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Lyophilized mRNA-lipid nanoparticles vaccine with long-term stability and high antigenicity against SARS-CoV-2

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Abstract

Advanced mRNA vaccines play vital roles against SARS-CoV-2. However, due to the poor stability, most current mRNA delivery platforms need to be stored at -20 °C or -70 °C. Here we present lyophilized thermostable mRNA loaded lipid nanoparticles, which could be stored at room temperature with long-term stability. We demonstrate the applicability of lyophilization techniques to different mRNA sequences and lipid components. Three lyophilized vaccines targeting wild-type, Delta and Omicron SARS-CoV-2 variant were prepared and demonstrated to be able induce high-level of IgG titer and neutralization response. In the Delta challenge *in vivo* experiment, the lyophilized mRNA vaccine successfully protected the mice from infection and clear the virus. This lyophilization platform could significantly improve the accessibility of mRNA vaccine or therapeutics, particularly in remote regions.

Keywords:

SARS-CoV-2, mRNA vaccine, Lyophilization, Lipid nanoparticles.

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1. Introduction

After years of research and development, mRNA delivery systems made their breakthrough and became the frontrunners that prevent coronavirus disease 2019 (COVID-19). Compared with inactivated vaccines and recombinant protein vaccine, mRNA vaccines could be easily and quickly updated to target different variants, and with comparable first-class protective efficacy as well [1]. The sequence-independent manufacturing process saved a lot of time and cost to develop a new vaccine, especially in the pandemic situation, just as the situation fighting against the new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2, 3].

Current mRNA therapeutics heavily depend on lipid or lipid-like delivery systems to improve their *in vivo* transfection efficacy [4]. Several lipid components form the nanoparticles carrying the mRNA to targeted organelles. Although mRNA therapeutics show such superiority, challenges about stability still impede their accessibility. Cryogenic preservation and transportation are needed for two current licensed mRNA vaccines, BNT162b2 (-80 °C ~-60 °C) and mRNA-1273 (-20 °C) [5]. The demanding requirements come from the complex interactions among multiple lipid components and the instability of mRNA, which is sensitive to oxygen, moisture, enzymes, pH and even more [6]. As the exact mechanisms have not been solved, how to improve the stability of mRNA therapeutics is still challenging [7].

Lyophilization is a process that removes water by sublimation under vacuum at a low temperature [8]. It's a relatively mild drying method and could improve the stability of vulnerable macrobiomolecules or colloidal nanoparticles [9]. The lyophilized mRNA could be stored at 4 °C or at room temperature for long time [10, 11]. However, drying of mRNA-lipid nanoparticles (mRNA-LNP) is more sophisticated, as the freezing and the dehydration process would induce mechanical force and deform the vehicle structure, leading to vehicle aggregation, mRNA breakage or leakage [12]. Moreover, some research showed that even the mRNA-LNP remained their integrity and encapsulation efficiency, the *in vivo* transfection efficacy was greatly reduced after lyophilization due to some unknown reasons [13].

Here we present an optimized lyophilization technique, which could effectively sustain the physiochemical properties and bioactivity of mRNA-LNP and achieve long-term storage at 2 °C ~8 °C. The improved thermostability was verified with LNPs containing different mRNA molecules, demonstrating its wide applicability. Furthermore, we utilized this technique to

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prepare the first lyophilized, thermostable mRNA-LNP vaccines with encoding the antigen of wild-type (WA1, Lyo-mRNA-WT), Delta (Lyo-mRNA-Delta) or Omicron COVID-19 variant (Lyo-mRNA-Omicron) and confirmed their high-leveled antibody responses and prevention ability. Noticeably, we optimized the mRNA sequences in theses lyophilized mRNA vaccines. The lyophilized vaccine could both induce strong immune response and produce high levels of neutralizing antibody titer. The mRNA-Delta and mRNA-Omicron vaccine also showed cross-protection ability against other variants. In the challenge study of mRNA-Dela vaccine against Delta strain, the vaccine also demonstrated to fully protect the mice from infection and clear the virus.

2. Materials and methods

2.1 Materials

Lipids were purchased from Xiamen Sinopeg Biotech Co., Ltd. Cholesterol was purchased from AVT (Shanghai) Pharmaceutical Tech Co., Ltd. Ethanol was obtained from Aladdin. Citric acid and trisodium citrate were obtained from Sigma-aldrich. SARS-COV-2 WA1, Delta and Omicron pseudovirus using recombinant replication-deficient vesicular stomatitis virus (VSV) vector that encodes Luciferase instead of VSV-G (VSVΔG-Luc) were obtained from Vazyme.

