Nanotechnology in COVID-19 Vaccines

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Abstract. Nanotechnology has been proposed in vaccine development as carriers and adjuvants time and again but received limited recognition before the development of vaccines for the COVID-19 pandemic. Nanotechnology has played a pivotal role in the triumph of the vaccines for the current pandemic caused by the SARS-CoV-2 virus. The first vaccine got approved within a year of the earliest reported case of the novel coronavirus. Presently, more than 35 vaccines are approved in at least one country, 10 out of which are sanctioned for emergency use by the WHO. The key categories include peptide vaccine, mRNA vaccine, inactivated & viral vector vaccines. Nanotechnology is a crucial component in the success of mRNA vaccines. Nanoparticles not only allow targeted drug delivery but also boost the pharmacokinetic profile and the immune response against the therapeutic. A few nanoparticles have received approval for use as adjuvants (MF59), and countless others (virosomes, PGLA) are under development for many vaccines for infectious diseases, for instance, Influenza, Hepatitis B, Human Papillomavirus, Malaria, Tuberculosis, etc. In this article, we will review the vaccines available against SARS-CoV-2 with a focus on the nanotechnology utilized in their development.

Keywords: SARS-COV-2 · Vaccines · Infectious Diseases · Nanotechnology · mRNA vaccine

1 Introduction

The initial reports of COVID-19 began in December 2019, and it was announced as a pandemic by March 2020. By late February 2020, the virus had started infecting people across the world at an alarming rate and was declared a global pandemic by 11th March 2020. The sequencing of the complete genome of SARS-CoV-2 was completed in January, 2020 [1], and the efforts to create a vaccine started right away. The development of vaccines saw unprecedented growth and within a year, the first vaccine was cleared for use by the Food and Drug Administration in the COVID-19 crisis. The candidates are a concoction of conventional and non-traditional approaches. Currently, more than 30 vaccines have been approved for emergency use in about 200 countries, while many more are in clinical and preclinical development [2]. Prime technologies employed include classical platforms, i.e. protein subunit vaccines and inactivated vaccines, and next-generation

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Table 1. The number of vaccines approved in at least one country for different categories as of 9th March, 2022

S.No.	Vaccine Type	Number of Candidates
1	Inactivated	13
2	Protein Subunit	11
3	Viral Vector	6
4	Nucleic Acid	4
5	Virus Like Particle	1

platforms, i.e. nucleic acid-based vaccines and viral vector vaccines. The World Health Organization has approved 10 vaccines-2 protein subunit vaccines, 2 mRNA vaccines, 3 non-replicating viral vector vaccines and 3 inactivated vaccines. 25 other vaccines have also been approved in at least one country. The coronavirus pandemic brought numerous firsts in the history of vaccinology, including the first plant-based vaccine, and DNA plasmid vaccine to be approved for mass vaccination.

Nanoparticles have long been proposed for vaccine delivery, however, the proposed vaccines rarely made past the development or trial stages. Most of the successful vaccines, and almost all the next-generation vaccines for SARS-CoV-2 employ nanotechnology in one way or another. It may be through the utilization of the nanoscale viral vector, a liposome, or nanoemulsion. From Moderna's SpikeVak to Oxford/Astrazeneca's Vaxzerveria, nanoparticle use is observed in majority of the vaccine formulations.

