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Recent updates on liposomal formulations for detection, prevention and treatment of coronavirus disease (COVID-19)

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ABSTRACT

The unprecedented outbreak of severe acute respiratory syndrome-2 (SARS-CoV-2) worldwide has rendered it one of the most notorious pandemics ever documented in human history. As of November 2022, nearly 626 million cases of infection and over 6.6 million deaths have been reported globally. The scientific community has made significant progress in therapeutics and prevention for the management of coronavirus disease (COVID-19), including the development of vaccines and antiviral agents such as monoclonal antibodies and antiviral drugs. Although many advancements and a plethora of positive results have been obtained and global restrictions are being uplifted, obstacles in efficiently delivering these therapies, such as their rapid clearance, suboptimal biodistribution, and toxicity to organs, have yet to be addressed. To address these drawbacks, researchers have attempted applying nanotechnology-based formulations. Here, we summarized the recent data about COVID-19, its emergence, pathophysiology and life cycle, diagnosis, and currently-available medications. Subsequently, we discussed the progress in lipid nanocarriers, such as liposomes in infection detection and control. This review provides critical insights into the design of the latest liposomal-based formulations for tackling the barriers to detecting, preventing, and treating SARS-CoV-2.

1. Introduction

1.1. Severe acute respiratory syndrome coronavirus 2: Emergence, pathophysiology and life cycle

Since the sudden emergence and spread of the novel coronavirus (first identified as 2019-nCoV) in December 2019, the virus has undoubtedly evoked the scientific community. This highly infectious disease, coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread across the globe in a relatively short period, riveting the World Health Organization (WHO) to officially announce it as a global pandemic on March 11, 2020 (Li et al., 2020). COVID-19 has devastated numerous countries, staggered various healthcare systems, and threatened the economic stability of many (Shang et al., 2021). As of November 2022, nearly 626 million cases of infections and over 6.6 million deaths caused by SARS-CoV-2 have been reported worldwide (World Health Organization, 2022). SARS-CoV-2 clinical presentations resemble influenza-like symptoms, including fever, cough, headache, taste and smell disturbance, and myalgia (Huang et al., 2020; Jiang et al., 2020; Yin and Wunderink, 2018).

Coronaviruses (CoVs) are positive-stranded RNA (+ssRNA) viruses that display spike proteins on the surface and a crown-like appearance (Brant et al., 2021). Historically, infection by coronaviruses in humans has been associated with human CoVs (HCoVs), HCoV-229E and HCoV-OC43, most commonly linked to mild upper respiratory tract infections, especially those causing the common cold. SARS-CoV-2 shares a similar disease progression with SARS-CoV-1 (2002–2003) and Middle East respiratory syndrome (MERS)-CoV (2012), causing respiratory disease. These viruses progressively affect other internal organs, simultaneously leading to organ failure (Brant et al., 2021; Brian and Baric, 2005; Krishnamoorthy et al., 2020; Naqvi et al., 2020). Although substantial efforts by the scientific community have led to better treatment and prevention strategies for SARS-CoV-2, halting the outbreak of SARS-CoV-2 and its variants has become a concern, as this virus continues to rapidly wreak havoc worldwide, mostly owing to the emergence of new variants of the virus (Shang et al., 2020; V’kovski et al., 2021).

Recent studies show that SARS-CoV-2 primarily transmits via infected respiratory droplets, where the viral infection is initiated directly or indirectly through contact with the oral mucosal, nasal or conjunctival
secretions (Rando et al., 2021). Upon contact, the viral particles in these droplets are deposited on the mucous membranes of the oral cavity, nasal passage, or conjunctiva. Subsequently, the absorption of droplets into the lungs initiates lower respiratory tract infections leading to mild to the severe acute respiratory syndrome. The most typical symptoms of COVID-19 infection are headache, flu, fever, cold, cough, sore throat, body fatigue, dyspnea, and loss of taste or smell, while severe symptoms often lead to pneumonia and internal organ malfunction (Harrison A et al., 2020; Rando et al., 2021).

