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Advances in the delivery of COVID-19 vaccines

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ABSTRACT

Several systems are being developed and investigated for the delivery of COVID-19 vaccines. A crucial challenge is the ability to maintain vaccine efficacy through the use of an effective delivery system. Some of these vaccine delivery systems such as lipid nanoparticles (LNPs) have been approved for the use by regulatory authorities in numerous countries. LNPs are currently used for the delivery of Moderna and Pfizer/BioNtech vaccines. LNPs consist of four constituents: Cholesterol for LNP stabilization, cationic lipids for the protection of messenger RNA (mRNA) molecules from nuclease degradation, and helper phospholipids that aid the formation and intracellular release of mRNA and PEGylated lipids that reduce nonspecific interactions. Researchers have also used virus-like particles (VLPs) for COVID-19 vaccine delivery. VLPs consist of several hollow viral proteins without the viral genome. VLPs are structurally identical to the native virus and can activate the human adaptive immune response. The nanosized VLPs self-assemblies have investigated as potential platforms for the delivery of COVID-19 vaccines. Liposomal vesicles are amphiphilic since the polar headgroups of phospholipids are oriented toward water molecules and the hydrophobic chains are in the internal area of the vesicles. The rationale behind the utilization of liposomes as vaccine delivery systems is their versatility and flexibility. Messenger RNA coding for SARS-CoV-2 spike protein can be entrapped into liposomes that are designed to remain stable in the bloodstream until their uptake by phagocytic cells. Other vaccine delivery approaches such as the use of microneedles and electroporation provide transdermal vaccine transport enable COVID-19 vaccines to cross the skin but not the cells of deep-lying tissues.

Keywords: Vaccines, SARS-CoV-2, COVID-19, Spike protein, Vaccine delivery

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the lethal COVID-19 pandemic.[1-3] COVID-19 is the fifth recorded pandemic after the 1918 flu[4] and SARS-CoV-2 is the second virus to cause a human pandemic in the 21st century.[5] COVID-19 has spread rapidly across the world due to its high transmissibility and pathogenicity.[6] The number of patients and deaths continues to rise.[7] Over 270 million infections and 5.3 million deaths have been caused by SARS-CoV-2.[8] COVID-19 is a global threat with severe economic losses and it is marked by tremendous human casualties.[9] The damaging economic fallout of COVID-19 in enormous with several countries stretching their health-care facilities and job losses occurring in several key industries.[10] SARS-CoV-2 is made up of a positive sense, single-stranded RNA ([+]ssRNA) genome (29891 nucleotides with 9860 amino acids) 29,903 base pairs (bps) long with genes encoding nucleocapsid protein, envelope protein, membrane protein, and spike protein, in addition to various other significant non-structural proteins (NSPs), including RNA-dependent RNA polymerase (RdRp, nsp12), helicase (nsp13), exonuclease (nsp14), as well as two

major cysteine proteases: The main protease 3-chymotrypsin such as protease (3CLpro or Mpro, nsp3) and the Papain-like protease (PLpro, nsp5).[11-13] The COVID-19 causing agent is a non-segmented RNA virus belonging to the Sarbecovirus subgenus, the Orthocoronavirinae subfamily, and the Coronaviridae family.[14] The Coronaviridae family is made up of the Torovirinae and the Coronavirinae subfamilies.[15] The Nidovirales order consists of the Roniviridae, the Coronaviridae, and the Arteriviridaen families.[15,16] The Coronavirinae subfamily is further divided into four genera: Gamma, delta, beta, and alphacoronaviruses. There are five subgenera of betacoronaviruses: Hibecovirus, embevovirus, nobecovirus, sarbecovirus, and merbecovirus.[17] Coronaviruses are nanoscopic in size measuring approximately 65–125 nm in diameter.[18] SARS-CoV-2 is a pleomorphic or spherical enveloped particle with a nucleoprotein within a capsid made up of matrix protein.[19]