1.1 Inactivated Vaccines

For the synthesis of inactivated vaccines, live viruses are cultivated in human cells and their genetic stability is evaluated. The stable viruses i.e. the ones showing least mutations are killed and used for vaccine development. Presently, there are 11 inactivated vaccines available for SARS-CoV-2 across the world. Covaxin, CoronaVac and Covilo developed by Bharat Biotech, Sinovac Biotech, and Sinopharm respectively, have been permitted for emergency use by the World Health Organization. For the development of Covaxin, stability of the candidate was observed by noting the change in the sequence of the NIV-2020-770 strains (0.075%) after 3 passages. Inactivated whole-virion vaccines were developed by using Algel-IMDG as adjuvants [3, 4]. CN2 variant of the SARS-CoV-2 was utilized for the formulation of CoronaVac (or PiCoVacc) by Sinovac Biotech [5] while HB02 strain was selected for Covilo (or BBIBP-CorV) by Sinopharm, owing to their genetic stability (no amino acid variations in 10 passages) [6]. Among other candidates, KCONVAC developed by Minhai Biotechnology uses the 19nCoV-CDC-Tan-Strain03 as the virion, cultivated in vero cells. Its inactivation is achieved by β-propiolactone and it uses aluminum hydroxide as an adjuvant [7]. Carnivak-COV, developed by Rosselkhoznadzor (Russia), only vaccine for carnivores including dogs, cats etc., against COVID-19 is also an inactivated vaccine [8]. No vaccine using a live-attenuated virus has crossed the trials yet. The main disadvantage of this method is the risk associated with the cultivation and processing of a live virus.

1.2 Protein Subunit Vaccines

In peptide vaccines, immunodominant proteins of the virus are expressed in a non-pathogenic cell (production host). Currently, there are 13 peptide vaccines available for SARS-CoV-2. Nuvaxovid (Novavax) and COVAVAX (based on Novavax formulation) are approved by the WHO [4, 9]. The spike protein, although modified in different ways, is the common component in all the current protein subunit vaccines. The vaccines designed by the Finlay Institute in Cuba- SOBERANA 02 and SOBERANA PLUS, contain the tetanus toxoid as an adjuvant, conjugated to the receptor-binding domain of spike protein to improve the immunogenic profile of the vaccine [10].

Covifenz, developed by Medicago uses a virus-like particle to mimic the spike protein of SARS-CoV-2. It is the first plant-derived vaccine in the world. RDB-Dimer vaccine produced by Anhui Zhifei Longcom has a tandem-repeat dimeric receptor binding domain protein [11]. It has an alum-based adjuvant and is permitted for distribution in China, Colombia, Indonesia and Uzbekistan. The Federal Budgetary Research Institution State Research Center of Virology and Biotechnology in Russia, developed EpiVacCorona, which employs chemically synthesized peptide immunogens having selective epitopes of SARS-CoV-2 Spike protein [12]. These peptides are conjugated to recombinant nucleocapsid peptide of the SARS-COV-2 envelope that acts as the carrier for the immunogen. It also uses aluminum hydroxide as an adjuvant. MVC-COV1901 vaccine candidate produced by Medigen makes use of the ExpiCHO system for overexpression of the S-2P proteins [13]. It is adjuvanted using alum and CpG 1018. CIGB-66 (commonly referred to as Abdala) expresses the receptor binding domain in yeast and utilizes alum as an adjuvant [14].

1.3 Viral Vector Vaccines

For the creation of this category of vaccines, replication-deficit viruses are used to express proteins from SARS-CoV-2. There are currently 6 viral vector vaccines approved for use in at least one country, all of which use an adenovirus for the delivery of the antigenic sequence. All of the 6 viral-vector vaccines approved use different types of adenoviruses for the development of vaccines. Convidecia, formulated by CanSino Biologics, uses a non-replicating Ad5 vector [15], Vaxzerveria and Covishield (Oxford/Astrazeneca formulation) use ChAdOx1, a chimpanzee virus, and Ad26.COV2.S, fabricated by Janssen (Johnson & Johnson) uses Adenovirus Type 26 [16].

1.4 Nucleic Acid Vaccines

The mRNA vaccines for COVID-19 contain mRNA coding for the antigenic peptide for SARS-CoV-2, enveloped by a lipid nanoparticle (or a liposome). As of now, there are 3 such approved vaccines including SpikeVak (Moderna) [17], TAK-919 (Moderna Formulation) and Comirnaty (Pfizer-BioNTech). The only DNA vaccine available at present is ZyCoV-D, developed by Zydus Cadilla that has an oligonucleotide coding for the spike protein cloned into a DNA plasmid. It is the earliest DNA plasmid vaccine that has been allowed to be given to humans [18]. The DNA plasmid is generally transported to the nucleus, expressed into RNA and then exported to the cytoplasm for translation.