As shown in Fig. 1, CoVs are enveloped with + ssRNA and the genome organization is structured in a + ssRNA of about 30 kb in length, equipped with a 5′-cap structure and 3′-poly-A tail (Brant et al., 2021; Brian and Baric, 2005). SARS-CoV-2 shares similar structural characteristics with other CoVs, and its genome encodes a set of four main structural proteins: spike (S), envelope glycoprotein (E), nucleocapsid (N), and membrane (M) protein and non-structural proteins (NSP). Like SARS-CoV, SARS-CoV-2 also uses human ACE2 (hACE2) as its receptor (Shirbhate et al., 2021). The initial entry of SARS-CoV-2 into host cells is mediated by the specific binding of its spike protein to the host cellular receptor, ACE2, followed by the S2 subunit, which mediates membrane fusion and subsequently releases its genetic material, viral RNA (Fig. 2). Once the viral RNA is released into the host cell, it indicates viral gene expression; the synthesis of polyproteins marks the initiation of the replication. Intracellularly, the expression and replication of viral genomes produce new copies for incorporation into newly assembled virions (Tortorici and Veesler, 2019). The classes of proteins that are encoded include two polyproteins that are cleaved into 16 NSPs for RNA synthesis, four structural proteins (S, E, M, and N) that are essential for the entry and assembly of the virus, and other additional proteins that are crucial for host immunity. After new virions are assembled, they are released by exocytosis to further infect and evade the replication machinery of other hosts (Trougakos et al., 2021; Wiersinga et al., 2020). ACE2 receptors are present in the excessive lining of the respiratory epithelium, such as type II alveolar epithelial cells, and are also located in other organs, such as the myocardial cells, proximal tubular cells of the kidney, and urothelial cells of the bladder (Salamanna et al., 2020; Yang et al., 2020); therefore, SARS-CoV-2 infections in other organs, such as the heart, kidney, and liver, are also observed. Therefore, categorizing the SARS-CoV-2 structure and its genomic organization is pivotal for tackling and addressing its pathogenicity.

1.2. Current management strategies for COVID-19 patients

The WHO has highlighted an appropriate guideline for COVID-19 testing for detecting infection and immunity. Diagnostic testing for infection includes real-time polymerase chain reaction (RT-PCR), antigen and antibody tests for detecting immunity against COVID-19. RT-PCR shows the highest accuracy for testing the infection in which the nucleic acid of the sample is amplified and quantified in real-time (Artik et al., 2022; Munne et al., 2021). Antigen testing by lateral flow assay of rapid antigen tests generated the fastest result within 15 min after saliva or nasal sample was loaded, making it the most accessible COVID-19 diagnostic test used at the moment (Mahmoudinobar et al., 2021; Peto et al., 2021). Antibody levels against COVID-19 can also be quantified using the blood samples of patients and serological enzyme-linked immunosorbent assay (ELISA) (Ravi et al., 2021). A summary of the diagnostic tests for COVID-19 approved by the WHO is shown in Fig. 3. In addition to containing the virus spread via early detection and
contact tracing, there is an urgent need for interventions to eradicate the virus spread before it is too late. Recently, numerous vaccines against COVID-19 have been designed and evaluated as preventive measures in clinical trials (Table 1). As of July 2022, 11 vaccines of different classes—messenger RNA (mRNA) vaccines, protein subunits, inactivated viruses, and adenovirus vectors—have been listed by the WHO under Emergency Use Authorization (EUA) as qualified COVID-19 vaccines, and more vaccines are still under ongoing clinical and preclinical studies.

In addition to the successful prevention of SARS-CoV-2 infection after vaccinations, the presence of therapeutics and treatment options to halt SARS-CoV-2 replication in infected patients, as well as reduce the number of hospitalized patients and death rates, is exceptionally pivotal. As of April 2022, a few treatment options have been outlined by the WHO and the U.S. Food and Drug Administration (FDA) in clinical studies to eradicate viral replication in infected patients. Treatment options include anti-viral therapy, anti-SARS-CoV-2 monoclonal antibodies (mAbs), and COVID-19 convalescent plasma (Jamshaid et al., 2020). Remdesivir and ritonavir-boosted nirmatrelvir (Paxlovid) are the only anti-viral candidates that have received emergency use authorization from the FDA. Meanwhile, five mAbs against SARS-CoV-2, including bamlanivimab plus etesevimab, bebtelovimab, casirivimab plus imdevimab, sotrovimab, and tixagevimab plus cilgavimab have been approved under the EUA (Liu et al., 2021; Pascarella et al., 2020).