The world has witnessed several forms of lockdown since March 2020, and even though countermeasures including masks have been adopted globally, COVID-19 has claimed millions of lives.[20] SARS-CoV-2 undergoes several mutations.[21] The emergence of the Omicron variant has deepened the challenge of overcoming this catastrophic pandemic. Omicron is the fifth "variant of concern" following the emergence of delta, gamma, beta, and alpha variants earlier.[22] There are more than 30 changes in the spike protein of omicron (B.1.1.529) variant.[22] Yang *et al*. reported several mutations for B.1.1.529 variant spike protein.[23] Despite alterations, researchers have documented highly conserved regions in the B.1.1.529 variant: The most potent S1 (non-RBD) protein domain missense substitution is the D614G followed by the G142D, while key (non) synonymous mutations that affect the critical receptor binding domain are the E484K, N501Y, K417N/T, and L452R.[21] Omicron variant is characterized by high transmissibility leading to increased infectivity and most likely elevated re-infection rates.[21] The need for safe and efficacious vaccines is obvious. Vaccines are required for the control and prophylaxis of the COVID-19 pandemic.[24] Four vaccines directed against SARS-CoV-2 have been approved by regulatory authorities in the USA and Europe.[25]

VIROLOGY AND PATHOGENESIS

Even though some vaccines are now available to combat COVID-19, the detailed molecular mechanisms underlying the pathogenesis of this disease remain largely unknown.[26] SARS-CoV-2 virus is the etiologic agent responsible for COVID-19.[27] The virus is transmitted mainly through respiratory droplets exhaled by infected individuals.[26] Subsequently, SARS-CoV-2 spike protein binds to the lung epithelial cells through the host cellular entry receptor angiotensin-converting enzyme 2.[26] SARS-CoV-2 lung entry requires priming of the spike protein by the transmembrane protease serine 2.[28] This is accomplished through cleavage of the S proteins at the S1/S2 and S2 sites. This cleavage step is necessary for host cell-virus membrane fusion and entry into the lung.[28] Following the unloading of the viral genome into the cytoplasm, the released positive-strand RNA virus ([+] ssRNA) utilizes host ribosome to translate open reading frames 1a and 1b into polypeptides pp1a and pp1ab [Figure 1].[13] These proteins are then cleaved and altered by the proteases PLpro and 3CLpro into 16 NSPs.[13] Proteins of the replication and transcription complex participate in structural protein synthesis and genome replication, leading to exocytosis of newly created virions.[13] Ultimately, immune cells release huge amounts of pro-inflammatory cytokines and chemokines, damaging distant organs, and leading to multiorgan failure and death.[26]

COVID-19 VACCINE DELIVERY

Lipid nanoparticles (LNPs)

Messenger RNA (mRNA) is an anionic and large (from 10^4 to 10^6 Da) molecule that cannot permeate the negatively charged lipid bilayer of cell membranes.[29] COVID-19 vaccines based on mRNA can transport genetic materials encoding the spike protein into the host cells, but due to the instability and restricted cellular uptake of naked mRNA, delivery systems are needed.[30] LNPs are currently used for the delivery of Moderna and Pfizer/BioNtech vaccines. Conventional vaccines require years of development, but development time for mRNA vaccines is significantly reduced, because mRNA can be speedily synthesized.[31] The two vaccines that have shown the most promising results and have been used widely in preventing COVID-19 infection consist of mRNA strands loaded into LNPs.[32] Researchers protect RNA from nuclease digestion by incorporating them into LNPs.[33] LNPs consist of four constituents: Cholesterol for LNP stabilization, cationic lipids for the protection of mRNA molecules from nuclease degradation, helper phospholipids that aid the formation, and intracellular release of mRNA and PEGylated lipids that reduce nonspecific interactions.[34,35] Ionizable cationic lipids consist of positively charged ionizable amine groups (at low pH).[35] A salient feature of LNPs is that cationic amines interact with anionic mRNA during particle formation and also promotes membrane fusion during the internalization process.[35] The uptake of LNPs by cells usually occurs through apolipoprotein E(ApoE)-dependent and/or ApoE-independent routes following the breakdown of PEG-lipid combination.[33] Once in the cell, protonated LNPs generate hexagonal phase structures, disorder the membranes, and deliver RNA molecules into the cytoplasm.[33] The discharged RNA molecules induce up regulation of spike proteins.[33] A principal limitation of mRNA-LNP COVID-19 vaccines is the very low temperatures required for their

