Vaccine design and delivery approaches for COVID-19

Kiana Shahzamani [a], Fatemeh Mahmoudian [b], Shahrzad Ahangarzadeh [c],
Mohammad Mehdi Ranjbar [d], Leila Beikmohammadi [e,f], Samira Bahrami [g],
Elmira Mohammadi [h,i], Sahar Esfandyari [j], Abbas Alibakhshi [k,*],
Shaghayegh Haghjooy Javanmard [h,**]

[a] Isfahan Gastroenterology and Hepatology Research Center (lGHRC), Isfahan University of Medical Sciences, Isfahan, Iran
[b] Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Razi Vaccine and Serum Research Institute, Agricultural Research, Education, and Extension Organization (AREEO), Karaj, Iran
[e] Department of Biochemistry, Erasmus University Medical Center, Rotterdam, the Netherlands
[f] Stem Cell and Regenerative Medicine Center of Excellence, Tehran University of Medical Sciences, 14155-6559 Tehran, Iran
[g] Biotechnology Department, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran
[h] Applied Physiology Research Center, Cardiovascular Research Institute, Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran
[i] Core Research Facilities, Isfahan University of Medical Sciences, Isfahan, Iran
[j] Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[k] Molecular Medicine Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

A B S T R A C T

COVID-19 is still a deadly disease that remains yet a major challenge for humans. In recent times, many large pharmaceutical and non-pharmaceutical companies have invested a lot of time and cost in fighting this disease. In this regard, today's scientific knowledge shows that designing and producing an effective vaccine is the best possible way to diminish the disease burden and dissemination or even eradicate the disease. Due to the urgent need, many vaccines are now available earlier than scheduled. New technologies have also helped to produce much more effective vaccines, although the potential side effects must be taken into account. Thus, in this review, the types of vaccines and vaccine designs made against COVID-19, the vaccination programs, as well as the delivery methods and molecules that have been used to deliver some vaccines that need a carrier will be described.

1. Introduction

Rapid transmission and asymptomatic spread of COVID-19 threaten human health, economy, and social life. Research and finding an effective and safe vaccine that is usually a powerful and potential solution to combat such diseases are urgent global needs in this critical situation. Currently, the pandemic has triggered an unprecedented race toward vaccine development against SARS-CoV-2 [1]. Researchers and commercial manufacturers are using all kinds of vaccines for COVID-19. The majority of vaccine projects are based on modern vaccine approaches, which are shifted away from live-attenuated and inactivated whole-pathogen vaccines towards purified antigens and epitopes. The

process of vaccine design has been revolutionized by reverse vaccinology, which focuses on finding potential vaccine candidates through bioinformatics analysis of the protein-coding genome (proteome) of pathogens.

The urgency of vaccine demand changed the long traditional process of vaccine development [2]. In just one year after identifying the SARS-CoV-2 virus and its genome, the extraordinary efforts of the scientific community around the world have led to the development of more than 100 vaccine projects in various stages of clinical trials in more than 10 cases successfully ended in Phase III. These different candidate vaccines can be clustered based on the technological platform and protective immune response production [3]. Having the opportunity to design and develop various vaccines based on different technologies, it is possible to select vaccines that can be most effective in certain epidemics, pandemics, and worldwide.

As announced by the WHO on July 15, 2020, more than 150 countries participate in the COVID-19 Vaccine Global Access Initiative (COVAX) to overcome the challenge of rapid, fair, and equitable access to COVID-19 vaccines worldwide [4].

The present review aims to provide the up-to-date status of various promising vaccine systems in study or different clinical phases or approved for public use by multiple institutions and how they are delivered by the diverse available strategies to combat the great threat of the COVID-19 pandemic.

2. Characteristics of SARS-CoV-2 and COVID-19

Coronavirus belongs to an enveloped virus with a positive-sense RNA genome. It is named because of the presence of crown-like spikes glycoprotein on its surface. The capability to infect multiple hosts and the cause of different diseases despite indicating acute respiratory infections in humans make this virus a complex pathogen [5,6]. The genomic sequence of SARS-CoV-2 shows close relation with bat-SL-CoVZC45 and bat-SL-CoVZXC21; its receptor-binding domain is similar to SARS-CoV, according to homology modeling [7]. SARS-CoV-2 genome is consisting of a non-structural region (genes encoding replicase complex) and a structural region (genes encoding Spike, Envelope, Membrane, and Nucleocapsid proteins) [8]. The spike protein can bind to angiotensin-converting enzyme 2 (ACE2) receptors and plays a critical role in virus entry to the host cell. The most variable part of the SARS-CoV-2 genome is the receptor-binding domain (RBD) located in the spike protein [9,10]. According to structural and biochemical studies, RBD can bind to ACE2 expressed on the cell surface in humans, cats, ferrets, and some other species with high receptor homology [9,11–14]. The inhibition of this attachment between spike and ACE2 can prevent the progression of the disease [15]. Experimental infections accompanied by different research groups have shown that hamsters, ferrets, cats, treeshrews, monkeys, fruit bats, and transgenic mice are sensitive; however, pigs, poultry, and dogs are resistant to this virus. Although, there is not any strong evidence for usual animal-to-human or continued animal-to-animal transmission of SARS-CoV-2 [16,17]. The clinical symptoms for COVID-19 range from mild to severe, including fever and/or respiratory appearance, dyspnea, failure in respiration, and some other symptoms that rarely occur [18]. The reverse transcription-polymerase chain reaction (RT-PCR) is the most important method to detect SARS-CoV-2 [19]. Besides, new approaches have been reported in recent studies, for example, using molecularly imprinted polymer (MIP)-based electrochemical sensor for detection of SARS-CoV-2 nucleoprotein (ncovNP), which can be a valuable substitute and a transferrable diagnostic platform for the rapid screening of COVID-19 [20]. The studies show that the pathogenicity of COVID-19 occurs in three sequential phases: pulmonary, proinflammatory, and prothrombic. The first phase consists of deficiency in ACE2 [21], RAS imbalance [22], and exacerbation of ACE2 deficiency, which occurs after ADAM metallopeptidase domain 17 (ADAM-17) over-activation by angiotensin II affected by ACE2 deficiency induced by the virus [23]. Suggested specific

