Molecular Therapy
Nucleic Acids
Commentary

PLA-PEG as an alternative to PEGylated lipids for nanoparticle-based DNA vaccination against SARS-CoV-2

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Recent studies have highlighted various novel ionizable cationic and helper lipids for nanoparticle-based DNA delivery.[1,2] However, there has been limited analysis on the impact of PEGylated lipids and lipid ingredient proportions on transfection efficiency and safety in DNA vaccines. The work of Huang and colleagues is of broader interest to the development of DNA vaccines, as they explored the use of a diblock PLA-PEG copolymer as an alternative to PEGylated lipids, such as DMG-PEG, in DNA-loaded lipid nanoparticles (LNPs). Their research demonstrated that these PLA-PEG-based LNPs could induce protective immunity against heterologous virus challenges and serve as effective boosters against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants (Figure 1).[3] This approach offers valuable insights into optimizing DNA vaccine delivery systems and enhancing their efficacy and safety.

LNPs have garnered significant attention as promising carriers for nucleic acids, especially following the success of LNP-based mRNA vaccines against COVID-19.[4] Despite their effectiveness, mRNA vaccines require ultracold storage and transportation, which poses logistical challenges for communities lacking cold-chain facilities. In contrast, DNA molecules offer high stability and do not necessitate ultracold storage, making DNA vaccines an attractive alternative. However, delivering DNA vaccines efficiently remains a major hurdle. Traditional delivery methods, such as viral vectors and electroporation devices, have notable limitations, including restricted transgene capacity, host immune responses, and possible cellular damage.[5]

Current research has explored the use of LNPs as a promising platform for next-generation DNA vaccine technology.[2,3] These investigations have demonstrated that LNP-encapsulated DNA can elicit enhanced protection and higher humoral responses compared to free DNA. Huang and collaborators formulated hybrid LNPs by combining varied molar ratios of the amphiphilic bioresorbable copolymer PLA-PEG with cholesterol, a helper lipid (phospholipid), and an ionizable cationic lipid through microfluidic mixing. In this approach, the conventional DMG-PEG used in approved mRNA vaccines was replaced by PLA-PEG. Huang and colleagues demonstrated that replacing PEGylated lipids with the bioresorbable copolymer PLA-PEG in LNPs enhanced biocompatibility *in vitro*, suggesting that the selection of surface-active agents may play a crucial role in reducing DNA-LNP-induced cell death. While PEGylated lipids typically serve as surface engineering agents to prevent nonspecific binding to proteins via steric repulsion, further studies are relevant to investigate additional contribution of PLA-PEG within LNPs, such as circulation time, endocytosis, and endosomal escape. Additionally, the hybrid LNPs, loaded with plasmid DNA encoding the luciferase protein, successfully induced luciferase expression in mice after intramuscular administration, with sustained expression for at least 1 month. These findings highlight the potential of PLA-PEG hybrid LNPs as a robust and biocompatible delivery system for DNA vaccines, highlighting the importance of surface-active agent selection in optimizing vaccine efficacy and safety.[3]

Huang and colleagues next evaluated whether encapsulating a plasmid DNA encoding a variant trimeric-spike gene of the Omicron variant, named TSomi-LNP, could confer protective immunity in hamsters challenged with the Wuhan strain of SARS-CoV-2. Hamsters vaccinated with TSomi-LNP demonstrated no significant weight loss and exhibited reduced viral load and inflammation in their lungs. However, since the plasmid DNA used encoded a SARS-CoV-2 Omicron variant antigen, further studies are relevant to determine the vaccine's effectiveness against the Omicron strain. Additionally, while hamsters are a useful model for mild COVID-19, it is important to consider other relevant models, such as K18-hACE2 transgenic mice (which express the human ACE2 as a receptor for SARS-CoV-2), for a more comprehensive assessment of vaccine efficacy, immunogenicity, and survival outcomes.

Moreover, the authors also evaluated whether Omicron-specific antibodies could be effectively generated by a hybrid-type vaccine booster. Hamsters were initially immunized with two doses of a Wuhan mRNA-LNP vaccine, which produced Wuhan-specific immunoglobulin G antibodies. At week 26, the hamsters received a booster dose of the TSomi-LNP vaccine. This boost elicited a strong neutralizing response against the Omicron variant, suggesting that the TSomi-LNP booster could provide effective protection against the virus.