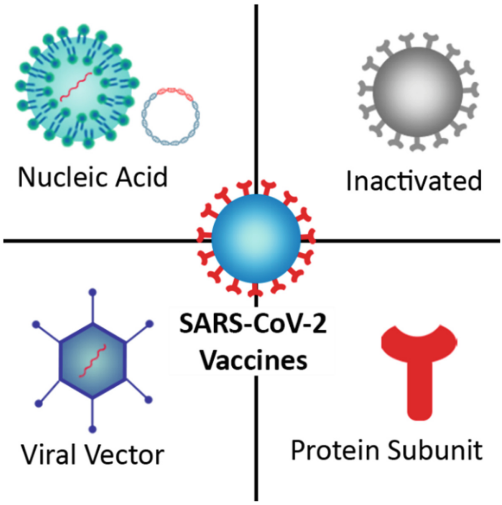

Fig. 1. Main categories of COVID-19 vaccines

On the other hand, mRNA is translated in the cytoplasm itself and can therefore be expressed more easily in the cell. The problems that might arise during the transport of the plasmid to the nucleus and its subsequent expression are also avoided [19] (Fig. 1).

2 Lipid Nanoparticles for SARS-CoV-2 Antigen Delivery

mRNA vaccines have an mRNA strand that is transported to the cell on a cargo. Although the direct injection of naked mRNA has been reported [20], it is an unstable molecule that is vulnerable to attack by the nucleases in the tissues or the matrix. Failed cellular uptake can also lead to ineffective mRNA vaccines or drugs [21]. Because of this, mRNA vaccines need an appropriate carrier that is safe, secure and efficacious. This carrier often presents itself in the form of a lipid nanoparticle (LNP). Although many mRNA vaccines have been designed for infectious diseases and were in the development and trial phases before the onset of the COVID-19 pandemic, the first mRNA vaccine to be sanctioned for human utility was the Pfizer/BioNTech vaccine candidate (now referred to as Comirnaty). The lipid nanoparticles are spherical particles that are generally composed of four main components: polyethylene glycol (PEG), cholesterol, phospholipid and an ionic lipid. PEG provides a longer half-life to the lipid nanoparticle, while cholesterol acts as a stabilizer to it [22, 23]. The phospholipid provides structural integrity to the LNP, initiates its formation, disruption and allows endosomal escape [24]. The cationic lipid is the crucial component of the liposome that performs the encapsulation of mRNA and also assists in the cellular uptake of the nanoparticle [25]. The lipid nanoparticles and the viral vectors act as carriers as well as adjuvants for the vaccines as they improve immunological profile of the vaccine in addition to allowing targeted drug delivery. Liposomes have been used for the delivery of anti-cancer drugs since 1990s. For instance, Doxil is the first approved liposomal drug, it contains