2.2 RNA synthesis

SARS-COV-2 NTD-RBD region were used as antigens in this work. The respective mRNAs were produced *in vitro* by standard T7 RNA polymerase-mediate transcription reaction and added with Cap1, then purified though Fast Protein Liquid Chromatography. Luciferase mRNA were synthesized as the same way and purified by MEGAclear™ Transcription Clean-Up Kit (Invitrogen).

2.3 Lipid nanoparticle (LNP) preparation and characterization

The lipid nanoparticles (LNPs) were prepared by mixing an aqueous phase containing the mRNA with an ethanol phase containing the lipid mixtures using a T-junction mixing device as reported previously [14]. In brief, the mRNA was dissolved in a 100 mM citric buffer (pH 4.0). The lipid mixture was dissolved in anhydrous ethanol with the molar ratio of ionizable lipid:1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC): cholesterol: PEG-lipid=46.3:9.4:42.7:1.6. The N:P ratio was kept as 6:1. And the ethanol and aqueous phases were mixed at a volume

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ratio of 3:1 in the T-junction device. After mixing, LNPs were dialyzed against a buffer at pH 7.4 for 6 hours. After that, LNPs were sterilized via a 0.22 μm filter and stored at 4 °C for further use. The average diameter, polydispersity (PDI) and zeta potential were measured with a NS-90Z device (Malvern Panalytical). The cryo-TEM images were obtained from Southern University of Science and Technology. The concentration of leaked mRNA (C_{leak}) was determined with a fluorescence detection kit, following the manufacture's protocols. LNP was also lysed with 0.4% Triton X-100 to determine the concentration of total mRNA (C_{total}). The encapsulation efficiency was calculated as the following equation:

$$\text{Encapsulation efficiency} = \frac{C_{total} - C_{leak}}{C_{total}} \times 100\%$$

2.4 Lyophilization

LNP solution was added with cryoprotectant and filled in a penicillin bottle, then the mixture was lyophilized with a freezer dryer (Pilot-2H, Boyikang) using a designed procedure. The resulting powder was collected, characterized and stored at 4 °C for further use. To measure the stability, lyophilized LNPs were incubated at either 4 °C, 25 °C, or 40 °C for different time. The mRNA integrity was measured with a gel retardation assay [15] and microfluidic capillary electrophoresis (Agilent Fragment Analyser) [16] , as previously reported.

2.5 Cell culture

HEK 293T/17 and ACE2-expressing 293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine, 10% FBS (sigma-aldrich) and 1% penicillin/streptomycin (BI) at 37 °C and 5% CO_2.

2.6 Animals

Female BALB/c mice and K18-hACE2 KI transgenic mice age of 6-8 weeks and body weight of 18-22 g were used. The mice were housed under a 12 h dark-light cycle at a constant temperature. The protocol and procedures used in the studies with animals were reviewed and approved by the Laboratory Animal Welfare and Ethics Committee in Wuhan University (Wuhan, China).

2.7 Luciferase transfection *in vivo*

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Luciferase mRNA loaded LNPs (mRNA-Luc LNPs) and its lyophilized product, Lyo-mRNA-Luc LNPs were examined with their *in vivo* transfection efficiency. The encapsulated mRNA concentration was adjusted to 100 μg/mL and freeze-dried. Lyo-mRNA-Luc LNPs were reconstituted using the same volume of nuclease-free water with before drying. Mice were injected with 100 μL LNP solution through tail vain injection. Post 24-hour injection, mice were narcotized with avertin solution (0.2%, 350 μL). Then the luciferin solution (1.4%, 200 μL) was administered intraperitoneally. And the mice were imaged with an IVIS Spectrum *in vivo* imaging system (FluoView400, Boluteng).

2.8 Immunization

Three kinds of LNPs with mRNA encoding the antigen of WA1 (Lyo-mRNA-WT), Delta (Lyo-mRNA-Delta) or Omicron COVID-19 variant (Lyo-mRNA-Omicron) were prepared and used for mice immunization experiment. 0.5 mL LNP solutions containing 0.1 mg/mL encapsulated mRNA were freeze-dried with a penicillin bottle. Lyophilized LNPs were reconstituted using 0.5 mL nuclease-free water. Mice were received two doses of LNPs through intramuscular injections of 20 μL, 50 μL, or 100 μL reconstituted LNPs. The dilution water was also nuclease-free. The blood samples were collected and centrifuged at 1200 g and 4 ^{o}C for 10 min. The supernatant serum was separated, aliquoted, and frozen at −80°C.