treatments for this phase are using RAS inhibitors [21], inhibition of virus entrance such as using antibodies against CD147 like meplazumab [24], inhibition of virus replication, for example, by using remdesivir and favipiravir, also using protease inhibitors [25]. The second phase includes innate immunity activation, Lymphopenia, and Cytokine storm [26]. Some treatments for this phase are using common inflammatory drugs, cytokine inhibitors, immunomodulatory drugs, complement pathway inhibitors, inhibitors of JAK-STAT signaling, and convalescent plasma therapy [23,26,27]. For the last phase, which is thrombosis activation, there are some reports about increased D-dimer (the product of cross-linked fibrin degradation) levels, extended prothrombin time, and little platelet counts in patients [28–31]. The specific therapeutics for this phase include the antagonist of vitamin K, anti-Xa agents, and antiplatelets [32,33]. Undoubtedly, designing and achieving a safe vaccine that prevents these symptoms can be the most important way to control the disease.

3. Vaccination for COVID-19: The process

3.1. Access

Equitable access to a vital life-saving vaccine is one of the basic human rights that depends on its widespread production, global supply chains, acceptability, accessibility, and cost-effectiveness. To realize these rights, a COVID-19 vaccination will almost surely be classified as an "essential medicine" by WHO, increasing national and international obligations to ensure access [34].

Large volumes of pre-purchases of rich countries and scarcity in supply create global access and timely delivery challenges. It may lead to insufficient support for people (about 25% of the world's population) access around the world to COVID-19 vaccines in days and months ahead, which can prolong the pandemic and increase the risk of subsequent mutations of the virus emerging and possibly weaken the effectiveness of existing vaccines [35,36].

3.2. Authorization

Recently, some of the COVID-19 vaccines have been approved or authorized by the U.S. Food and Drug Administration (FDA) for emergency use authorization (EUA) that is a system to simplify the use and accessibility of medical equipment and facilities within public health emergencies, such as the current pandemic (1).

At first, the Pfizer-BioNTech COVID-19 vaccine was authorized by FDA on December 11, 2020, for use in persons aged 16 years and older. In the following, FDA approved the Moderna and the Janssen COVID-19 vaccines on December 18, 2020, and February 27, 2021, respectively, for use in persons aged 18 years and older to COVID-19 prevention (2).

A large number (about 289) of experimental COVID-19 vaccines were being developed on February 3, 2021, that 66 number of them were in different phases of the clinical trial. Among them, only five vaccines – Pfizer-BioNTech, AstraZeneca-Oxford, Moderna, Gamaleya, and Sinopharm-Beijing – have been authorized by WHO or other stringent regulatory authorities as per WHO criteria. Another five vaccines – from Russia, China, India, and Kazakhstan– have been authorized by other regulatory agencies for emergency use [36].

3.3. Timelines

The vaccine production process usually takes between 5 and 10 years to implement various steps from designing to approval and manufacturing. Due to the acute situation of the current pandemic, the national and international protocols for approval and production of COVID-19 vaccines and drugs must be accelerated. To this, the time course of COVID-19 vaccine production has reduced (less than 18 months) in comparison with conventional vaccines [37].

3.4. Production

Normally, only after authorization or approval of a drug by precise regulatory authorities, the pharmaceutical companies start production that may last months to years [38]. There is a fantastical achievement about COVID-19 vaccines that have developed in less than 12 months by several manufacturers, whereas the development of new vaccines usually takes a decade or more [38,39]. Governments and non-profit organizations have invested in clinical trials, construction, and production facilities to provide the rapid roll-out of vaccines (14).

Scaling up production to meet the global requirement is a monumental challenge and requires expansive technology to enable the development of manufacturing capacity. Ten manufacturers have mentioned they will be able to produce 1 billion doses or more this year, while nine developers have set production targets at most 700 million doses each. It is noteworthy that during this period, none of the companies will be able to supply all countries alone [36].

3.5. Prioritization

In the United States, after authorization of a vaccine by the FDA, the Centers for Disease Control and Prevention (CDC) recommends how to prioritize groups for vaccination. For the COVID-19 vaccine, this center recommends that the healthcare personnel and residents of long-term care be given priority in vaccination. The Acting Secretary has commanded that teachers, school staff, and child care workers are qualified for COVID-19 vaccinations on March 2, 2021, and issued a directive to expand COVID-19 vaccine competence to all Americans by May 1, 2021 (2).

Recently, the WHO SAGE (Strategic Advisory Group of Experts on Immunization) values framework has been developed to provide a values foundation for prioritization decisions about specific COVID-19 vaccines and proposes guidance globally on the allocation of these vaccines. In this document, there is a "Values to Priority Groups" section that indicates the values, principles, and objectives for the COVID-19 vaccination. In this section, some groups, such as health care workers, display more than once in the table that indicates their various prominent roles in several values objectives [36].