2. Liposomal formulations for the detection, prevention, and treatment of COVID-19

Although several alternatives and options for the management and treatment of COVID-19 patients have been listed by the WHO as of November 2022, the drawbacks and side effects of these options for COVID-19 remain unresolved. For example, RT-PCR analysis, considered the gold standard for diagnosing COVID-19, may yield false negatives. RT-PCR using the samples from the upper respiratory tract of a patient only depicts the viral infection that occurs in the early stages of infection and rapidly deteriorates; however, the positivity remains high in the lower respiratory tract samples, particularly in the late stages of the disease (Williams et al., 2022). Therefore, the RT-PCR results may complicate the interpretation process and not effectively detect the viral infection in other infected body parts (Olofsson et al., 2011). The other drawbacks associated with the available methods for diagnosing COVID-19 include untargeted delivery of vaccine active materials, inability to
escape endosomal clearance, and unwanted toxicity of antiviral agents to the organs (Aygün et al., 2020; Crommelin et al., 2021; Elhissi, 2017; Mohammad Zadeh et al., 2021; Tada et al., 2015).

Lipid or polymeric nanoparticles delivery systems can be formulated to enhance pharmacological properties as well as therapeutic effects of antiviral agents and other biomolecules to be administered into the body. These nano-delivery systems have been reported to address the drawbacks that hinders the efficacy of drugs or vaccines in clinical applications. These systems include, lipid carriers such as liposomes, micelles, polymersomes, and other (Aldosari et al., 2021; Large et al., 2021; Rudokas et al., 2016). In this review article, we aim to discuss the most recent studies on liposomal formulations for the detection, prevention, and treatment of COVID-19.

2.1. The versatility of liposomes and their ideal characteristics

Liposomes are identified by their bilayer membrane properties and are known to spontaneously form by the dispersion of phospholipids in an aqueous solution. Liposomes have numerous properties that make them ideal carriers for drugs, active ingredients, and antigens (Guimarães et al., 2021). These properties include biocompatibility, low toxicity, ease of preparation, ability to entrap both hydrophilic and hydrophobic drugs, and their close resemblance to mammalian biological membranes to enable easy penetration of drugs through cell membranes, consequently improving the bioavailability of drugs (Khan et al., 2008). In terms of the incorporation of charged molecules into liposomes, liposomes are easily complexed with both positively and negatively charged molecules owing to the versatility of their lipid composition (Filipczak et al., 2020). Liposomes may also be conjugated with ligands to precisely locate targeted cells, improve their pharmacokinetics, and reach their highest concentrations inside cells. To date, liposomes have been the most widely used formula for clinical applications in the treatment of various diseases, including liposomal doxorubicin and Doxil®, an anticancer drug against ovarian cancer, to improve the efficacy and safety of chemotherapeutic agents compared to the original formulation (Barenholz, 2012; Leonard et al., 2009).

2.2. Recent advancements in liposomal formulation for the detection of COVID-19

Infected cells secrete extracellular vesicles (EVs) with pathogenic factors in response to protection from hydrolases, allowing them to reside in circulation. Viral genomes have been reported in previous studies to infect cells via EV-mediated transfer (Purvinsh et al., 2021; Schwab et al., 2015). Therefore, SARS-CoV-2 has been suggested to share the same mechanism. Based on this, Ning et al. (2021) formulated a liposome-mediated assay that resembles an ELISA for detecting viral infections to detect SARS-CoV-2 RNA-positive EVs in the plasma. Upon the binding of an antibody to the EV surface protein cluster of differentiation 8 (CD8), EVs are captured from the plasma and incorporated together with liposomes containing reagents for reverse transcriptase (RT), including recombinase polymerase amplification (RPA) and
To characterize administration routes other than intramuscular injection, Huang et al. (2021) reported a liposomal formulation clustered regularly interspaced short palindromic repeat (CRISPR)-Cas12a. Ning et al. (2021) postulated that EVs extracted from plasma that contains SARS-CoV-2 RNA can be detected at the initial phase post-infection and showed that the developed test method yielded positive results for the samples that were detected as negative using RT-qPCR assays of nasal samples. The major advantage of this formulation is it does not require any extraction procedure, with the ability to detect plasma EV from SARS-CoV-2 RNA as an early indicator of systemic infection. This analysis method is also more advantageous than the analysis of total plasma RNA for SARS-CoV-2, as RNA from captured EVs may be less degraded or off-target RNA than extravesicular RNA compared to RT-PCR samples. This detection method eliminates the laborious, time-consuming sample preparation required by other analyses, which may induce different yields and purities of the samples. This study has shown an approach for detecting SARS-CoV-2 infection by utilizing the ability of liposomes to encapsulate various molecules, which is highlighted in the study, encapsulation of the reagents needed for diagnostic purposes. The other liposome-based detection methods for SARS-CoV-2 are presented in Table 2.