2.9 Enzyme-linked Immunosorbent Assay (ELISA)

96-Well ELISA Microplates (Greiner) were coated with 2 ng/μL RBD protein (NOVOPROTEIN) in coating buffer (Dakewe) at 4°C for 15 h. After washing and blocking, gradiently diluted mouse serum were incubated in plate at 4°C for 2 h. Following washes, the secondary antibody, goat Anti-Mouse IgG H&L conjugated HRP (Abcam), was incubated in plate at room temperature for 1 h. And 3,5,3’5’-tetramethylbenzidine (TMB) (Dakewe) was used as the substrate to detect antibody responses. Data collection was performed using a MOLECULAR DEVICES microplate reader and SoftMax Pro software version 7.1.0. Endpoint titers were calculated as the dilution that absorbance exceeding 4X background (protein and secondary antibody).

2.10 Pseudotyped virus neutralization assay

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Neutralizing antibody titers were tested according to the reported methods [17]. Briefly, serum samples were diluted and mixed with a certain amount (1.3×10^4 $TCID_{50}$/mL) of pseudotyped virus and incubated at 37℃, 5% CO_2 for 30 min. After that, the 293T-ACE2 cells are added and incubated for 24 h. Then the Bio-Lite luciferase detection reagent (Vazyme) were mixed and incubated for 2 min. The luminescence values (RLU) were detected with the microplate reader immediately, and the amount of pseudotyped virus entering cells was calculated by detecting the expression of luciferase, thus to obtain the neutralizing antibody content of the sample. Luminescence readout data are collected and the half maximal inhibitory concentration (IC50) are calculated for the tested samples.

2.11 SARS-CoV-2 Delta virus neutralization assay

SARS-CoV-2 Delta-neutralizing antibody was determined by in vitro inhibition of cytopathic effect (CPE), and was performed in the ABSL-3 lab at Center of Laboratory Animal Sciences, Wuhan University (Wuhan, China). Heat-Inactivated sera from K18-hACE2 KI mice were diluted in cell culture medium, and serial 3-fold dilutions starting from 1:100 to 1:218700. The diluted samples were mixed with a SARS-CoV-2 Delta virus suspension of 100 plaque-forming unit (PFU), followed by 1 h incubation. The virus-serum mixtures were added to Vero-E6 cells seeded in 24-well plate and cultured in a 5% CO_2 incubator at 37 °C for 3 days. The inhibitory capacity of each sample dilution was assessed for cytopathic effect (CPE), and the neutralization titer was calculated as the reciprocal of serum dilution required for 50% neutralization of viral infection.

2.12 SARS-CoV-2 Delta challenge in K18-hACE2 KI transgenic mice

The SARS-CoV-2 Delta challenge in K18-hACE2 KI transgenic mice to evaluate Lyo-mRNA-Delta vaccines of the study was performed at the Biosafety Level 3 (BSL-3) in Center of Laboratory Animal Sciences, Wuhan University (Wuhan, China). 36 female K18-hACE2 KI transgenic mice, 6 weeks old, were divided into 4 groups, namely the negative control group(n=6), blank lipid nanoparticles (blank LNP) control group(n=6), low-dose vaccine group (n=12) (5 μg/dose) and high-dose vaccine group (n=12) (10 μg/dose). The latter three groups of animals were intramuscularly immunized with 0.1ml blank LNP, 5 μg Lyo-mRNA-Delta and 10 μg Lyo-mRNA-Delta, respectively, at Day 0 (D0) and Day 14 (D14). At Day 28 (D28, 14 days after the second immunization), mice immunized with Lyo-mRNA-Delta or only blank LNP were challenged with

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2.5×10^3 PFU SARS-CoV-2 Delta virus intranasally. The challenged mice were observed clinical symptoms and death, and weighed on day of infection and daily for up to 14 days post infection (dpi). At days 7 and 14 dpi, six mice were sacrificed under anesthesia from each vaccination, and 7 dpi from blank LNP control group. The lung and brain tissues were collected to determine viral titer by qPCR.

2.13 Statistical analysis

Data were collected at least six independent experiments for *in vivo* experiment. All other experiments were performed at least three times independently unless specified. Statistical analysis was mainly processed using Prism software version 8.3 (GraphPad Software Inc., San Diego, CA) and analyzed by one-way or two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistically significant differences were considered when $p < 0.05$.

3. Results and discussion

3.1 LNP preparation and lyophilization

Four kinds of LNPs used in this paper were prepared through a classic T-junction mixing process [14]. LNPs containing Luc mRNA (mRNA-Luc LNPs), WT mRNA (mRNA-WT LNPs), Delta mRNA (mRNA-Delta LNPs) or Omicron (mRNA-Omicron LNPs) all showed narrow size distribution and high encapsulation efficiency (EE), as listed in **Table 1**. From the cryo-TEM images (**Figure 1**A), we could observe the uniform, lamellar and vesicular structure of the LNPs. After lyophilization, the dried LNPs looked like a white fluffy cake (**Figure S1**, supporting information) and could be dissolved in water easily and quickly (<10 seconds). The reconstituted solution was uniform and translucent, just as the freshly-prepared LNP solutions. The size, polydispersity index (PDI) and encapsulation efficiency of LNPs (Table 1) only changed slightly, indicating that the optimized lyophilization process didn't changed their basic physical properties. The mRNA integrity was also well-maintained (>90%, **Figure S2**, supporting information), demonstrating the lyophilization process didn't cause damage to the mRNA structure.