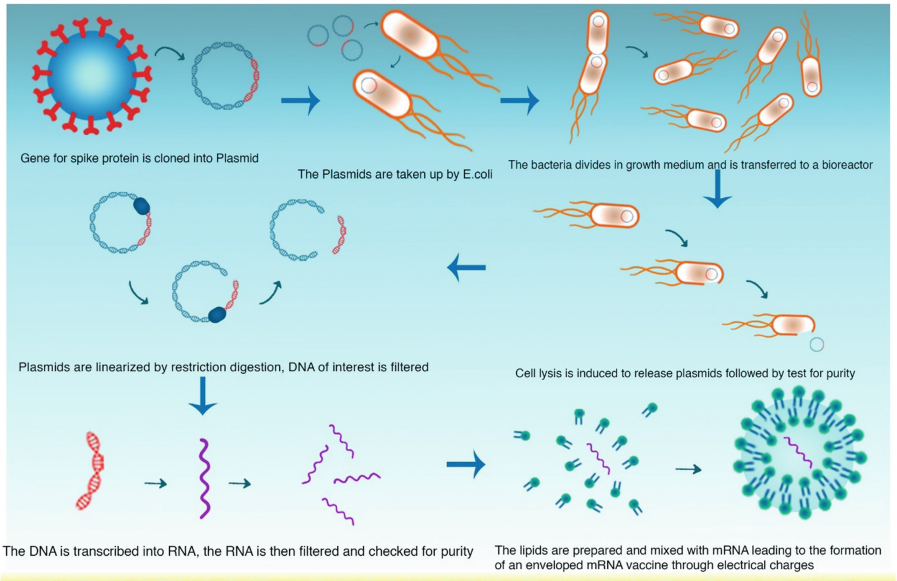

Fig. 2. Generalized overview of the mRNA manufacturing process

doxorubicin for the treatment of cancer [23]. Lipofectin, developed in 1989, containing a combination of cationic lipids DOPE (dioleoyl phosphotidylethanolamine) and DOTMA (N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride) is used for transfection of oligonucleotides into endothelial cells and is a common cargo for in-vitro cell-culture experiments [26]. Patisiran (or Onpattro) was the first therapeutic using small interfering RNA to receive approval in 2018 by the FDA. It is used to treat polyneuropathy which has been caused by a type of hereditary transthyretin amyloidosis, through selective delivery of the siRNA to parenchymal cells of the liver i.e., the hepatocytes [27]. Patisiran LNP contains Cholesterol, DPSC {1,2-distearoyl-sn-glycero-3-phosphocholine}, PEG2000-C-DMG {α-(3′-{[1,2-di(myristyloxy)proponoxy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene} and DLin-MC3-DMA {(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate}[28]. The LNP surrounds a 2'OMe-modified siRNA [29]. Onpattro's nanoparticles have a long shelf life (about 3 years) when stored at 2-8^{o} C, augmenting their viability as therapeutic cargos [30].

The indispensable role of LNPs in the victory of the mRNA vaccines in COVID-19 has made its applications evident. Pfizer/BioNTech's Comirnaty and Moderna's Spikevak are one of the most successful COVID-19 mRNA vaccines today. TAK-919, another approved vaccine produced by Takeda is based on Moderna's formulation of SpikeVak. The mRNA in Moderna's mRNA-1273 is a nucleoside modified molecule that codes for the S-2P antigen, where 2P are two proline substitutions at 986 and 987 positions of the peptide.

These mutations make the pre-fusion conformation of the spike protein stable, making it a more suitable for vaccine design and development. The ratio of mRNA to lipids

is fixed for the formation of the LNP [17, 31]. The LNP of SpikeVak consists of four lipids-1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) {the phospholipid}, 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000 DMG) {the PEGylated lipid}; Cholesterol, and most likely cationic lipid SM-102 (heptadecan-9-yl 8-{(2-hydroxyethyl)[6-oxo-6 (undecyloxy)hexyl]amino}octanoate) {The ionic lipid} [32]. SM-102 (C44H87NO5) is an ionizable- cationic lipid that was patented by Moderna in 2017. It is a chemically synthesized amino lipid that is pH sensitive. Although it is uncharged near the physiological pH (7.40), it acquires a positive charge at lower pH ranges [33]. The charge allows efficient binding of the mRNA to LNP and targeted delivery to the lung cells, as changing the ratio of the cationic lipids in the LNP can change the cell that is targeted for delivery. For instance, decreasing the ratio of the cationic lipids has shown to shift the delivery target from lungs to spleen [34].

ALC-315 ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) is an ionizable lipid patented by Acuitas Therapeutics in the year 2017. It acts as the cationic lipid component of the LNP of the Pfizer/BioNTech's BNT162b2 candidate. The opposite polarity of ALC-315 lipid and the mRNA allows their interaction and binding, thus enabling the compaction of the mRNA, facilitating its delivery into the cells. The other components of Comirnaty lipid nanoparticle are 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159)-PEGylated lipid; Cholesterol and 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC). The mRNA is nucleoside modified with 2 proline substitutions in the central helix of the S antigen [35]. The overview of manufacturing procedure for the Pfizer/BioNTech's Comirnaty is outlined in the Fig. 2. The vaccine production takes about 60 days and involves many quality-control steps and transport to different facilities [36].

