

Opinion

Role of nanotechnology behind the success of mRNA vaccines for COVID-19



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ARTICLE INFO

Article history:

Received 2 January 2021

Received in revised form 19 March 2021

Accepted 22 March 2021

Available online 26 March 2021

Keywords:

COVID-19

mRNA

Lipid nanoparticles

BNT162b2

mRNA-1273

ABSTRACT

The emergency use authorization (EUA) by the US-FDA for two mRNA-based vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) has brought hope of addressing the COVID-19 pandemic which has killed more than two million people globally. Nanotechnology has played a significant role in the success of these vaccines. Nanoparticles (NPs) aid in improving stability by protecting the encapsulated mRNA from ribonucleases and facilitate delivery of intact mRNA to the target site. The overwhelming success of these two mRNA based vaccines with ~95% efficacy in phase III clinical trials can be attributed to their unique nanocarrier, the "lipid nanoparticles" (LNPs). LNPs are unique compared with bilayered liposomes and provide improved stability of the cargo, possess rigid morphology, and aid in better cellular penetration. This EUA is a major milestone and showcases the immense potential of nanotechnology for vaccine delivery and for fighting against future pandemics. Currently, these two vaccines are aiding in the alleviation of the COVID-19 health crisis and demonstrate the potential utility of nanomedicine for tackling health problems at the global level.

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Introduction

It is the first time in history that two mRNA-based vaccines developed using lipid nanoparticles (LNPs) have been given emergency

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use authorization (EUA) by the US FDA for clinical therapeutics against the COVID-19 [1]. This undermines the skepticism on the potential of nanotechnology based approaches. Nanoparticles (NPs) offer many unique advantages compared to other conventional drug carriers including tailored drug release profile, enhanced surface area, protection of the cargo from degradation and modulation of drug pharmacokinetics [2–9]. Nanotechnology has fast tracked the development of mRNA-based COVID-19 vaccines invented by Moderna and Pfizer/BioNTech [10–12]. On 16th November 2020, Moderna officially shared the preliminary data of the phase III clinical trial of its COVID-19 candidate vaccine mRNA-1273 followed by Pfizer-BioNTech on 18th November, 2020 with the clinical trial outcome of its COVID-19 candidate vaccine BNT162b2. Efficacy is an important

Table 1
Salient features of Pfizer-BioNTech and Moderna mRNA vaccines.

Key features	Pfizer-BioNTech (BNT162b2)	Moderna (mRNA-1273)
Active ingredients: Messenger ribonucleic acid (mRNA)	<ul style="list-style-type: none"> ■ Nucleoside modified messenger RNA (modRNA) encoding the viral spike glycoprotein (S) of SARS-CoV-2. 	<ul style="list-style-type: none"> ■ Synthetic mRNA encoding the pre-fusion stabilized spike glycoprotein (S) of SARS-CoV-2 virus.
Fats: Encases & protects the fragile mRNA	<ul style="list-style-type: none"> ■ Cholesterol ■ 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide ■ ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) ■ 1,2-distearoyl-sn-glycero-3-phosphocholine 	<ul style="list-style-type: none"> ■ Cholesterol ■ Sphingomyelin-102(SM-102) ■ Polyethylene glycol [PEG]2000 dimyristoyl glycerol [DMG] ■ 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC]
Saline solution: Buffer to maintain the pH level close to our body	<ul style="list-style-type: none"> ■ Dibasic sodium phosphate dihydrate ■ Monobasic potassium ■ Potassium chloride ■ Phosphate ■ Sodium chloride ■ Sucrose 	<ul style="list-style-type: none"> ■ Acetic acid ■ Sodium acetate ■ Sucrose ■ Tromethamine ■ Tromethaminehydrochloride
Storage temperature	<ul style="list-style-type: none"> ■ (-80 to -60 °C storage) 	<ul style="list-style-type: none"> ■ (-25 to -15 °C storage)
Stability	<ul style="list-style-type: none"> ■ Stable for 6 months at -80 °C ■ Stable for 5 days at 2-8 °C 	<ul style="list-style-type: none"> ■ Stable for 6 months at -20 °C ■ Stable for 30 days at 2-8 °C
Dosing	<ul style="list-style-type: none"> ■ 0.3 mL (containing 30 µg vaccine), 2 doses (first priming shot followed by a second booster shot), 21 days apart 	<ul style="list-style-type: none"> ■ 0.5 mL (containing 100 µg vaccine), 2 doses (first priming shot followed by a second booster shot), 28 days apart
Efficacy in phase- III clinical trial	<ul style="list-style-type: none"> ■ 95% 	<ul style="list-style-type: none"> ■ 94.5%

parameter for vaccines and is defined as the percentage reduction in disease incidence among the vaccinated group during the clinical trial compared with an unvaccinated control group under similar conditions [13]. The primary data revealed BNT162b2 and mRNA-1273 have an efficacy of 95% and 94.5% against SARS-CoV-2, respectively [14,15]. The Moderna vaccine is based on a stabilized mRNA of the viral spike protein, [16,17] and the BNT162b2 is based on a nucleoside modified RNA (modRNA) of the SARS-CoV-2 virus. Table 1 lists the chemical composition of both the vaccines and Fig. 1 depicts the key delivery carrier.