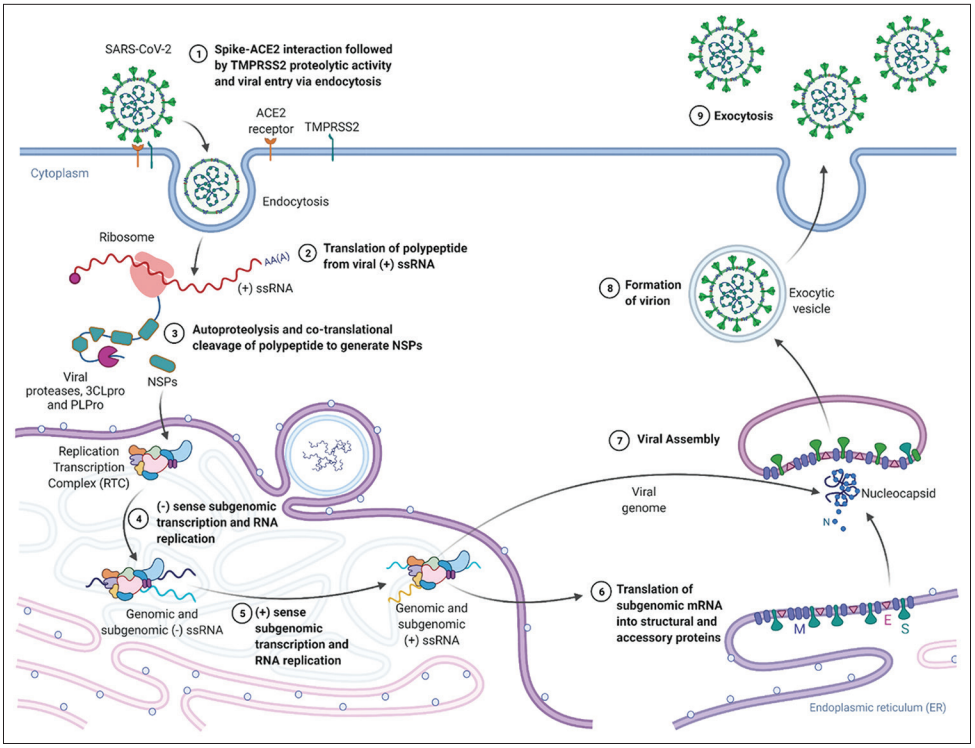

Figure 1: The SARS-CoV-2 viral life cycle (reproduced with permission from reference[13]).

storage.[32] Tozinameran (Pfizer/BioNTech vaccine) needs shippers to keep the storage temperature at −70 ± 10°C for up to 15 days.[24] Following thawing, the vaccine is stored at 2–8°C for up to 5 days.[24] The storage temperature for Moderna vaccine (elasomeran) is −30°C.[36] Several other mRNA vaccines that are delivered by LNPs are in clinical trials.[33]

Solid lipid nanoparticles (SLNs) have particle sizes between 10 and 1000 nm.[37] SLNs are formulated from biodegradable and physiological lipids as well as other materials that are largely recognized as safe.[37] SLNs can be prepared using high-speed stirring or high-pressure homogenization without using toxic solvents.[37] SLNs are considered the first generation of LNPs prepared from a solid matrix.[37] The key difference between SLNs and nanostructured lipid carriers (NLCs) is that SLNs are prepared from solid lipids, whereas NLCs are formulated from liquid lipids.[37] Comirnaty® (tozinameran) developed by Pfizer/BioNTech has been granted full approval by the Food and Drug Administration (USA) for the prophylaxis of COVID-19.[38] Emergency use authorization was granted to Moderna for the use of Spikevax® (elasomeran) for COVID-19 prevention.[33] Ho *et al.* documented the use of squalene nanoparticles (SQ@NP) for the delivery of SARS-CoV-2 subunit spike (S) protein.[39] The nanoparticles were formulated through a high shear microfluidized process from Span®85, lipid squalene, and a bioresorbable polymer suspended in PBS buffer. Before vaccination, the investigators mixed SQ@NPs 1:1 v/v with SARS-CoV-2 antigen.[39] It was demonstrated that both SQ@NP and aluminum phosphate can be used as vaccine adjuvants for generating humoral immune responses (adaptive immunity through the generation of antibodies by B lymphocytes) against SARS-CoV-2.[39] Nevertheless, SQ@NP was a more efficient vaccine adjuvant for the propagation of T-cell immunity.[39]