The main disadvantages of the mRNA vaccines are their cold chain requirements and the possibility of an anaphylactic shock. The mRNA vaccines have stringent cold chain requirements and are needed to be kept at -70 °C freezer. Due to allergic reactions observed in rare cases, both Pfizer/BioNTech's Comirnaty and Moderna's Spikevak vaccines in use carry N1-methylpseudouridine rather than uridine to lessen cytotoxicity, the chances of the mRNA inducing an allergic reaction are low [37, 38]. The constituents of the LNP may be allergens, where the lipids are the most likely cause [39]. The PEGlyated lipids could be the cause of these reactions, as hypothesized previously [40]. Such limitations can hamper development of these vaccines.

3 Nanoparticles as Adjuvants in COVID-19 Vaccines

Adjuvants are vaccine components that play a role in instigating a powerful immune response from the system. The most common adjuvants are aluminum salts including alum, aluminum hydroxide, alumina oxide etc. Non-replicating viral vectors and RNA based vaccines do not require adjuvants but peptide and inactivated vaccines are immensely reliant on them. Other than aluminum salts, some of the approved adjuvants are AS02, AS04, AS01B, and MF59. AS02 has MPL (3-O-desacyl-4'-monophosphoryl lipid A) along with saponin QS21. MPL is a TLR4 agonist and is a constituent of two vaccines: Cervarix, the vaccine developed by GlaxoSmithKline targeting carcinogenic forms of Human Papillomavirus-HPV-16 and HPV 18 and Fendrix, also designed by GlaxoSmithKline targeting Hepatitis B [41].

Among the COVID-19 vaccines, the adjuvants for EpiVacCorona, KCONVAC, and BBIBP is aluminum hydroxide, Covaxin has Algel-IMDG and CoronaVac, Sinopharm along with few others use alum. MVC-COV1901 (Medigen), a peptide subunit vaccine with S-2P as the main component, uses CpG 1018 (750 μg) along with aluminum hydroxide (375 μg) as adjuvants [13, 42]. Aluminum hydroxide imparts long term immunological memory by activating macrophages. It does not interact with Toll like Receptors (TLRs) generally and achieve dendritic cell activation through macrophages [43]. CpG 1018 is an adjuvant developed by Dynavax Technologies. It is a short oligonucleotide made up of 22 residues of cytosine phosphoguanine that was initially used in Heplisav-B vaccine. It is an TLR9 (Toll-Like Receptor 9) agonist, but also induces a strong TH1 helper response. Through the stimulation of the TLR9, B-cells and dendritic cells are also activated [44].

Nanoemulsions often act as great adjuvants. Nanoemulsions such as AS03 and MF59 have been utilized in influenza vaccines earlier. MF59 is a nanoemulsion of oil-in-water created by Novartis in 1990s with a size of about 165 nm containing squalene, tween 80 and span 85 as the main components. It was also used as adjuvant in the Fluad® vaccine for influenza [45]. AS03 is an adjuvant system created by GlaxoSmithKline that contains squalene, polysorbate 80 and DL-α-tocopherol. It was a component of the Pandremix vaccine that was used for the H1N1 pandemic flu [46]. The Covifenz vaccine against SARS-CoV-2, produced by Medicago, is a protein subunit vaccine that contains virus-like particles, and is adjuvanted by AS03. The spike protein used for this vaccine is expressed using a plant-based expression system, making it the first plant-based vaccine in the world. Nuvaxovid & COVOVAX makes use of Matrix M™ as the adjuvant, which comprises of cholesterol, phospholipids and saponin extracted from *Quillaja saponaria.* Together, these components form 40 nm nanoparticles that improve the response of the lymphocytes greatly [47].