Over the past one year, some more vaccines have been developed and are now being used clinically on regular a basis. The major new vaccines include inactivated/live attenuated virus, recombinant protein, recombinant viral vector, DNA vaccine, and messenger RNA (mRNA)-based vaccines [7,18]. COVID-19 vaccines based on conventional technologies include vaccines developed by Oxford/AstraZeneca (UK), Johnson and Johnson (USA), Gamaleya Research Institute (Russia), Bharat Biotech (India), SinoVac (China), Sinopharm (China) and others [19–25]. These conventional vaccines based on inactivated/live viruses offer advantages like robust immune response, ease of storage and shipping [26], while the disadvantages include difficulty in manufacturing at sufficient titers, cost per dose and the need for multiple doses to achieve immunity [27]. However, mRNA-based vaccines were granted prioritized clinical approval as the technology ensures the stability of the mRNA, together with enhanced delivery efficiency to ferry the mRNA inside the host cell. mRNA vaccines are both non-infectious, and thus safer, and do not require penetration into the nucleus, which is difficult to achieve [28]. Further, these vaccines can be produced rapidly, which is a major advantage in the existing pandemic, where billions of doses are needed in a short time to vaccinate the world population [29–32]. mRNA uptake by a cell is a very challenging task, firstly, due to presence of RNA degrading enzymes, which debase every RNA molecule they encounter [33,34], and secondly, being negatively charged, mRNA cannot easily cross the negatively charged cell membrane. Hence, to address this challenge, researchers have designed LNP based carrier molecules to preserve mRNA integrity and foster its uptake inside the cell [35]. LNPs are complex systems, which aid effective delivery of siRNA or mRNA into the host cells. Being a US-FDA approved carrier, LNPs are now extensively used for delivering antigen-encoding mRNA, encapsulating viral antigens against influenza, rabies, human immunodeficiency virus (HIV), cytomegalovirus (CMV) and others [36,37].

The focus on delivery: key advantages of lipid nanoparticles

In the late 1960s, liposomes were anticipated as a novel drug delivery system (NDDS) and since then have been improved for disease targeting [38]. LNPs present a novel colloidal drug delivery system, and differ from liposomes in that they form micellar structures within the core that can be modified based on formulation and synthesis parameters [39]. The structure of LNPs consists of a solid core made up of lipid, which is composed of triglycerides or any other glyceride mixture. A typical LNP has four parts: (i) an ionizable lipid portion that allows self-assembly, enhances the rate of mRNA encapsulation, and aids endosomal escape, (ii) a stabilizing agent for stability and membrane fusion (cholesterol or a sphingolipid), (iii) a phospholipid that stabilizes the bilayer, encapsulating the lipid structure [40], and (iv) polyethylene glycol (PEG), a lipid-based stabilizing agent that reduces nonspecific binding to proteins, increases half-life, and boosts circulation time by aiding escape from first pass metabolism or reticulo-endothelial system (RES). Their rigid morphology and kinetic stability are key advantages, making LNPs, a carrier of choice over liposomes. Their potential to transport a diverse group of therapeutic cargos from therapeutic drugs to nucleic acids (mRNA, siRNA, DNA) making them an appealing drug delivery system [41–43]. Once mRNA enters inside the cytoplasm from the endosome, it translates into the encoded immunogenic protein against specific antigens [44,45]. This recent discovery and integration of nanotechnology have shown various advantages in safer delivery of next generation RNA vaccines.

In addition to their simple synthesis method, small size and serum stability, the efficacy of LNPs in the delivery of nucleic acids into cells makes them superior to other carriers. Since biological membranes and nucleic acids are negatively charged, it is difficult to deliver mRNA across this barrier. LNPs offer an ideal platform for delivering nucleic acid therapies as the ionizable lipids are near-neutrally charged at physiological pH. However, in acidic endosomal compartments (pH-4.5), they become ionized, promoting endosomal escape for effective intracellular delivery [46,47]. Hence, LNPs achieve high encapsulation rate for nucleic acids with improved transfection efficiency. Furthermore, LNPs have relatively low cytotoxicity and immunogenicity compared to liposomes, thus favoring delivery of nucleic acid based therapeutics [48–50].