3.6. Allocation

One of the essential foundations of the COVID-19 vaccination challenge is ensuring adequate global equitable allocation [40]. In April 2020, WHO informed the creation of the COVID-19 Vaccine Global Access (COVAX) Facility, a global allocation mechanism, in partnership with CEPI (Coalition for Epidemic Preparedness Innovations) and Gavi COVAX is a colligated preparation initiative that seeks to provide low-cost vaccines for all countries during the acute phase of the pandemic in 2021. Low-income and middle-income countries can purchase vaccines from COVAX at significantly low prices, whereas high-income countries can get procurement vaccines at higher average prices [36]. According to the COVAX approach, all participating countries would receive an initial proportional allocation of COVID-19 vaccines to cover 20% of their populations, then a follow-up phase to expand coverage to other populations as needed (8).

3.7. Distribution

An ethical, equitable, and efficient distribution of COVID-19 vaccines is imperative to minimize the risk of new variants of the virus and protect the population from SARS-CoV-2 [36,41].

The board of the ASHP (American Society of Health-System Pharmacists) approved some principles for distributing the COVID-19 vaccine on August 25, 2020. ASHP offers effective communication, cooperation, and coordination with local and international public health organizations, regulatory agencies, and health departments to implement a framework for the ethical and equitable global distribution of COVID-19 vaccines. For the effective distribution of COVID-19 vaccines, the organization also proposes the best practices for proper storage methods and the use of vaccines with maximum shelf life, minimized decadence, and no wastage by temperature control, from distribution to administration [41].

4. Vaccination for COVID-19: How COVID-19 vaccines work

While the aim of usual medical care is the treatment of disease after emerging symptoms, vaccines are designed to prevent the development of disease in persons without any symptoms of disease [42]. Vaccine strategy is considered a major public health success in the last century and annually is assessed to rescue 2–3 million people globally [43]. Vaccines train the immune system to recognize and overcome the pathogen safely without the risk of disease occurrence. They stimulate the immune system to recruit lymphocytes and secrete antibodies. Therefore, if an infection occurs, the immune system can overcome the infection [43].

Firstly, vaccines initiate an innate immune response as the first line of defense, and it leads to activation of the adaptive immune response as the second line. However, innate immunity is developed during few hours but has no memory and specification for particular antigens. Adaptive immunity is determined by various specialized lymphocytes and antibodies, which can eliminate pathogens by activating cellular and humoral immunity. Then, immunological memory is created, leading to a more strengthened response following a later encounter with the same antigen. This is a way for long-term protection against infections and the final aim of vaccine strategies [44].

The effectiveness of a vaccine against COVID-19 is demonstrated by its potency to inhibit the disease and its minimal adverse effects in a short time [45]. Immune deficiency negatively affects vaccine efficacy, especially in senescent people, because various factors are associated with their weakened immune systems. These factors also cause different diseases like obesity, metabolic syndrome, type II diabetes, and immune-mediated cancers. The reasons for developing these diseases include reduction of immune cell levels and their function, a weak affinity for antigen recognition, the increased time required for humoral immune responses, and defect of memory cells [42]. Moreover, the administration of immunosuppressive medicines [46] and habitation in low-income countries with a low socioeconomic position lead to more mortality compared with high-income countries [47]. Other important factors that impact the ineffectiveness of the COVID-19 vaccine are the high rate of obesity because of increased secretion of IL-6 and decreased levels of IgG, pneumonia, and parasitic infections [42].

Globally, different types of vaccines are developing against COVID-19 which introduce antigens to the immune system in various manners. These include weakened or inactivated vaccines, viral vector vaccines, protein-based vaccines, and RNA and DNA vaccines [43]. It is demonstrated that weakened or inactivated vaccines have functioned well because of having two required signals to stimulate the immune system: the antigen and the natural adjuvant. The antigen induces a specific adaptive immune response for the special pathogen, and the adjuvants induce the innate immune response via pattern recognition receptors (PRRs) recognizing pathogen-associated molecular patterns (PAMPS) [48]. An antigen could be immunogenic if it contained PAMPS inducing PRRs like Toll-like receptors (TLRs) and viral nucleic acid sensors (RIG-I and cGAS) in antigen-presenting cells. Since dendritic cells are the major type of cells for inducing an adaptive immune response, involvement of different expressed PRRs on a particular subset of dendritic cells leads to activation and migration of these cells to lymph nodes where the lymphocytes of the adaptive immune response are recruited to fight with a particular pathogen [49]. There are some FDA-approved adjuvants for human vaccines such as alum, TLR9, etc. For COVID-19 vaccines, several adjuvants are developing in different clinical trial phases [48]. It is important to mention that adjuvant safety

and purity and limited knowledge of the association of innate and adaptive immune response to combat specific pathogens affect vaccine efficacy negatively [50,51].

5. Types of COVID-19 vaccines

Multiple new and traditional strategies have been adopted in the development of COVID-19 vaccines, and efficiency and potency depend on antigen selection, delivery system, formulation, adjuvants, and immunization routes (such as aerosol, intramuscular (I.M.), and subcutaneous (S.C.) routes) [52]. Until now (April 2, 2021), the number of vaccines for COVID-19 in pre-clinical and clinical testing are 184 and 85, respectively. Based on WHO's draft landscape on April 2, 2021, the most marketed vaccines in the first line are inactivated vaccines, mRNA-based vaccines, non-replicating viral vectors, and then protein subunit and DNA vaccines. In the third line, a VLP (virus-like particles) vaccine was authorized (Fig. 1).