We then evaluated the thermostability of Luc LNPs by incubating then at 4 ^{o}C, 25 ^{o}C, or at 40 ^{o}C for different time (Figure 1B, 1C). The products didn't exhibit any change and sustained the size and encapsulation efficiency at 4 ^{o}C and 25 ^{o}C after 18 days, indicating the high stability of the product. At 40 ^{o}C, the size increased, but the encapsulation efficiency and mRNA

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integrity (Figure 1D) were maintained. It could be estimated that the lyophilized LNPs could be stored at a 2~8 °C refrigerator for a long time.

The *in vivo* transfection efficiency of lyophilized LNPs was first evaluated with Luciferase mRNA. As seen in Figure 1E, mice treated with lyophilized mRNA-Luc LNPs (Lyo-mRNA-Luc LNPs) showed comparable total fluorescent intensity (6.91±0.22 $\times 10^7$p/s) with those treated with freshly-prepared Luc LNPs (9.43±0.66 $\times 10^7$p/s). This result indicated the mRNA-Luc LNPs maintained its bioactivity well after lyophilization. Next, we investigated the immunogenicity of mRNA-WT LNPs after lyophilization, following a prime-boost regimen. Mice were immunized with mRNA-WT LNPs or Lyo-mRNA-WT LNPs at day 0 and day 7, through intramuscular injection, then the serum was collected at day 21. As shown in **Figure 2**A, all vaccines induced high IgG titers after immunization. The bioactivity of the antibodies was then tested through a pseudotyped virus assay (Figure 2B). Neutralizing response was also observed at this time point, titers against WA1 for the groups treated with 5 μg mRNA-WT LNPs (1991) and 5 μg Lyo-mRNA-WT LNPs (1973) showed no significant difference, demonstrating the lyophilization process didn't affect their bioactivity.

Table 1. Main physical characteristics of LNPs before and after lyophilization

Samples	Fresh			Lyophilized		
	Size (nm)	PDI	EE (%)	Size (nm)	PDI	EE (%)
mRNA-Luc LNPs	82.1	0.12	95.2	116.8	0.13	87.9
mRNA-WT LNPs	92.4	0.14	92.1	117.7	0.16	87.0
mRNA-Delta LNPs	85.1	0.12	93.8	106.3	0.14	95.6
mRNA-Omicron LNPs	77.6	0.09	94.3	88.4	0.16	86.2

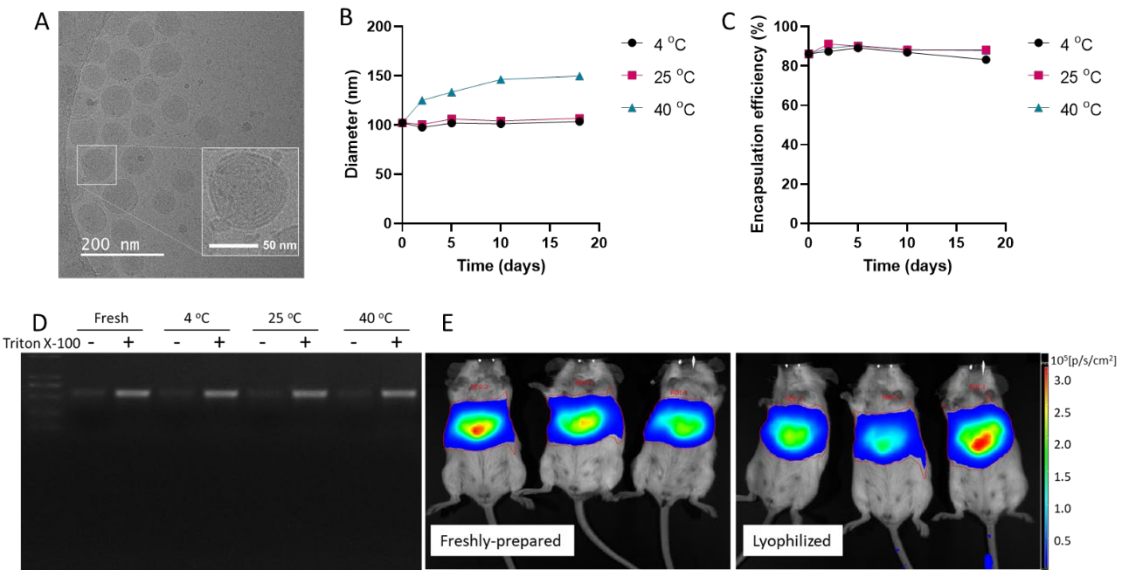

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Figure 1. (A) Cryo-TEM image of mRNA-Luc LNPs. Change of (B) size and (C) encapsulation efficiency of Lyo-mRNA-Omicron LNPs at 4 °C, 25 °C, or 40 °C after being incubated for different time. (D) Stability of Lyo-mRNA-Omicron LNPs at 4 °C, 25 °C and 40 °C after 10 days. Triton X-100 was used to release mRNA from LNPs. (E) Bioluminescent images of mice after treated with freshly-prepared or lyophilized Luc LNPs.