Lipids used in formulating these nanoparticles are biocompatible and are well endured by fatty acids, triglycerides, waxes and steroids. In addition, selecting a good combination of emulsifiers could

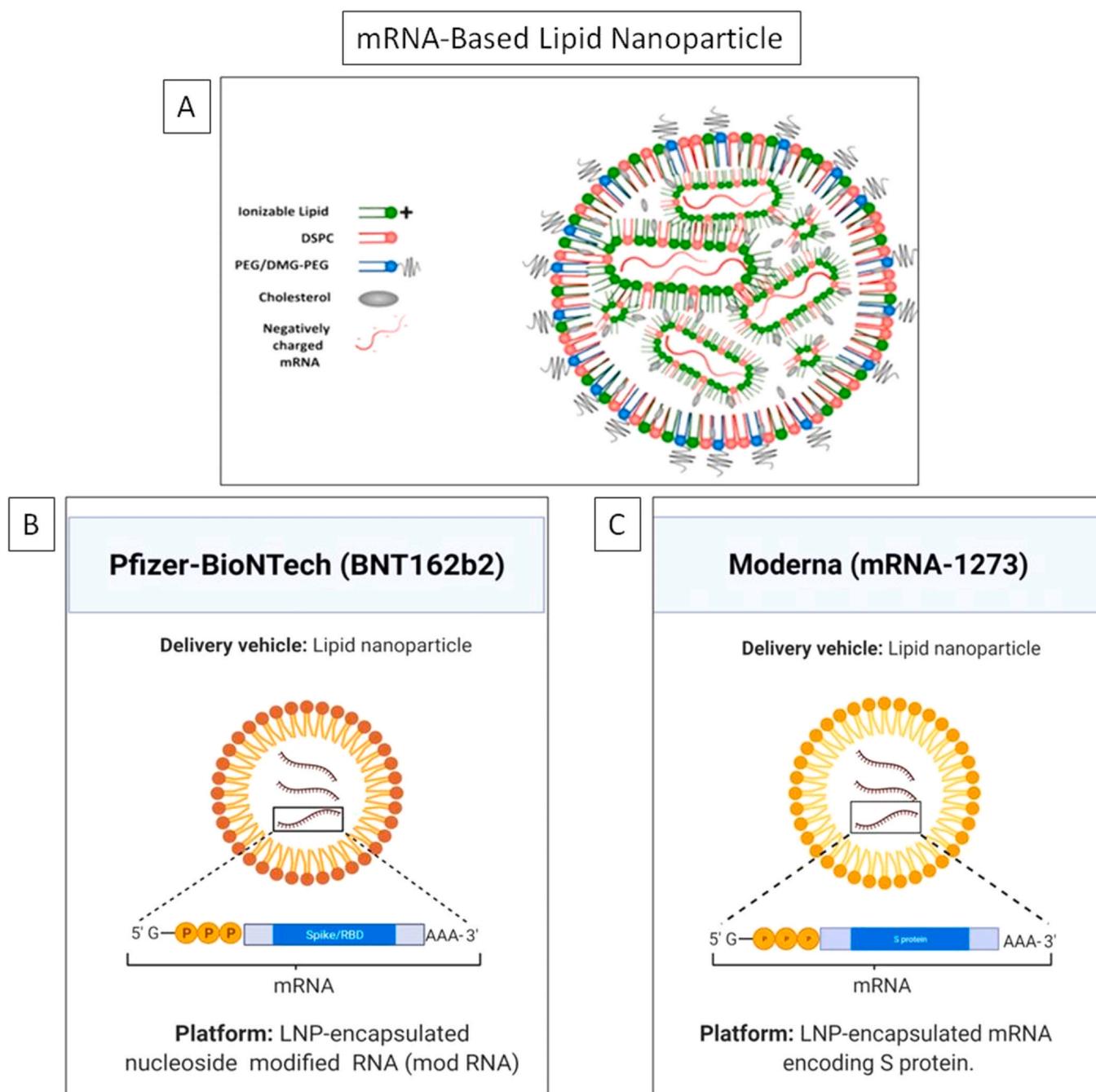


Fig. 1. A) The general structure of lipid nanoparticle (LNP) showing the major components and the unique mRNA cargo of, B) Pfizer-BioNTech, and C) Moderna vaccines. Figure A) Reprinted (adapted) with permission from [53] and is Copyright (2021) of American Chemical Society. Figure B) and C) were created with BioRender.com.

make the formulation more stable with higher efficiency [52]. In terms of industrial scale up, LNPs offer multiple advantages compared with other carrier systems, such as large scale production using microfluidics or T-junction mixing, stability, and low cost of raw materials [52–54]. The most significant parameters in LNPs characterization are particle size, their size distribution, degree of polymorphism, zeta potential, crystallinity, drug loading, drug release and entrapment efficiency [55]. Drug release from LNPs mostly relies on the type of matrix used and the position of drug in the matrix formulation. The ingredients of lipid matrix, manufacturing parameters and surfactant concentrations, such as rate of stirring, temperature etc. can modulate the drug release profile [56]. In a nutshell, the most fundamental rationale of using LNPs, as an

alternative to polymeric nanoparticles, is the simplicity of large-scale manufacturing and their low toxicity [57,58].