In non-whole cell technologies, studies have mainly focused on S protein. Also, nanotechnology-based formulations have been introduced in current coronavirus vaccine developments for increasing uptake antigen by antigen-presenting cells (APCs) [53]. Approximately all platforms and technologies are embedded based on previous SARS, MERS, animal coronaviruses, Influenza, and Ebola vaccine developments. Furthermore, an advantage over traditional technologies, in subunits, mRNA and peptide vaccines, bioinformatics and immunoinformatic methods have extensively accelerated COVID-19 vaccine development [54]. However, the results of such methods, especially for predicted immunodominant peptides, need to be confirmed by wet-lab experiments [54].

Since the onset of the disease, the technologies that have been introduced for COVID-19 vaccination include mRNA based, recombinant DNA (Full length S protein), viral vector (full-length Spike or S1 inserted in modified adenovirus vector or vaccinia vectors), subunits (full-length Spike, S1, RDB, nucleocapsid), virus-like particles (VLP) (RDB, S or co-expressing of S1, M, and E produced in baculovirus) and protein/peptide vaccines [55–57] (WHO draft landscape, 2020). In comparison, new vaccines have saving time, creating a better financial margin, and do not need isolation and culturing of virus (accessibility and feasibility concerns) and also direct exposure to live virus (safety concerns) [56,57]. Here, we review comprehensively developing technologies and platforms for potential COVID-19 vaccine candidates. Table 1 shows the Covid-19 vaccines that have passed the clinical phases until mid-2021. Table 2.

5.1. Traditional Whole-Cell antigens (WCAs) technologies

If the selected antigen is a traditional WCA, killed/inactivated vaccines or live-attenuated vaccines (LAV) are raised.

5.1.1. Killed (inactivated) vaccine.

The inactivated vaccine, as usual, is made by exposure of the virulent virus to chemical or physical treatments to eliminate virus infectivity. In this vaccine type, the virus as inactivated is injected to induce immune system factors (Fig. 2). By comparing different vaccine platforms and technologies on SARS-CoV-2, the traditional killed vaccine has superiority and is advisable [56], though it may cause changes in immunogenicity, hypersensitivity, possible Th2-bias, and needs large amounts of antigens to elicit antibody responses. Fortunately, most of these disadvantages have been improved by replacing suitable adjuvants, changing the dose of the vaccine, immunization schedules, and route of administration [58,59]. Previously, inactivated vaccine efficacy and potency is proved by successful studies on seasonal influenza and SARS vaccines [58,59].

Large-scale isolation and culture of SARS-CoV-2, similar to the SARS virus by different research groups around the world, has helped to provide the substrate for inactivated vaccines with advantages of easy to prepare, safe, and high-titer production of neutralizing antibodies. The methods used include β-propiolactone, formaldehyde, and ultraviolet (U.V.) radiation [58,60]. Currently, The Chinese Centers for Disease Control and Prevention (China CDC) and several other institutes and universities in China, the USA, and India have successfully isolated SARS-CoV-2 and have started exploits for different vaccine development strategies.

5.1.2. Live-attenuated virus (LAV).

Similar to the SARS vaccine, the LAV candidate vaccine is achieved by isolation and culturing of SARS-CoV-2. The consequences of these low pathogenic mutants include the diminished limited neutrophil influx, minimal lung injury, and increased anti-inflammatory cytokine expressions [61]. LAV vaccines efficiently stimulate T and B cell responses. However, LAVs are sometimes not suitable choices for vaccination due to issues such as virus recombination (especially homologous recombination) and phenotypic or genotypic reversion to a virulent strain [58,62].
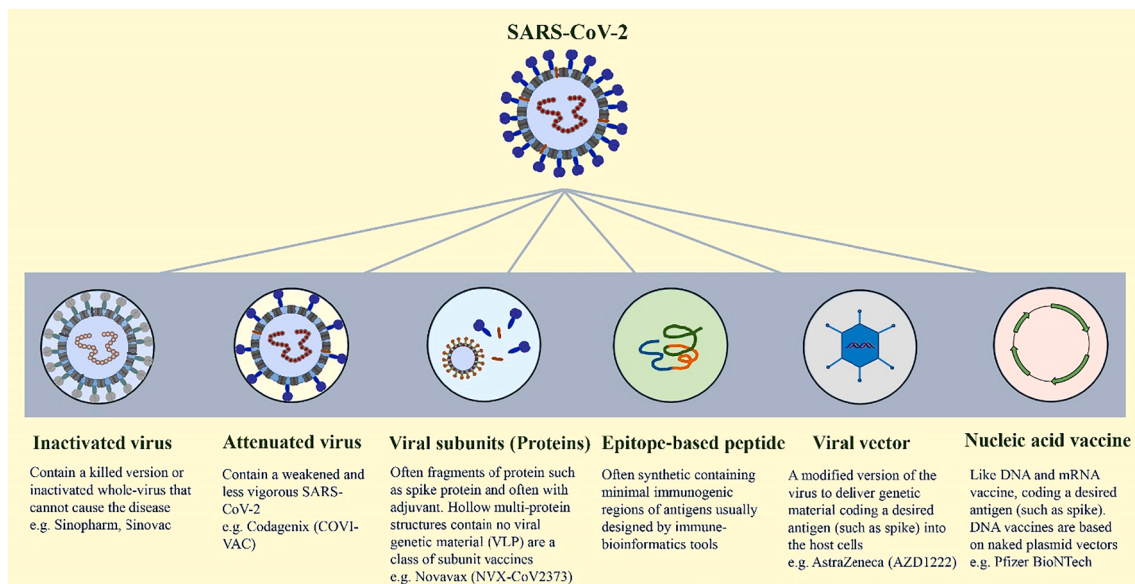

Fig. 1. Types and characteristics of viral vaccines used against SARS-CoV-2.