Liposomes

Liposomes are uni- or multi-lamellar lipid vesicles that are typically prepared from phospholipids, cholesterol, other lipids, and polymers.[40] Liposomal bilayers are amphiphilic since the polar headgroups of phospholipids are oriented toward water molecules and the hydrophobic chains are in the internal area of the vesicles.[40] The rationale behind the utilization of liposomes as vaccine delivery systems is their versatility and flexibility.[41] Messenger RNA coding for SARS-CoV-2 spike protein can be entrapped into liposomes that are designed to remain stable in the bloodstream until their uptake by phagocytic cells.[42] Indeed, Huang *et al.* constructed receptor binding domain-encoding mRNA (RBD-mRNA) incorporated into liposomes (LPX/RBD-mRNA) and studied the effect of administration routes on the immunogenicity of the delivery system.[43] The liposomes were formulated through a modified thin-film dispersion technique.[43] The authors dissolved 1,2-dioleoyloxy-3-(trimethylammonium)

propane chloride and cholesterol in ethanol and removed the organic solvents by utilizing a rotary evaporator to produce a thin film of lipid.[43] The lipid film was hydrated with RNase-free water and sonication was subsequently used for liposome formation.[43] The authors reported that LPX/RBD-mRNA expressed SARS-CoV-2 RBD *in vivo* and successfully induced specific antibodies in the vaccinated mice, which efficiently neutralized SARS-CoV-2.[43] Liposomes can also be utilized as vaccine adjuvants, Abhyankar *et al.* prepared a SARS-CoV-2 spike subunit vaccine formulation from dual TLR ligand liposome adjuvant.[44] Dipalmitoylphosphatidylcholine, GLA, PEGylated dipalmitoyl phosphatidylethanolamine, 3M-052, cholesterol, and α-tocopherol were mixed in chloroform. The authors used a rotary evaporator to remove the chloroform and then hydrated the lipid film with 25 mM ammonium phosphate buffer (pH 5.8).[44] A microfluidizer and sonicator were subsequently used to obtain liposomes.[44] The adjuvanted vaccine induced systemic and local anti-Spike IgA which is necessary for a COVID-19 vaccine.[44]

Virus-like particles (VLPs)