The path forward

To harness the full potential of mRNA therapeutics in future, the following challenges need particular attention: (i) the intrinsic immunogenicity of mRNA; (ii) the propensity to enzymatic and thermal degradation; and (iii) the inability to cross negatively charged cell membranes. Nucleoside modification, sequence engineering by codon optimization and uridine depletion are some of the methods used to reduce inherent immunogenicity, protect from enzymatic degradation and facilitate cellular uptake of the mRNA

platform [59–63]. The mean half-life of mRNA vaccines decreases with increasing temperature, which is a challenge for their long term storage. However, chemical modification by applying an outer coating of nonionic or an ionic surfactant enhances the thermal stability of mRNA. These chemical alterations change the dimensions of a nanoparticle and aid in the effective carrying of mRNA with higher thermal stability. Some of the reported allergic reactions to LNP nanovaccines can be minimized by using PEG complexed with lipids, which have improved biocompatibility. Hence, in future mRNA-based nanovaccines should possess an improved safety profile, higher stability (for storage at higher temperature), and enhanced cellular penetration. The next generation RNA vaccines will be more personalized and the tools of genetic engineering will play a crucial role in exploiting the full potential of the mRNA platform.

Concluding remarks

The EUA of the Pfizer-BioNTech and Moderna vaccines has undoubtedly brought great hope for the years ahead. However, it remains challenging to decide how to prioritize the allocation of vaccines to the population. While the EUA of these mRNA vaccines brings hope for developed countries, these vaccines remain largely out of reach of developing and underdeveloped nations on economic grounds and need for special storage conditions. At high or at room temperature, mRNA has poor stability, and thus these mRNA-based vaccines need to be stored at such a low temperature. The Pfizer-BioNTech vaccine needs to be stored at -80°C to -60°C and the Moderna vaccine at -25°C to -15°C to avoid degradation of mRNA encased inside the LNPs. Further, as it is common to all vaccines, mRNA vaccines are not devoid of side effects, including pain at the site of administration, fever, chills, fatigue, headache, muscle pain, and joint pain. In addition, some patients have experienced allergic responses, which has been linked to the presence of PEG in the formulation [64]. The adverse effects, duration of protection and storage at very low temperature are critical issues that require careful consideration for the upcoming COVID-19 nanovaccines [54].

The Oxford-AstraZeneca vaccine has also been approved and is in widespread use in the UK and elsewhere. It is the frontline vaccine candidate for the mass inoculation of global population along with the other conventional vaccines being developed around the globe. Nonetheless, the two mRNA vaccines represent a great success for biotechnology and molecular therapeutics. It motivates materials scientists who have developed and optimized nanoformulations for drug and vaccine delivery over the past two decades and encourages their acceptance in nanomedicines. Until the COVID-19 crisis, oncology had been the major area where nanotechnology based drug carriers had been widely explored. These two mRNA-based vaccine formulations will serve as a stepping stone for future applications of nanomedicine. These nanocarrier based vaccines highlight the importance of the nanoscale and the ability of nanoscale delivery systems to protect payloads from degradation, provide tailored biodistribution and cellular delivery.

CRediT authorship contribution statement

Amit Khurana: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Prince Allawadhi:** Conceptualization, Methodology, Writing - original draft. **Isha Khurana:** Conceptualization, Methodology, Writing - original draft. **Sachin Allwadh:** Conceptualization, Methodology, Writing - original draft. **Ralf Weiskirchen:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Anil Kumar Banothu:** Conceptualization, Methodology, Writing - original draft. **Deepak Chhabra:** Conceptualization, Methodology, Writing - original draft. **Kamaldeep Joshi:** Conceptualization, Methodology,

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors sincerely convey their gratitude to Dr. Kannan Govindaraj, the University of Twente, Enschede, The Netherlands for his kind help in proofreading the manuscript. The authors are grateful to the Dean, College of Veterinary Science, PVNRTVU, Hyderabad, Telangana, India.

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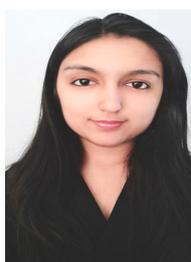
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