Table 1
Some COVID-19 vaccines behind the clinical phases until mid-2021.

Name	Company/Sponsor	Country of origin	Type	Phase	Status	FDA approved	Injection route	Stability
Pfizer-BioNTech	BioNTech, Pfizer	United states, Germany	mRNA	2,3	Approved in several countries.Emergency use in U.S., E.U., other countries.	Yes	Muscle injection	Freezer storage only at –25 °C to –15 °C
Moderna	Moderna, NIAID, BARDA, CEPI	United States	mRNA	3	Approved in Switzerland. Emergency use in U.S., E.U., other countries	Yes	Muscle injection	30 days with refrigeration, 6 months at –20 °C
Sputnik V	Gamaleya Research Institute of Epidemiology and Microbiology	Russia	Ad26, Ad5	3	Early use in Russia. Emergency use in other countries.		Muscle injection	Freezer storage
Oxford-AstraZeneca	University of Oxford, AstraZeneca, CEPI	United Kingdom, Sweden	ChAdOx1	2, 3	Approved in Brazil. Stopped use in Denmark. Emergency use in U.K., E.U., other countries.		Muscle injection	Stable in refrigerator for at least 6 months
Convidecia	CanSino Biologics	China	Ad5	3	Approved in China. Emergency use in other countries.		Muscle injection	Refrigerated
Janssen	Johnson & Johnson	United States, Netherlands	Ad26	3	Emergency use in U.S., E.U., other countries. Paused in some countries. Stopped use in Denmark.	Yes	Muscle injection	Up to two years frozen at –20° C, and up to three months refrigerated at 2–8° C
EpiVacCorona	BEKTOP	Russia	Protein	3	Early use in Russia. Approved in Turkmenistan			
Novavax	Novavax, CEPI	United States	Protein	3	United Kingdom, Canada, Australia and South Korea.		Muscle injection	Refrigerated
BBIBP-CorV, Sinopharm	Sinopharm, China National Pharmaceutical Group Corporation	China	Inactivated	3	Approved in China, U.A.E., Bahrain. Emergency use in other countries.		Muscle injection	
CoronaVac	Sinovac	China	Inactivated	3	Approved in China. Emergency use in other countries.		Muscle injection	Refrigerated
Covaxin	Bharat Biotech	India	Inactivated	3	Emergency use in India, other countries.			At least a week at room temperature
CureVac	CureVac, CEPI	Germany	mRNA	3			Muscle injection	At least 3 months at 2–8 °C
ZyCoV-D	Cadila Healthcare	India	DNA	3			Skin injection	Room temperature for three months
ZF2001 (ZIFIVAX)	Anhui Zhifei Longcom Biopharmaceutical Co.	China	Adjuvanted protein	3	Use in Uzbekistan and China			Refrigeration temperatures (2 °C-8 °C)
CoviVac	Chumakov Centre	Russia	Inactivated	3				Refrigeration temperatures (2 °C-8 °C)
Minhai	Minhai Biotechnology Co.	China	Inactivated	3				Refrigeration temperatures (2 °C-8 °C)
AG0302	AnGes	Japan	DNA	3			Skin injection	Over a year at room temperature
GRAd-COV2	ReiThera, Lazzaro Spallanzani National Institute for Infectious Diseases	Italy	Adenovirus	2/3				
ZFSW	Anhui Zhifei Longcom and the Chinese Academy of Medical Sciences	China	Adjuanted RDB	3	Emergency use in China		Muscle injection	
Soberana 02	Finlay Institute	Cuba	Subunit (conjugate)	3	Early use in Cuba, Iran			
CoVLP	Medicago, GSK	Canada, United Kingdom	VLP	3			Muscle injection	Stable in refrigerator
Abdala	Center for Genetic Engineering and Biotechnology	Cuba	RBD	3				
MVC	Medigen Vaccine Biologics, Dynavax Technologies	Taiwan	Adjuvanted protein	3				
QazCovid-in (QazVac)	Research Institute for Biological Safety Problems	Kazakhstan	Inactivated	3	Early use in Kazakhstan			Refrigeration temperatures (2 °C-8 °C)
BIOKANGTAI	Shenzhen Kangtai Biological Products Co	China	Inactivated	3				

Table 1 (*continued*)