VLPs consist of several hollow viral proteins without the viral genome.[45] VLPs are structurally identical to the native virus and can activate the human adaptive immune response.[45] The nanosized VLPs self-assemblies can be utilized as vaccine antigen delivery systems.[46] They are non-replicating and non-infectious systems, because they do not contain a genetic component.[34,46] VLPs can directly stimulate immune cells by modeling the three-dimensional conformation of native viruses, but they do not contain infectious genetic material, which renders them comparatively safer than inactivated or attenuated viruses.[47] Apart from possessing efficient cell entry, VLPS present both intracellular T-cell epitopes and high-density B-cell epitopes for antibody production; thus, they can generate strong cellular and humoral immune responses.[47] Several expression systems, such as cells from bacteria, yeast, mammals, plants, or insects can be used to fabricate VLPs.[47] CoVLP, which is developed by Medicago, is in in phase 2/3 clinical trials (NCT04636697).[46] It is a vaccine based on VLP obtained through temporary transfection of tobacco plants with Agrobacterium.[46] The VLPs are used to deliver stabilized pre-fusion spike protein trimmers.[46] It is important to note that trivalent VLPs displaying the spike protein from several coronavirus strains are presently in pre-clinical development.[46] Dubé *et al.* recently formulated a plant-derived recombinant virus-like particle vaccine candidate directed against SARS-CoV-2.[48] The authors assessed adverse prenatal and postnatal action of AS03-adjuvanted CoVLP in Sprague-Dawley female rats during gestation (gestation days) and before cohabitation for mating.[48] VLPs containing S protein trimmers were isolated from a plant matrix (Nicotiana benthamiana) and subsequently purified. The AS03 adjuvant system was an oil-in-water emulsion consisting of 10.69 mg squalene, 11.86 mg DL-α-tocopherol, and 4.86 mg Polysorbate 80 (PS 80) per human dose of 0.25 Ml.[48] The authors documented a robust immune response in female rats before mating as well as at the end of gestation and lactation. Significantly, the use of AS03-adjuvanted CoVLP (twice during gestation and twice before mating) did not lead to any significant reproductive or developmental toxicity.[48] Another interesting project entailed the formulation of a triple antigen virus-like particle vaccine candidate (PRAK-03202), through the cloning and transformation of SARS-CoV-2 gene segments into a S. cerevisiae-based platform (D-Crypt™).[45] Antigen-specific (envelope, spike, and membrane proteins) humoral response and neutralizing antibodies were generated following vaccination with three doses of PRAK-03202.[45]

Electroporation

Electroporation is the use of high voltage pulses to temporarily create pores in cell membranes.[49] Although electroporation has been utilized widely for drug and vaccine delivery, the exact mechanism of this technique has not been completely elucidated. It has been postulated that aqueous pores are generated in cell membranes following electroporation.[50] Similar to microneedles (MNs), electroporation is mainly applied to the skin, thus allowing vaccines to enter into the bloodstream through a transdermal route. Changes in the stratum corneum lipid ultrastructure following the application of high voltage pulses occur probably due to the interaction between electric field and water dipole.[50] There is normally an enhanced but transient membrane permeability when cells are exposed to high voltage electric pulses.[51] When the electric field attains a certain amplitude, there will be an increase in plasma membrane permeability.[51] This process of electroporation can be employed for synthetic DNA delivery. One advantage of synthetic DNA is that it does not require cold chain and it is thermostable, which makes it different from approved RNA and vector vaccines especially in developing countries that may not have adequate resources.[25] Remarkably, injection of DNA plasmid into the skeletal muscle followed by a short electrical stimulation (electroporation) enhances DNA uptake and gene expression by several 100-fold.[25] Conforti *et al.* conducted preclinical investigation of a COVID-19 vaccine candidate that was developed through electroporation of engineered, synthetic cDNA encoding a viral antigen.[25] The authors utilized DNA electroporation with an IGEA Cliniporator® through a needle electrode.[25] Cell-mediated and antibody immune response were analyzed at different time points. Several DNA vaccines were formulated, and their immunogenicity evaluated in animal models.[25] A DNA plasmid encoding a secreted monomeric form of SARS-CoV-2 S protein receptor-binding domain (COVID-eVax) generated the strongest immune responses including a robust T-cell response.[25] It has been

observed that a key limitation with electroporation is pain and discomfort at the application site in comparison with traditional injections.[50,52]