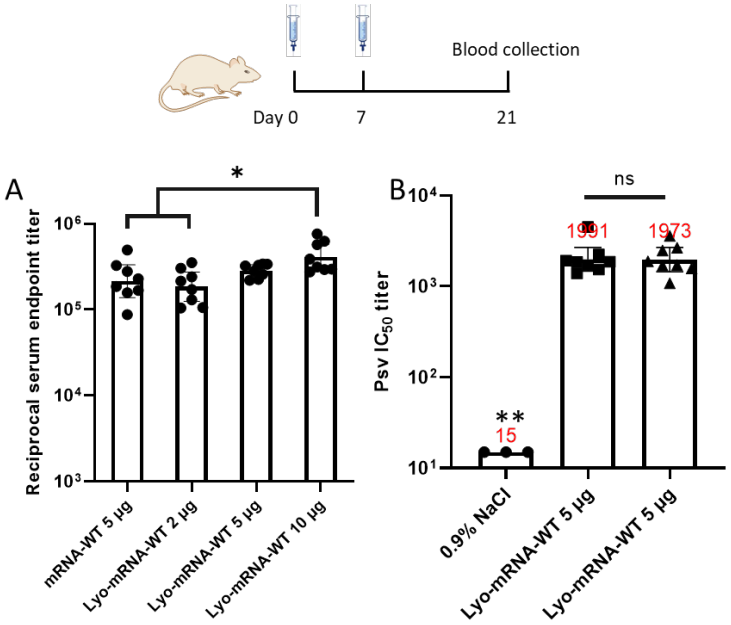

Figure 2. (A) Total IgG titer and (B) Pseudotyped IC_{50} titer of mice post-immunized twice with freshly-prepared mRNA-WT LNPs or Lyo-mRNA-WT LNPs. ns: no significant difference; *p<0.05, ** p<0.005.

3.2 High neutralizing antibody induced by lyophilized SARS-COV-2 mRNA vaccine

Next, a mRNA-Delta vaccine was prepared to further evaluate its efficacy against SARS-COV-2 Delta and other variants based on the lyophilized mRNA-LNP formulation (Lyo-mRNA-Delta). As show in **Figure 3**A, after immunizing intramuscularly twice with 5 μg dosage at a 2-week interval, the sera obtained 14 days after the second vaccination showed a very strong Delta-RBD specific IgG antibody response in the six-week-old female BALB/cJ mice, with a geometric mean titer of 2.37×10^6. Potent neutralizing activity was also induced at this time point, titers against Delta were up to 9340 reciprocal ID50, while cross against Omicron, WA1 were 3278 and 30874, respectively, as showed in a vesicular stomatitis virus-based SARS-CoV-2 pseudovirus neutralization assay (Figure 3B). In contrast to the phenomenon of 20- to 130-fold neutralization reduction for ancestral spike vaccines elicited sera [18-22], the titers of lyophilized mRNA-Delta vaccine against Omicron were still high, consistent with data from

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other studies [23, 24]. Interestingly, the mRNA-Delta vaccine stimulated much higher neutralizing antibody titers to wild-type SARS-COV-2 pseudovirus than Delta itself.

We also developed a lyophilized mRNA vaccine against Omicron (Lyo-mRNA-Omicron) since it has a significant transmission advantage over Delta and become the dominant variant of concern in the current wave of COVID-19 [25]. To investigate its antigenicity, mice were two-dose injected intramuscularly by an accelerated immunization process at day 0 and day 7. Sera were collected one week after the second dose and pseudovirus titers were analyzed. Neutralizing antibody titers against Omicron increased to a high value as 4758. Still, it could induce certain neutralization response against Delta though lower than Omicron.

In summary, our lyophilized mRNA vaccines were able to trigger robust antibody response against SARS-COV-2 virus.