2.3. Preventing SARS-CoV-2 infection by the liposomal vaccine formulations

To date, the most widely used vaccine technology for the prevention of COVID-19 is lipid-based mRNA vaccines owing to their ease of manufacturing, immunogenic capability, and excellent safety (Jackson et al., 2020; Rosa et al., 2021). The need for mRNA vaccines to fully exert their therapeutic effects is often hampered by their untargeted delivery and inability to escape endosomal clearance; hence, a myriad of materials have been incorporated into mRNAs for their safe and effective delivery, including polymers and lipid nanoparticles (Crommelin et al., 2020; Rosa et al., 2021). Various pharmaceutical products that utilize lipids have been employed to prevent a myriad of diseases, including hepatitis A and influenza (Chatzikleanthous et al., 2021).

Table 1
A list of vaccines against COVID-19 that have obtained Emergency Use Authorization (EUA) as of January 12, 2022 (World Health Organisation, 2022; Park and Lee, 2021).

| Vaccine name | Class of Vaccine | Active Material | Manufacturer | Date of approval |
|--------------|------------------|----------------|--------------|-----------------|
| BNT162b2 Comirnaty | mRNA vaccine | mRNA-1273 | Pfizer (New York, USA) and BioNTech (Maine, Germany) | December 31, 2020 |
| mRNA-1273 | mRNA vaccine | mRNA-1273 | Moderna (Cambridge, USA) and NIAID (North Bethesda, USA) | April 30, 2021 |
| AZD1222 | Adenovirus vector | Replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein | AstraZeneca (Cambridge, UK) and Oxford University (Oxford, UK) | February 16, 2021 |
| Janssen/Ad26.COV2.S | Adenovirus vector | Replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 spike glycoprotein | Johnson & Johnson (Beerse, Belgium) | March 12, 2021 |
| Ad5-nCoV | Adenovirus | Human type 5 adenovirus encoding SARS-CoV-2 Spike (S) glycoprotein | CanSino Biologics (Tianjin, China) | February 25, 2021 |
| COVISHELD | Adenovirus | Replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein | Serum Institute of India | January 3, 2021 |
| BBIBP-CoV | Inactivated virus | Inactivated SARS-CoV-2 antigens and aluminum hydroxide adjuvant | Sinopharm (Beijing Institute of Biological Products) | May 7, 2021 |
| CoronaVac | Inactivated virus | Inactivated SARS-CoV-2 virus (CZ02 strain) and aluminum hydroxide adjuvant | Sinovac Biotech (Beijing, China) | June 1, 2021 |
| BBV152 COVAXIN | Inactivated virus | Inactivated whole virion vaccine based on Asp614Gly variant | Bharat Biotech (Hyderabad, India) | November 3, 2021 |
| Nuvaxovid (NVX-CoV2373) | Protein subunit | Recombinant SARS-CoV-2 spike protein nanoparticle administered as a co-formulation with the adjuvant Matrix-M | Serum Institute of India and Novavax (Gaithersburg, USA) | December 17, 2021 |

Table 2
A list of liposome-mediated detection methods reported for detecting SARS-CoV-2.

| Detection Strategies | Lipid composition | Reference |
|----------------------|-------------------|-----------|
| Liposome-mediated detection of SARS-CoV-2 RNA-positive extracellular vesicles in plasma | 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC) | Ning et al., 2021 |
| Temperature-responsive liposome-linked immunomodorbent assay for the Rapid detection of SARS-CoV-2 using immunoliposomes | 1,2-dipalmitoyl-sn-glycero-3-phosphatic acid sodium salt (DPPA) | Hu et al., 2022 |
| Engineering viral genomics and nano-liposomes in microfluidic platforms for patient-specific analysis of SARS-CoV-2 variants | Not stated | Satta et al., 2022 |
encapsulating the mRNA vaccines to induce immunological responses. In this study, mRNA encoded by the receptor-binding domain (RBD-mRNA) of SARS-CoV-2 was encapsulated in liposomes (LPS/RBD-mRNA), and the immunogenicity of the liposomal formulations was characterized via multiple routes of administration. In addition, the neutralization ability of the reported formulations was also evaluated. Mice immunized with LPX/RBD-mRNA were reported to elicit RBD-specific IgG antibodies and efficiently neutralize the SARS-CoV-2 pseudotyped virus. C57BL/6 mice were then treated with LPX/RBD mRNA via five injection routes: intravenous, intramuscular, inhalation, intraperitoneal, and intradermal. This study concluded that compared to intravenous (i.v.) and intramuscular (i.m.) administrations, intradermal (i.d.) administration elicited specific IgM more rapidly in the early immunization on day 15. The five injection routes for LPX/RBD-mRNA vaccination induced similar levels of RBD-specific IgG and IgM antibodies. However, the i.d. administration route showed greater efficacy in neutralizing the pseudotyped virus than the i.m. administration route. These data provide insight into the efficacy of vaccines in preventing viral infection. The immune tolerability assay demonstrated the greatest increase in immune function index via i.m. administration compared to i.v. administration, while i.p. administration resulted in the lowest increase and systemic cytokine production. In addition, the efficiency of producing non-neutralizing antibodies, especially Th1- and Th2-biased cellular responses is an important characteristic of the vaccines; LPX/RBD-mRNA vaccination via inhalation, i.d. and i.p. injections induced Th1-biased immune responses. In summary, Huang et al. (2021) demonstrated the potential of the liposome-based mRNA vaccine as a safe and effective vaccine candidate for SARS-CoV-2, with suitable administration routes designed to enhance its efficacy.