MNs

There is significant interest in the use of MNs for vaccine delivery. MNs are usually fabricated in the form of an array possessing an adhesive backing layer.[53] MNs can be used for vaccine administration. Vaccines are typically administered as intramuscular or subcutaneous injections, but these techniques frequently result in low compliance, the risk of infection and the need for highly trained professional staff.[54] In contrast, MNs do not have these limitations and are currently being investigated by several laboratories worldwide for the delivery of vaccines. MNs are microscopic projections that are long enough to permeate the skin but too short to cause pain.[55] MN arrays can be fabricated from a wide range of materials including stainless steel and polymers.[56] The use of MNs is a minimally invasive approach to transdermal drug delivery.[57] Significantly, MNs can solve problems associated with the logistics and distribution of vaccines.[58] There are five types of MNs: Solid, coated, hollow, dissolving, and hydrogel-forming. Researchers traditionally use solid MNs to pre-treat the skin before applying the medication in the form of solution or ointment.[59] Hollow MNs, made out of materials such as silicon, stainless steel, or gold, have bores within the individual needles which permit the delivery of fluids.[53] A coated MN system can deliver active pharmaceutical ingredients gradually across the skin.[60] Coated MNs can also be employed for vaccine delivery. The human skin is immunologically reactive and harbors an enormous amount of immune-accessory and antigen presenting cells with innate immune function.[61] Kim *et al*. formulated MN-mediated SARS-CoV-2 subunit vaccines.[61] Obelisk-shaped dissolving MNs were prepared from carboxymethylcellulose at room temperature using polydimethylsiloxane micromolds.[61] Tip-loaded spike protein in the molds was overlayered with carboxymethylcellulose hydrogel to form mechanically robust MN arrays and backing layers.[61] Significantly, the SARS-CoV-2 S1 subunit vaccines delivered through dissolving MNs generated potent antigen-specific antibody responses.[61]

Micelles

Micelles are self-assembling nanoconstructs formulated from amphiphilic copolymers.[62] They possess a core-shell structure and can be utilized for the delivery of nucleic acids.[62] An interesting feature of polymeric micelles that lead to widespread use in drug and vaccine delivery is their ability to solubilize therapeutic or prophylactic agents within their core, thus enhancing their bioavailability.[58] Shinde *et al*. formulated micelles for the delivery of a COVID-19 vaccine.[46,63] The vaccine (NVX-CoV2373) consists of the amphiphilic detergent PS 80, saponin-based Matrix-M1 adjuvant, and full-length SARS-CoV-2 spike glycoprotein.[46,63] The vaccine was made by engineering a baculovirus that carries a gene encoding pre-fusion stabilized S protein.[63] The authors used PS 80 to formulate protein nanoparticles.[63] NVX-CoV2373 was found to be 96% effective against wild-type SARS-CoV-2.[46,63] Two 5 μg dose regime induced serum IgG and nAb titers that were significantly greater than those found in convalescent individuals.[46,64]

Recombinant viral vectors

Recombinant viral vectors are typically used to express antigens.[20] Delivery of target antigen through this platform mimics natural infection and thus can generate strong T-cells responses.[20] Chimpanzee adenovirus vector (ChAd) encoding a pre-fusion stabilized spike (S) protein has been studied as a nasally delivered vaccine against SARS-CoV-2.[65] Most importantly, ChAd expressing the SARS-CoV-2 spike protein is a COVID-19 vaccine granted Emergency Use Authorization by the UK's Medicines and Health-care Products Regulatory Agency.[66] ChAd is a non-replicating ChAd, which has been used to expresses the SARS-CoV-2 spike protein with a tissue plasminogen activator signal sequence.[67] Elevated levels of neutralizing antibodies as well as T-cell responses were observed following a single vaccination against SARS-CoV-2.[20]

In a separate study, Bos *et al*. used a recombinant replication-incompetent adenovirus type 26 carrier to deliver SARS-CoV-2 spike protein.[68] The investigators reported that constructs with wild-type signaling peptide and stabilizing substitutions (two proline residues in the hinge region of the S2 subunit of SARS-CoV-2 spike protein (686–1273 amino acid residues) and furin cleavage site mutations increased the ratio of neutralizing and non-neutralizing antibody binding in comparison with constructs made with tissue plasminogen factor signal peptide.[68] Interestingly, CD4 and CD 8 T-cell responses were achieved following vaccination with the Johnson and Johnson Ad26.CoV.S single dose vaccine despite past infection.[69]

CONCLUSION

There is a frantic race to develop efficacious vaccines directed against SARS-CoV-2. Delivery systems have a key role to play in this research. Several vaccine delivery systems have been described in this review. It is hoped that with the right delivery system, more effective vaccines will be deployed to combat this global health scourge.

Declaration of the patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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