Huang and colleagues demonstrated the immunogenicity of DNA-LNP vaccines in hamsters, showing their ability to induce

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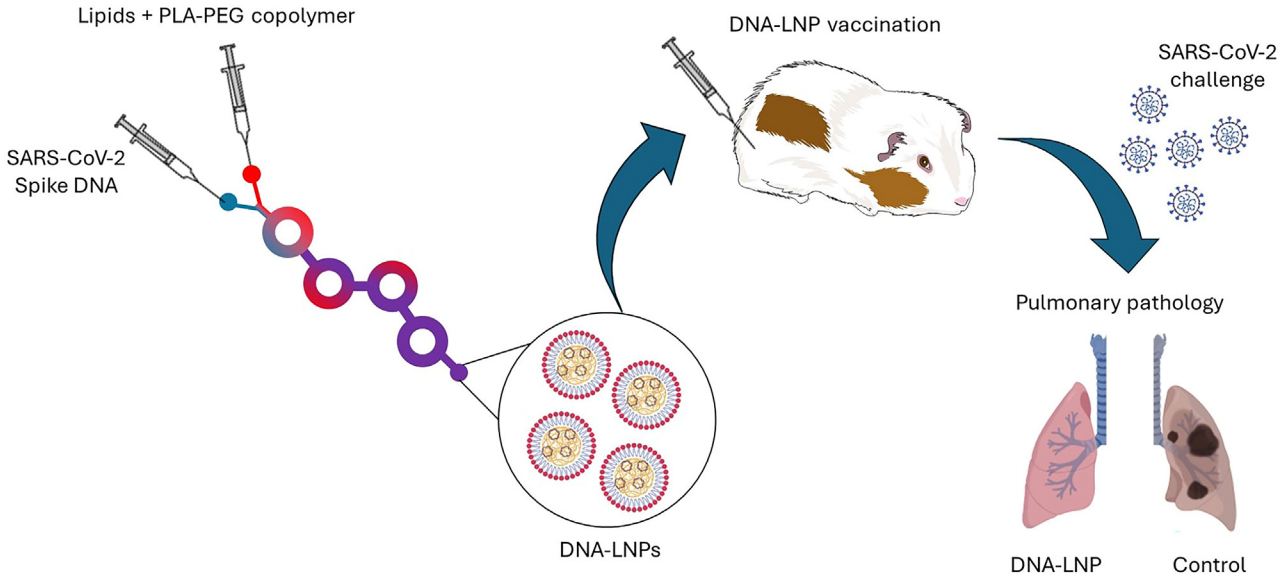

Figure 1. Formulation of DNA-LNPs involved mixing DNA encoding a SARS-CoV-2 Omicron variant antigen with an ethanolic phase containing cholesterol, phospholipids, the ionizable lipid SM-102, and the amphiphilic copolymer PLA-PEG through a toroidal channel mixer

The resulting DNA-LNPs were characterized and used to vaccinate hamsters, which were subsequently challenged with the SARS-CoV-2 Wuhan strain. Post-challenge pulmonary analysis demonstrated the protective effects of the DNA-LNPs by reduced viral load and inflammation. Figure adapted with permission from Yang et al.[3]

protective immunity against heterologous virus challenges and serve as effective boosters against SARS-CoV-2 variants. Their findings provide valuable insights into the relationship between LNP composition, manufacturing processes, and vaccine efficacy. This contributes to the development of advanced DNA-LNP formulations to enhance vaccine effectiveness against current and potential infectious diseases and pathogens.

Furthermore, integrating surface-engineered LNPs into DNA vaccine strategies could significantly impact vaccine development, opening possibilities to other immunotherapies that could benefit from nucleic acid delivery. Future studies could explore optimizing PLA-PEG modifications to increase immune responses for various antigens and patient cohorts. Advancements in LNP technology could also lead to customized DNA vaccines based on individual immune profiles, thereby enhancing their effectiveness and safety.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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