Nevertheless, as mRNA vaccines are well known for their instability, which constrains their distribution during a pandemic, assessing the stability of the developed vaccines and their ease of manufacturing are also important. Liposomes can be tailored to address these stability issues, as lyophilization of liposome formulations retains almost the same immunogenicity and neutralization capability of molecules when reconstituted. This potential of liposomes has been demonstrated in delivering antigens or biomolecules (Nguyen et al., 2016; Suzuki et al., 2021). To support the aforementioned characteristics of liposomes, Mabrouk et al. (2021) developed a lyophilized liposomal formulation incorporating cobalt porphinyl-philosopholipid (CoPoP), monophosphoryl lipid A (MPLA), and saponin-derived immunogenic adjuvant (QS-21), with SARS-CoV-2 RBD, displayed on the liposomal surface, and assessed its immunogenicity and neutralization capabilities. The incorporation of CoPoP enables the presence of cobalt ions in between the lipid bilayer to enable the stable formation of donating bonds to form bonds with histidine imidazole rings, which anchor the display proteins. MPLA and QS-21 were included in the liposomal formulation to increase the immunogenicity of the vaccine (Duthie et al., 2011; Welshy et al., 2017). Lyophilization of the liposomal formulation was intended to improve

The storage stability of vaccines bound to the spike glycoprotein and RBD. In this study, the lyophilized powder was reconstituted to analyze the integrity of liposomes using cryo-transmission electron microscopy, which revealed the retention of the spherical morphology of the liposomes. To test the thermostability of the liposomal formulations in liquid and lyophilized forms, S protein and RBD-encapsulating liposomes were incubated for up to 14 days at 40 °C and 60 °C and their ability to bind ACE2 was evaluated. Both formulations showed high stability and remained functional compared to the liquid formulations that were compromised on day 3 for RBD and day 7 for Spike at 40 °C. However, at 60 °C, liquid formulations of RBD and S protein were unstable as early as 1 d, suggesting that lyophilization of the formulation does not require a very low temperature for the storage of vaccines, facilitating the transportation of the vaccines. The lyophilized liposomal formulations were then used to immunize K18 hACE2 transgenic mice with a prime-boost dose within 14 days through i.m. injections of 0.1 μg antigen (containing 0.4 μg CoPoP, 0.16 μg PHAD, and 0.16 μg QS-21). Both vaccines induced neutralizing antibodies against spike protein and RBD post-boost immunization. On the contrary, IgG isotyping also indicated a balanced T helper cell 1 (Th1/Th2) response. Mice were then infected intranasally with a high dose of SARS-CoV-2 and with the same antigen dosing and immunization protocols; the immunized mice showed a high IgG titer against the S protein and RBD. Viral load assessment in the nasal turbinate and lungs revealed almost undetectable levels of the virus, a 100 % survival rate, and no significant weight loss compared to control mice immunized with phosphate-buffered solution and QS-21 lacking antigen. To recapitulate, this lyophilized liposome displayed S or RBD vaccine formulation may be suitable for addressing the limited stability of vaccines. It also demonstrated the potential of the formulation in inducing protection against SARS-CoV-2 in the K18-hACE2 mouse model with a low dose of 0.1 μg antigen.