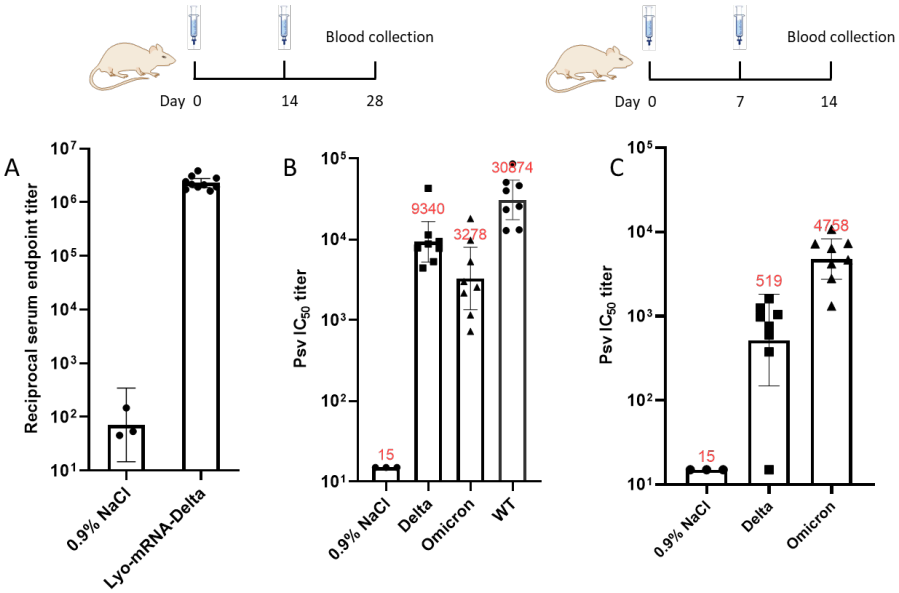

Figure 3. Lyophilized mRNA vaccine elicited robust binding and pseudovirus neutralizing antibody responses in BALB/c mice. Mice were immunized at day 0 and day 14 with Lyo-mRNA-Delta, and sera were collected 14 days post-immunization. The (A) IgG titer and (B) neutralizing antibodies against Delta, Omicron or WT SARS-CoV-2 pseudovirus were analyzed. Other mice were immunized with an accelerated immune process at day 0 and day 7 with Lyo-mRNA-Omicron, and sera were collected 7 days post-immunization. (C) The neutralizing antibodies against Delta and Omicron SARS-CoV-2 pseudovirus were analyzed.

3.3 Protective efficacy of lyophilized mRNA-Delta vaccine in K18-hACE2 KI transgenic mice

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The immunogenicity and protective efficacy of Lyo-mRNA-Delta were evaluated in K18-hACE2 KI transgenic mice. The results showed that mice received 5 µg and 10 µg Lyo-mRNA-delta vaccine were sufficient to induce robust neutralizing activity before the challenge, the GMT of neutralizing antibodies against the SARS-CoV-2 Delta virus reached 4627 and 9044, respectively (**Figure 4**A). After challenge, K18-hACE2 KI mice received one-dose blank LNPs were highly susceptible to SARS-CoV-2 Delta infection. Beginning at 2 dpi, mice demonstrated clinical symptoms, including lethargy, ruffled fur, arched back, drowsiness. At 6 dpi, body weight reduction was seen in blank LNP control mice, and showed approximately 10% weight loss by 7 dpi, and one-thirds mice (two mice) died, and the remaining mice were moribund, humanely euthanized according to ethical principle. In contrast, mice received two-dose of reconstituted Lyo-mRNA-Delta showed no clinical abnormity, with no difference in body weight comparing to the negative control mice (Figure 4B, C). For these groups, half of the mice (six mice) were euthanized 7 dpi, and the other half (six mice) euthanized 14 dpi. After euthanasia, brain and right lung samples were collected from each animal for viral load quantification, and left lung were fixed for histopathological evaluation. The results showed that lyophilized mRNA-Delta vaccination protected the mice from SARS-CoV-2 Delta infection. The viral load in right lung tissue of Lyo-mRNA-Delta vaccinated animals remained at the level of low limit of quantification 7 dpi. In contrast, a high level of viral load existed in the right lung tissue of animals in blank LNP control group, which up to approximately 1011 copies/g (Figure 4D). Importantly, the viral load in right lung tissue had almost undetectable in all Lyo-mRNA-Delta vaccinated mice 14 dpi (Below detection limit). The viral load in brain tissues in 5 µg animal groups displayed similar patterns as those in lung tissues. And 10 µg Lyo-mRNA-Delta showed remarkable protective effect on viral dissemination and amplification (Figure 4E).

Together, these data suggest that lyophilized mRNA-Delta vaccine is highly immunogenic and can fully protect the challenged mice from SARS-COV-2 infection.