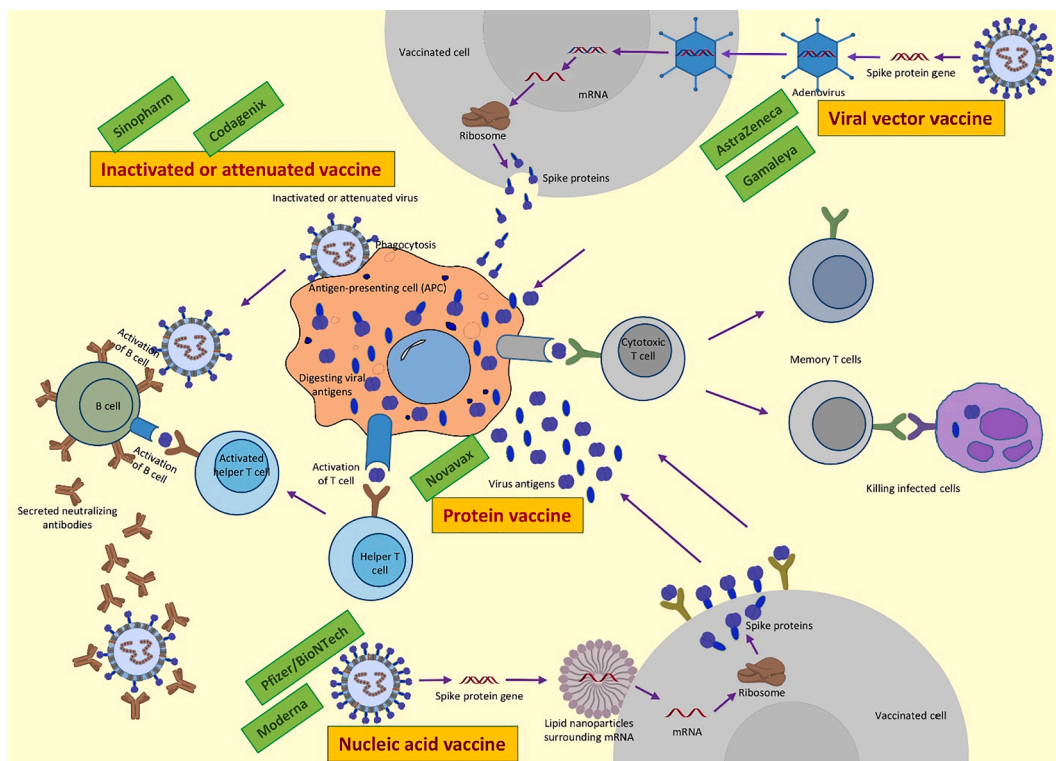| Name	Company/Sponsor	Country of origin	Type	Phase	Status	FDA approved	Injection route	Stability
Valneva	Valneva SE	France	Inactivated	3	Approved for phase III clinical trial in the Philippines Early use in United Kingdom			
COVIran Barekat	Barakat Pharmaceutical Group, Shifa Pharmed Industrial Group	Iran	Inactivated	3				Refrigeration temperatures (2 °C-8 °C)

Fig. 2. Molecular mechanisms of different types of COVID-19 vaccines.

Both inactivated and attenuated virus vaccines have different advantages and disadvantages, and it seems that more attention should be paid to new recombinant vaccines prepared by different strategies. Complexity in the composition of WCAs caused stringent quality control evaluations and longtime screening tests for obtaining strains with low (attenuated) or without pathogenicity. It is important to note that for preparing WCAs vaccines, SARS-CoV-2 needs to be isolated and cultured in cell lines. SARS-CoV-2, and betacoronavirus genus, were isolated and proliferated from the clinical specimens using various cell lines (https://web.expasy.org/cellosaurus/sars-cov-2.html), such as human airway epithelial cells, Vero cell (kidney epithelial cells from monkeys), Vero C1008 (or Vero E6), Vero-CCL81, Huh-7 (human liver cells), human airway epithelial cells, FRhk4 (Fetal Rhesus Kidney-4), Caco-II cell (human colon epithelial cancer cell line), Calu-3 (human lung cancer cell line), LLC-MK2 (a rhesus monkey kidney epithelium cell line and engineered VeroE6/TMPRSS2. Cytopathic effects (CPE) were varied in mentioned cell lines and observed from 24 to 96 h after inoculations. In comparison, VeroE6 expressing TMPRSS2 surface receptor produced higher titers (a tenfold more than other cells), and viral copies were greater than 100 times greater than VeroE6 cells in the supernatant of culture [63]. TMPRSS2 is a transmembrane serine protease that helps in viral entry to Vero cells [63]. COVI-VAC is a single-dose intranasal, live attenuated vaccine against SARS-CoV-2 generated by Codagenix

biotechnology company that has been reported to be in its clinical stages. This vaccine can be easily cultured in cell culture and produced in large quantities [64].

5.2. Subunit vaccines

Virus genome and SARS-CoV-2 gene sequences (especially S) in GenBank facilitate the rapid development of subunit and recombinant vaccine designs. In recent decades, subunit vaccines have shown high safety, consistency in production, induction of both cellular and humoral immune responses, and high-titer neutralizing antibodies. Nevertheless, it requires high cost and facilities, lower and limited immunogenicity, need to repeated (booster) injections and strong adjuvants, and sometimes may fail to yield protective response when tested clinically [55,62,65]. Target antigens for subunit COVID-19 vaccines are full-length S, S1, RDB, and nucleocapsid, formulated with various adjuvants [66,67]. NVX-CoV2373 is a protein-based COVID-19 vaccine candidate produced by the Novavax company and has good efficacy in phases of clinical research [68]. The subunit vaccines are categorized into a few classes.

5.2.1. Virus-like particles or virosomes or pseudo typed virions.
VLPs are hollow multi-protein self-assembly structures without any

genetic component (so defective in replication), imitate genuine viruses, and are obtained by *in vitro* expression in a eukaryotic system [69]. Medicago and Nevada used their experiences with SARS-CoV and MERS-CoV V.L. nanoparticles vaccines. They have suggested virus-like nanoparticle technology for COVID-19, and now their projects are in clinical trial stages. Their methodology is the purification of full-length S protein of COVID-19, which forming ~ 25 nm diameter particles and is combined with Matrix M1 adjuvant. Also, VLP consist of RDB, S, N, or Co-expressing of S1, M, and E structural proteins, which, if expresses in a baculovirus system, can help to multimeric antigen display and preserve virus particle structure but require optimum assembly condition.