In addition to the design of appropriate administration routes and advancements in improving the thermostability of vaccines by exploiting liposome properties, liposomes can be further tailored to incorporate several adjuvants to enhance vaccine immunogenicity (Tandrup Schmidt et al., 2016). Wang et al. (2022) developed an adjuvant-protein conjugate and displayed conjugates on the liposomal surface to induce an immune response against SARS-CoV-2. This conjugation involves an immune activator, α-galactosylceramide (αGalCer), an effective natural killer T cell (iNKT) agonist with the N-terminus of the RBD of SARS-CoV-2. α-GalCer exerts a myriad of immune effector characteristics. When activated by α-GalCer, iNKT cells release copies of cytokines and address dendritic cells (DCs) to increase their ability to initiate humoral and cellular responses. Moreover, these iNKT cells help B cells undergo proliferation and maturation (Osada et al., 2004; Zhang et al., 2019). \cite{Wang et al., 2022a} reported a significantly improved adjuvant efficacy, enhanced humoral responses, and cellular responses in mice in the αGalCer-RBD conjugate mixture compared to the unconjugated RBD/αGalCer mixture. Moreover, the antisera from αGalCer-RBD-immunized

### Table 3

A list of recent liposomal formulations as a preventive measure for SARS-CoV-2.

| Vaccine strategies | Vaccine technology | Lipid composition | Administration route | Reference |
|--------------------|--------------------|-------------------|---------------------|-----------|
| Self-adjuvating lipoprotein conjugate αGalCer-RBD induces potent immunity against SARS-CoV-2 and its variants of concern | Protein subunit | 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) | Subcutaneous | Wang et al., 2022a |
| Lyophilized, thermostable Spike or RBD immunogenic liposomes induce protective immunity against SARS-CoV-2 in mice | Protein subunit | 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) | Intramuscular | Mabrouk et al., 2021 |
| MPLA-Adjuvanted Liposomes Encapsulating S-Trimer or RBD or S1, but Not S-ECD, Elicit Robust Neutralization Against SARS-CoV-2 and Variants of Concern | Nucleic-acid based | 1,2-distearyl-sn-glycero-3-phosphocholine (DSPC) | Subcutaneous | Wang et al., 2022b |
| The investigation of mRNA vaccines formulated in liposomes administered in multiple routes against SARS-CoV-2 | mRNA-based | 1,2-dioleoyl-sn-(trimethylammonium)propane (DOTAP) | Inhalation, intravenous, intraperitoneal, inhalation, intradermal | Huang et al., 2021 |
| Development of COVID-19 vaccine using a dual Toll-like receptor ligand liposome adjuvant | Protein subunit | 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) | Subcutaneous | Abhyankar et al., 2021 |
maximum therapeutic efficacy, consequently causing unwanted toxicity (Zadeh et al., 2021). These issues have been reported in remdesivir, to organs, such as the liver and kidney (Aygün et al., 2020; Mohammad, 2020).

2.4. Utilizing liposomes in improving the current treatment strategies

Inhalation

Fig. 4. Schematic illustration of Rdv-lips inhalation route. The atomized Rdv-lips suspension was inhaled to the lungs to improve drug accumulation and deposited into the alveolar surface. Once deposited, the Rdv-lips diffused through the alveolar epithelial cells due to the great biocompatibility of the liposomal formulation. Herein, a higher concentration of drugs was sustained in the lung (illustration), adapted from Li et al., 2021.

Another study on liposomal vaccines was performed by Liu et al. (2020), where the authors developed a liposomal formulation comprising cationic lipids to accommodate the anionic S1 subunit of SARS-CoV-2 on the liposome surface. The liposomal formulation also included two adjuvants: amphiphilic monophosphoryl lipid A (MPLA) (for toll-like receptor (TLR) 4) and CpG oligodeoxynucleotide (for TLR 9). Structurally, CpG oligodeoxynucleotides are loaded into the core structure, whereas MPLA is entrapped within the lipid bilayer. Then, anionic S1 was adsorbed onto the cationic surfaces of the liposomes by electrostatic interactions between the cationic lipid and anionic S1. Animal studies were performed to evaluate the immunogenicity of the formulations in mice. Serum from immunized mice revealed superior IgG2a levels in MPLA/CpG-loaded liposomes compared to other combinations. Simultaneously, mucus collected from the nasopharynx of mice showed excellent humoral and mucosal immunity in the liposomal vaccine formulation.

In addition to the liposomal vaccine formulations discussed above, numerous studies have also highlighted the role of liposomes as vaccine carriers against SARS-CoV-2 (Table 3), demonstrating excellent advancements in liposomal formulations for vaccine delivery.