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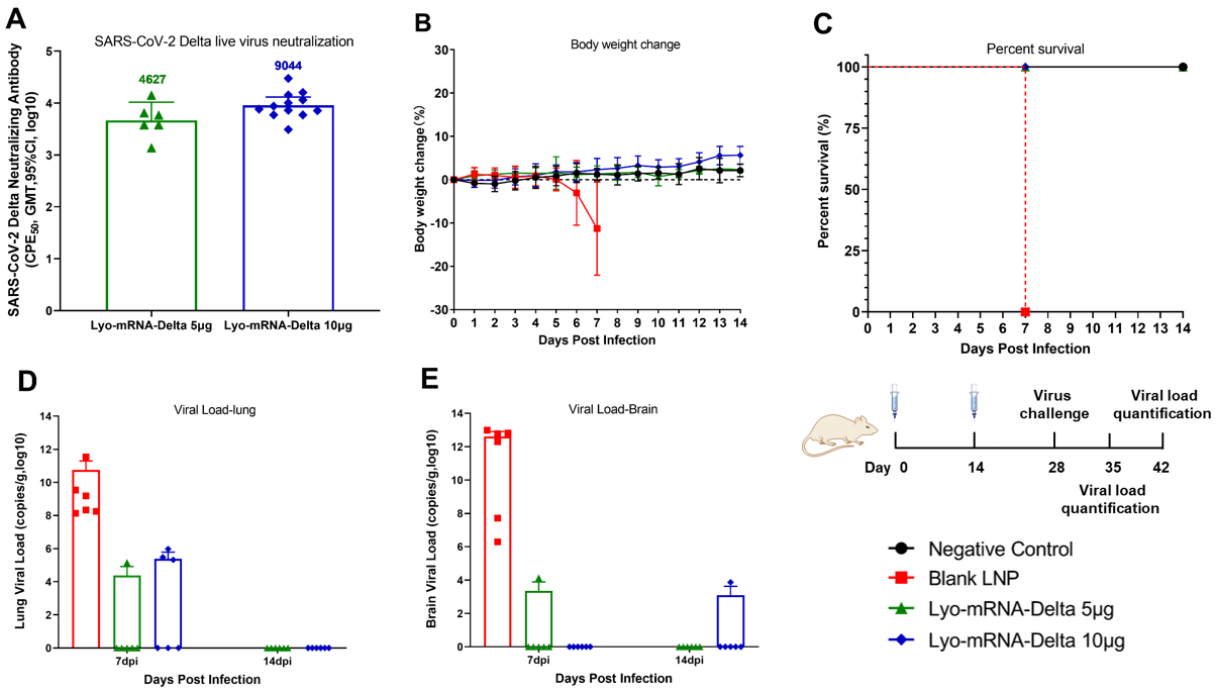

Figure 4. Protective efficacy of Lyo-mRNA delta vaccine in K18-hACE2 KI transgenic mice. Mice were intramuscularly inoculated with 5 μg or 10 μg Lyo-mRNA delta vaccine twice, separated by 14 days, and two weeks after the second dose, 2.5×10^3 PFU SARS-CoV-2 Delta was administrated to the animals intranasally. (A) Neutralizing antibody to SARS-CoV-2 delta virus induced by Lyo-mRNA-delta vaccine. (B) Changes in body weight after challenge. (C) Animal survival curve after challenge, all blank LNP animals died or were euthanized 7 days after challenge. (D) and (E) Viral load in lung and brain 7 and 14 days after challenge.

4. Conclusion

The mRNA vaccine has several advantages, such as rapid design of antigen, potential for quick, inexpensive and scalable manufacturing, and inducing strong humoral and cellular immunity, which make it a powerful weapon against infectious diseases, especially against those caused by virus that are easy to mutation. The advantage and efficacy of mRNA vaccines has also been proved by two approved and marketed mRNA vaccines against COVID-19, Comirnaty and Spikevax. While the storage and transport of the current approved mRNA vaccines require ultracold temperature, which limit its accessibility.

According to data in Our World in Data as of Feb. 6, 2022, 61.4% of the world population has received at least one dose of a COVID-19 vaccine. 10.22 billion doses have been administered globally. However only 10% of people in low-income countries have received at least one-dose vaccine [26]. Therefore, it is particularly important to develop a mRNA vaccine that can be

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transported in conventional cold-chain and be stored at 2°C~8 °C, or at room temperature stably for broad application.

In this study, we successfully achieved the lyophilization of mRNA-LNP vaccine. The particle size, encapsulation efficiency, poly dispersity index and zeta potential after reconstitution only changed slightly compared with freshly-prepared LNPs. And the bioactivity and immunogenicity were well-maintained, meeting the mRNA vaccine specification. The lyophilized mRNA-Delta and mRNA-Omicron vaccine could both produce high levels of neutralizing antibody titer. In the challenge study of Lyo-mRNA-Dela vaccine against Delta strain, it was confirmed that the vaccine could fully protect the mice from infection and clear the virus. Moreover, the lyophilized mRNA-LNPs showed long term storage stability at 4 °C and 25 °C, addressing the storage and transportation issue of current mRNA vaccines.