4 Nanoscale Viral Vectors in COVID-19 Vaccines

The vector of choice for vaccine development is the Adenovirus. Adenoviruses belong to the family Adenoviridae, a family of icosahedral unenveloped viruses with a double-stranded DNA genome. Although Adenoviruses have been actively applied for gene therapy for a couple of decades, their utilization in vaccines is a relatively recent development. Adenoviruses have a ~40 kb genome and induce a strong innate and adaptive immune retaliation. The innate response include the recognition by pattern recognition receptors, pro-inflammatory cytokines and interferon activation [48, 49]. The thermostability, cost, production and the ease of manipulation of their genome present adenoviruses as suitable delivery vehicles [50].

The regions of the virus that are manipulated frequently to generate a suitable vector include E1, E3 and E4 regions. The E1 region products are involved in replication, E3 products are involved in modulation of the immune response while E4 proteins are involved in the management of viral replication and gene expression [50]. The deletion of the E1 region leads to replication deficit in the virus, E3 deletions lead to the increase in viral capacity of up to 5 kb. However, such vectors exhibit inefficient growth in human cell lines. E4 modifications can often assist in overcoming this limitation.

Gamaleya National Center of Epidemiology and Microbiology developed Sputnik Light and Sputnik V vaccines, which use two recombinant vectors namely adenovirus type 5 (rAd5) and adenovirus type 26 (rAd26) [51]. Ad26 belongs to the Group D of the human serotypes while Ad5 is a part of group C. Both carry SARS-CoV-2 full-length glycoprotein S gene. Sputnik V is lyophilised (Gam-COVID-Vac-Lyo) or frozen (Gam-COVID-Vac) and has 1011 viral particles of both vectors per dose. Sputnik Light often acts as the booster dose for Sputnik V. The vaccine developed by Cansino Biologics uses an E1 and E3 deleted-Ad5 vector that is inept at replication to produce the spike protein attributed to the SARS-CoV-2 with the tissue plasminogen. The vector was modified with the help of the Admax system developed by Microbix Biosystem, from Toronto, Canada [15]. Vaxzerveria expresses the trimeric prefusion conformation of the S protein in the chimpanzee adenovirus ChAdOx1 [52]. ChAdOx1 vector was developed by The Jenner Institute in 2012 [53]. It is a modified simian adenovirus with Y25 serotype. It has been associated with the human serotype E, which includes HAdV-4 [54]. While designing the vector of the vaccine, the E1 and non-essential E3 regions of the virus were deleted, and the E4 region was modified to improve its efficiency as a vector. For the development of the ChAdOx1 vector, the Orf6, Orf6/7 and Orf4 of the E4 were reformed with the Ad5. Ad26.COV2.S utilizes a full-length S, with a wildtype signal peptide having proline substitutions and furin cleavage site mutations. The Ad26 vector was designed using the AdVac system, a vaccine technology platform designed by Janssen [16].

The primary concern with Adenovirus-based vectors is that most of the humans have been infected by Adenoviruses at least once in their lives, thus the presence of neutralizing antibodies against the virus affects the efficacy of the vaccines negatively [55]. Additionally, Th1 responses that exhibit cross-reactivity are also observed [56].

5 Conclusion

Nanoparticles have significant applications in vaccine development. In fact, nanotechnology-based approaches have proven to be much more effective than conventional approaches for vaccine development like inactivated vaccines. One of the risks involved with inactivated vaccines are associated with the cultivation and processing of a live virus. In terms of cost, safety, transportation and production too, nanoparticles offer a better alternative. However, improvements can be made to formulate nano particles that may prevent allergic and adverse reactions in the body. Additionally, the requirement of cold chain for the storage of mRNA vaccine hinders their application and use in third world countries due to lack of these facilities. Inspection of somewhat unexplored nanoparticles like dendrimer nanoparticles as adjuvants can also be attempted as they have shown to activate dendritic cells *in vitro* [57]. Polymeric nanoparticles modified with amino acids are also being explored to improve vaccine efficacy [58].

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