2.4. Utilizing liposomes in improving the current treatment strategies against SARS-CoV-2 infection

Although a myriad of treatment options highlighted by the WHO have been clinically proven to elicit antiviral properties in patients with COVID-19, their therapeutic effects are often hindered by adverse side effects. These side effects include low solubility, poor accumulation of the therapeutics in the targeted locations, and rapid renal clearance, leading to a high dose of drugs needed to be administered to realize maximum therapeutic efficacy, consequently causing unwanted toxicity to organs, such as the liver and kidney (Aygün et al., 2020; Mohammad Zadeh et al., 2021). These issues have been reported in remdesivir, hydroxychloroquinone, favipiravir, and ribavirin, the drugs that have been approved (Saha et al., 2022). Therefore, the biocompatibility, composition, and ability to encapsulate hydrophilic or lipophilic therapeutic molecules are important characteristics pertaining to the delivery of treatments to infected COVID-19 patients.

Remdesivir, the first antiviral drug approved in March 2020 by the U. S. FDA under EUA, has been reported in clinical studies to show remarkable antiviral potency against SARS-CoV-2. Remdesivir inhibits viral replication by binding to RNA-dependent RNA polymerase and terminating the addition of new nucleotides in the growing chain (Gordon et al., 2020; Tchesnokov et al., 2019). To tackle the issues pertaining to poor accumulation and activation of drugs at targeted locations and low solubility of remdesivir, a study (Li et al., 2021) developed a liposomal remdesivir formulation that can be administered via inhalation to bypass rapid renal clearance and increase drug concentration in the lungs, the most affected organ in COVID-19 infection. Liposomes were chosen as carriers for their biocompatibility with alveolar surfactants because the major components are lipids; thus, more drugs can be localized in the lungs, reducing the amount of drug dosage needed and subsequently reducing side effects (Elhissi, 2017; Rudokas et al., 2016). Liposomal remdesivir (Rdv-lips) was lyophilized and reconstituted into a suspension for pulmonary delivery, and its efficacy was compared to that of commercialized remdesivir (Rdv-cyc); the strategy is illustrated in Fig. 4.

To assess the improvements in the pharmacokinetics of Rdv-lips and their in vitro efficacy when administered via pulmonary delivery, the pharmacokinetics of NTP in the lungs were determined. In the control group, Rdv-lips were administered via i.v. injection (commercialized by Gilead Sciences, Inc.) for comparison with Rdv-lips administered via inhalation. Owing to the direct delivery of Rdv-lips to the lungs, the accumulation of remdesivir was outstanding for the two aerosolized formulations. Rdv-lips administered via inhalation showed an approximately 100- and 77-folds increase in NTP concentration compared to Rdv-cyc administered via i.v. injection and vis inhalation, respectively. These data prove the incomparable advantage of pulmonary delivery compared to the commercial injection route of the liposomal formulation. Furthermore, the liposomal formulation revealed a more rapid transition of remdesivir to NTP when the peak concentration reached the maximum ($T_{\text{max}}$), 1 h post-administration, compared to the control group, which reached its $T_{\text{max}}$ 4 h post-administration. The authors hypothesized that the data could be deduced from the high drug loading
and excellent biocompatibility of liposomes with the cell membrane, thereby increasing the cell uptake of remdesivir. To evaluate the in vivo behavior of NTP, the tissue distribution of NTP was assessed, which showed the highest NTP concentration in the lungs of the Rdv-lips inhalation group compared to the Rdv-cyc i.v. and Rdv-cyc inhalation groups, further demonstrating the increased pulmonary accumulation of Rdv-lips. This study highlighted the improved concentration of Rdv-lips administered via inhalation as an effective strategy to overcome the limitations of remdesivir, especially to enhance its therapeutic efficacy. However, this study focused mostly on the properties, safety, and distribution of the developed formulations in vitro and in vivo, and the neutralization capability of the formulations against SARS-CoV-2 infection was not assessed. Therefore, it did not reflect the real antiviral properties of the Rdv-lips administered via inhalation.

Although inhalation administration of liposomal therapeutics has been the preferred option in ongoing studies for COVID-19, liposome composition and charge can be manipulated to target the lungs via i.v. injection. One study developed “stealth” cationic liposomes to treat COVID-19 by utilizing small interfering RNAs (siRNAs) and delivered the formulations intravenously to directly target the lungs. Previous studies have shown that increased concentrations of 1,2-dioleoyl-3-trimethylammonium-Propane (DOTAP) with DLin-MC3-DMA (MC3) incorporated into lipid nanoparticle (LNP) formulations can improve lung targeting. However, the authors of this study attempted to compare their modified formulation from a previous study, with reduced concentrations of 40% DOTAP with MC3 (dmLNP), to deliver siRNAs with antiviral effects on SARS-CoV-2 in vivo to the K18-hACE2 mouse model (Idris et al., 2021). In the initial phase of the study, candidate siRNAs were selected and screened against SARS-CoV-2, and chemical modifications were performed to stabilize the siRNAs. The top candidates are
siHe1, siHe2, UC7, and sUTR3. The ability of these siRNAs to repress viral replication in vitro was evaluated, and the results showed that these siRNA candidates could potentially target and halt viral replication of SARS-CoV-2, either individually or in combination.