5.2.2. Molecular clamp or fusion-protein approach.

This method has been previously developed for class I and class III enveloped viruses such as influenza, respiratory syncytial virus (RSV), SARS, and SARS-CoV-2. In normal, "pre-fusion" S protein on the SARS-CoV-2 surface acts as a metastable fusion protein and facilitates virus entry into the cell. S follows structural rearrangements to a highly stable post-fusion conformation. The pre-fusion conformation of S protein is more important since studies have revealed the pre-fusion conformation of viral envelope fusion proteins covering immunodominant epitopes that are not recognized in the post-fusion conformation. When S protein is removed or prepared by the recombinant method, it partially loses its conformations [70]. The University of Queensland has developed a technique that different proteins or subunits of SARS, influenza and Ebola are stabilized by the innovative molecular clamp method [70,71]. In such a way, S protein (or a part of this protein) will be highly stabilized by attaching the clamp polypeptide (or also called a chimeric polypeptide) to its body to mimic the protein conformations found on the live virus. Clamp polypeptide motif is made up of amino acids in a pattern that repeats itself after every seven residues and must be at least 14 residues in length [70].

5.2.3. Optimized adjuvant vaccines.

The polypeptides alone are not strong immunogenic and need an adjuvant along with repeated administration. Moreover, using an unchanged alum-adjuvant candidate alters the immune response to TH2 cell-like responses that are unpleasant for human defense against COVID-19. Hence, Novavax and Glaxo Smith Kline (GSK) use subunit COVID-19 vaccines along with Matrix-M and AS03 (an oil-in-water emulsifier containing alpha-tocopherol, squalene, and Tween 80) adjuvants, respectively [72–74]. Moreover, CEPI, in collaboration with GSK and the University of Queensland, is investigating a subunit vaccine based on a new AS01 (a liposome adjuvant containing 3-O-desacyl-4′-monophosphoryl lipid A (MPL) or saponin QS-21) or AS02 (an oil-in-water emulsifier containing MPL and QS-21) or AS03 and/or AS04 (an aluminum adjuvant containing MPL) against COVID-19 [75]. It reduces the amount of antigen dose injection and is faster and safer to overcome early clinical development when the protective efficacy of antigen may not be strong enough [76].

5.3. DNA vaccines

DNA vaccines are based on naked plasmid vectors (such as; pVax1TM and pVRC8400) carrying full-length spike or S1 and administrated by intramuscular injection (I.M.) followed by electroporation [77,78]. These have the advantages like easy design, manipulation, preparing and harvesting in large quantities, relative stability, and high safety, triggering both cellular and humoral antigen-specific immunity and producing high-titer neutralizing antibodies. Disadvantages of these vaccines are the need for an efficient delivery system, low immune responses compared with live vaccines, and the possibility of toxicity due to repeated injection doses [77,78]. At least six projects produce a DNA vaccine using the S protein gene against COVID-19 at various stages of the clinical trial to evaluate the safety, tolerability, and immunogenicity of the vaccine [79].

5.4. mRNA based vaccines

Prophylactic RNA vaccines exhibit characteristics of subunit vaccines, DNA vaccines, and live-attenuated. Some time ago, the WHO acknowledged mRNA as a novel and effective therapeutic tool [80]. Nowadays, mRNA vaccines have gained considerable attention as they are easier to design and have short production cycles. They also show a high degree of adaptability, high potency, strong induction of immune responses, low-cost manufacturing, and safe administration [78,81]. However, they are very unstable under physiological conditions. In the mRNA vaccine approach, instead of proteins, mRNA is given to the person that allows the body to manufacture the proteins itself. This leads to bypassing the step of expression or/ and producing pure viral proteins that cause save a lot of time which is usually spent on standardization and ramping up the mass production [80].

Two main types of RNA vaccines are derived from non-replicating mRNA or in-vitro transcribed mRNA (IVT-mRNA) and self-amplifying mRNA or self-replicating mRNA (Rep mRNA) [81]. Conventional mRNA-based vaccines containing 5′ and 3′ untranslated regions (UTRs) encode the antigen of interest. In contrast, self-amplifying RNAs, in addition to the antigen of interest, also encode the viral replication machinery, which leads to intracellular RNA amplification and abundant protein expression (Fig. 3) [81].

Formulation of nanoparticles with an optimal adjuvant plays a crucial role in the efficacy of viral antigens as vaccines. Moderna vaccine candidate, mRNA-1273, is a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes a full-length, perfusion stabilized S protein of SARS-CoV-2 [82]. The Pfizer-BioNTech vaccine (known as BNT162b2) with a demonstrated efficacy rate of over 90 percent is also a mRNA encoding SARS-CoV-2 spike glycoprotein RBD, wrapped in oily bubbles made of lipid nanoparticles. After injection, the particles fuse to cells and release mRNA into the cells (Fig. 2). The mRNA sequence is read and spike proteins are expressed. The protruding proteins will be then recognized by the immune system including antigen-presenting cells (APCs) [83] (Fig. 3). Generally, DNA and mRNA-based vaccines are easier to design and rapidly develop into clinical trials.

