 4°C for 12 weeks did not impact immunogenicity, and storage at both temperatures for 24 weeks yielded a modest decrease in the resultant immunogenicity. Storage of lyophilized LNPs at 42°C resulted in a considerable drop in luciferase production and immunogenicity after storage for all tested timepoints. Together, these results demonstrate that mRNA-LNP activity is clearly dependent on storage temperature and time. The lyophilization procedures used here enabled storage of LNPs at ambient temperature for 12 weeks or at 4°C for at least 24 weeks with no impact to immunogenicity.

Currently, mRNA-LNP vaccines against COVID-19 are stored frozen in their liquid form without lyophilization. This is a great challenge for the long-term storage and transportation of LNPs, both for the COVID-19 vaccines and toward the development and implementation of LNPs for other diseases. The two major mRNA-LNP vaccines, SpikeVax and Comirnaty, have stability reported at ambient temperature for only 12 h and 6 h, respectively.[12] In this article, lyophilization enabled stable storage of the lyophilized PR8 HA mRNA-LNPs for 12 weeks, or reconstituted LNPs for 24 h, at ambient temperature. This is a substantial improvement over the current standard. The stability studies conducted in this article provide important guidelines on the long-term storage, stability, and delivery efficacy of mRNA-LNPs. Moving forward, it will be valuable to compare multiple lyophilization conditions, such as the type and concentration of cryoprotectant, to enable longer storage at ambient temperature, as lyophilization techniques have been shown to impact LNP stability and performance.[8] These additional studies, combined with the findings in this article, are critical to the widespread development and implementation of LNPs for long-term disease management. Further, storage at ambient temperatures would enable easier transportation and greater access to LNP therapeutics worldwide.

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Emerging infections and pandemics: The critical importance of global health equity action

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"No man is an island entire of itself; every man is a piece of the continent, a part of the main…"[1]

The coronavirus disease 2019 (COVID-19) pandemic has demonstrated the stark reality of how unprepared the United States and the rest of world were and are for emerging infections that can lead to pandemics. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is unlikely to be the final pathogen that leads to a multi-country epidemic or a global pandemic. A host of other pathogens are waiting in line, ranging from zoonotic

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