In conclusion, the lyophilized mRNA-LNP vaccine has the advantage of both high immunogenicity and accessibility, and very suitable for prevention of epidemic such as SARS-COV-2. We are actively promoting its clinical trial application.

Supporting Information

Supporting Information is available from the author.

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Supporting Information

Lyophilized mRNA-lipid nanoparticles vaccine with long-term stability and high antigenicity against SARS-CoV-2

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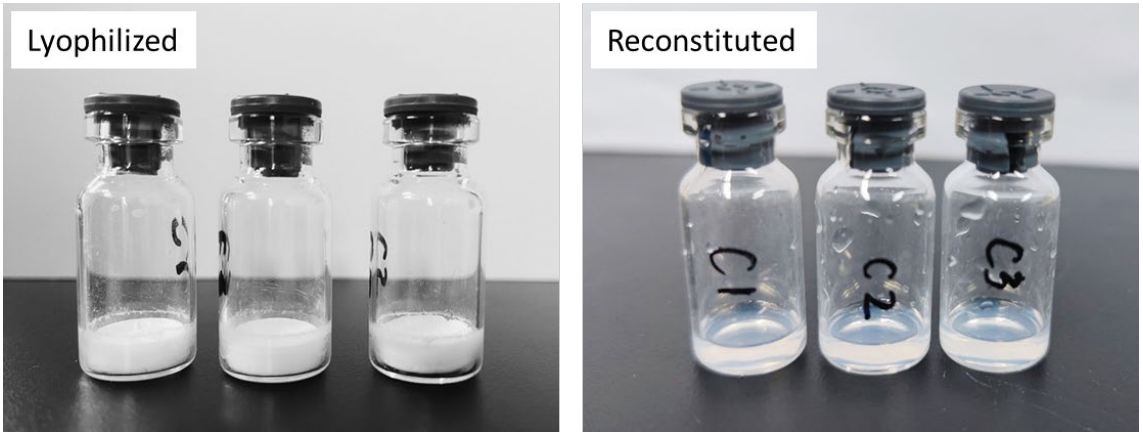

Figure S1. Images of lyophilized mRNA-LNPs and the reconstituted solutions.

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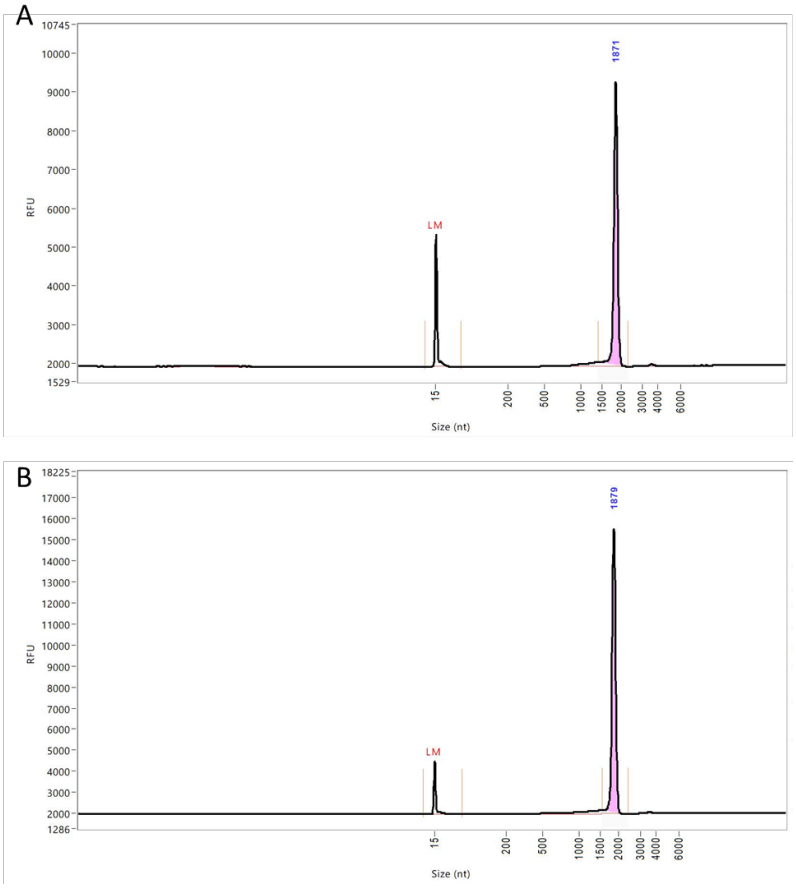

Figure S2. Microfluidic capillary electrophoresis analysis of (A) Omicron mRNA and (B) reconstituted Lyo-mRNA-Omicron.