To further determine the anti-viral effects of the LNP formulations in vivo, K18-hACE2 mice were first inoculated with 4 × 10^5 plaque-forming units of virus and treated with 1 mg/kg in 100 μL LNP-siRNA formulations through i.v. injections in the retro-orbital 1 day before and 2 days after infection. The design and result of the in vivo studies are illustrated in Figure 5. DOTAP 50 % was initially incorporated into the formulation as the standard concentration for cationic lipids. Promising results of viral repression and survival advantages post-infection were observed in the in vitro studies. To evaluate the in vivo efficacy, the K18-hACE2 mice were administered intravenously, even with the incorporation of MC3, suggesting that DOTAP is a pivotal component in targeting the lungs.

In the distribution studies, the dmLNP-siRNAs containing 40 % DOTAP and 25 % MC3 also targeted the lung, liver, and spleen. These dmLNP-siRNAs of 40 % DOTAP exhibited reduced lung targeting when administered intravenously, even with the incorporation of MC3, suggesting that DOTAP is a pivotal component in targeting the lungs.

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Table 4: A list of recent liposomal formulations for the treatment of SARS-CoV-2.

| Treatment strategies | Lipid composition | Intended administration route | In vivo study (Animal model) / In vitro study | Reference |
|----------------------|-------------------|-----------------------------|---------------------------------------------|-----------|
| DPPC-liposomes co-loaded with corticosteroids MPS and NAC | 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) | Intravenous and intratracheal | Preclinical (C57BL/6 mice) | Arber Raviv et al., 2022 |
| Liposomal Remdesivir (Rdv-lips) | 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) | Inhalation | Preclinical (BALB/c mice) | Li et al., 2021 |
| Liposomal Hydroxychloroquine (liposomal-HCQ) | Dipalmitylophosphatidylcholine (DPPC) | Inhalation | Preclinical (Sprague-Dawley rats) | Tai et al., 2021 |
| siRNA-LNP | 1,2-dioleoyloxy-3-(trimethylammonium) propane (DOTAP) | Intravenous | Preclinical (K18-hACE2 mice) | Idris et al., 2021 |
| Flavonoids Liposomal Formulation (FRF-Lip) | Not stated | Inhalation / pulmonary delivery | In vitro study | Owis et al., 2021 |
| Propolis-liposomes (PP-lip) | Phosphatidylcholine | Not stated | In vitro study | Relaat et al., 2021 |
| Liposomal lactoterrin (LF) | Phosphatidylcholine | Oral | In vitro study | Serrano et al., 2020 |
| Oozinated oil in liposomes eyedrop gel (OED) | Not stated | Ocular | In vitro study | Rizzo et al., 2021 |

The SARS-CoV-2 pandemic has ravaged the healthcare system around the globe and affected millions of lives. Although the scientific community has reported several diagnosis tools, vaccines, and treatment strategies to contain this deadly virus, current management strategies still possess major drawbacks and loopholes that should be given attention. Furthermore, with a large number of similar viruses circulating in bats and camels, and the rapid emergence of variants, the possibility of additional outbreaks poses a major threat to global public health.

Nevertheless, advanced nanotechnology-based strategies, such as liposomes or lipid-based drug formulation or LNP carriers, that have been used as delivery vehicles to stabilize antigens, proteins, and drugs, overcoming barriers to cellular and tissue uptake can be utilized to curb the calamity. Owing to their importance, liposome-based formulations have achieved considerable success in recent decades. In the near future, with the rapid development of materials science, biotechnology, genetic engineering, medical technology, and other scientific fields, people will have a more extensive and in-depth understanding of the unique properties of liposomes and their derivatives. We hope that more efficient liposomal formulations for detection, prevention, and prevention will be designed by developing novel material preparation strategies and more advanced formulation methods. We believe that the discussions in this review will provide better insight for future research to be adapted to the treatment and management of SARS-CoV-2 patients, reducing the number of deaths and hospitalizations and preparing for future viral outbreaks.

CRediT authorship contribution statement

Nur Dini Fatini Mohammad Faizal: Conceptualization, Writing – original draft, Visualization. Mohd Cairul Iqbal Mohd Amin: Supervision.

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.

Data availability

No data was used for the research described in the article.

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