5.5. Viral vector-based vaccines

Genetically Modified Viruses (GMVs) vector-based vaccines are comprising one (mainly S or part of it) or more immunogenic SARS-CoV-2 proteins in the context of an attenuated viral backbone (Fig. 2) [84]. Such recombinant vectors are created by replacing the viral structural protein-coding regions with genes of interest (GOI). This vaccine enters cells by the virus and induces strong humoral and cell-mediated immune responses.

Classification of viral vectors is mainly based on genetic material (DNA or RNA), whole particle size, transgenic and packaging capacity, replicative or non-replicative, and cell type tropism [60,84]. Some common GMVs vector-based vaccines in coronaviruses include [60,84]: Adenovirus vectors, Modified Vaccinia Ankara (MVA) vector, Live modified horsepox (LMH) vector, Venezuelan equine encephalitis (VEE), and ChAdOx1 (chimpanzee adenoviral vector 1), and rhabdovirus-based vector. Due to safety concerns, the replication-defective viruses gained more attention for entry to clinical trials.

5.5.1. Adenovirus (Ad) vectors.

The typical characteristics of Ad vectors are large genome size and packaging capacity, double-stranded DNA with icosahedral-shaped capsid, non-enveloped, without integrating into hosts' genome, and ease of manipulation [85,86]. They are used for a variety of pathogens [84,87] and also are effective carriers for the delivery of coronavirus antigens into the host cells to elicit strong immune responses. Ad vectors, in comparison with other viral vectors, have a highly immunogenic profile with an ability to induce both major immune responses in hosts. However, pre-existing neutralizing antibodies (previous infection by
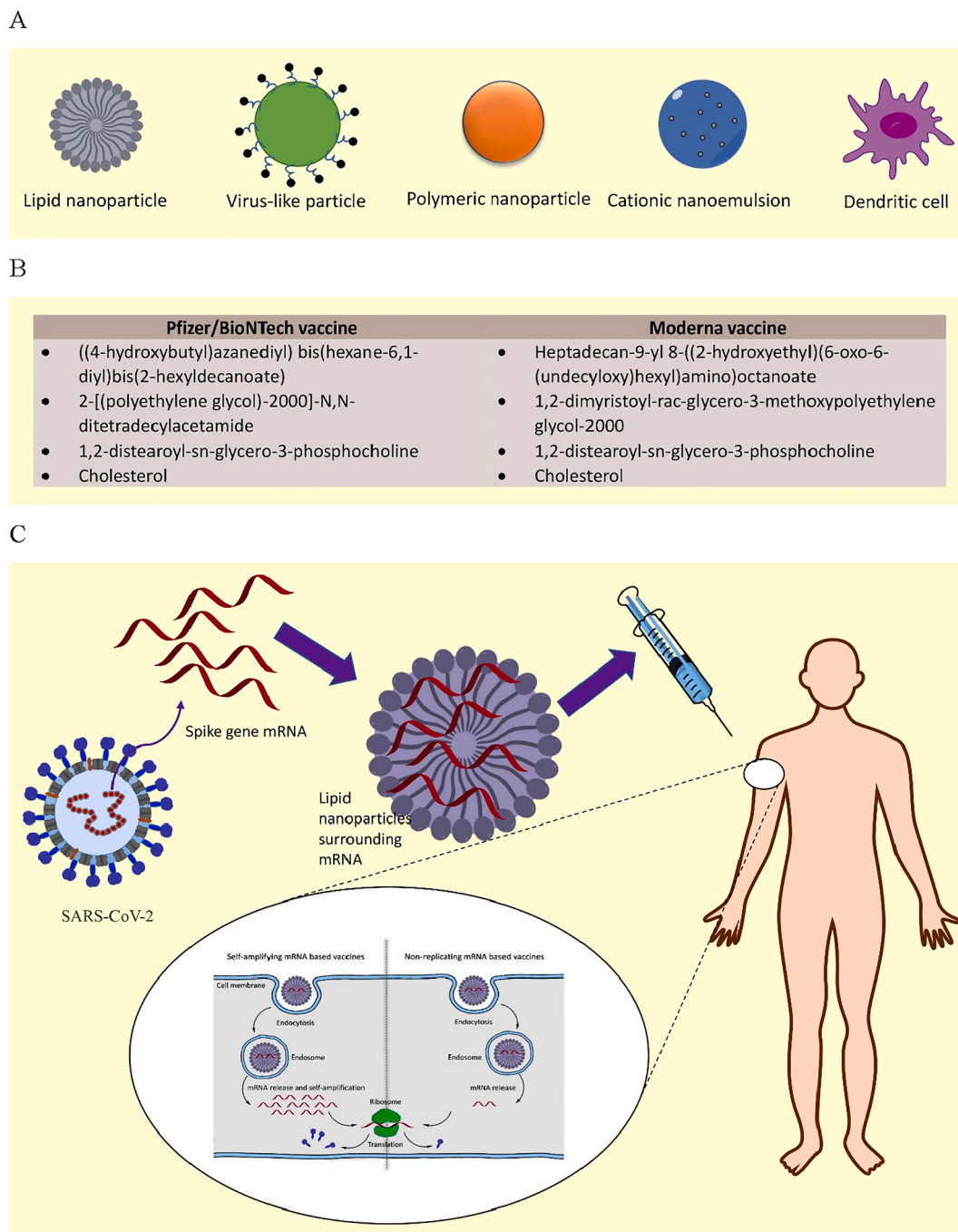
A

B

Pfizer/BioNTech vaccine	Moderna vaccine
• ((4-hydroxybutyl)azanediyl) bis(hexane-6,1-diyl)bis(2-hexyldecanoate) • 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide • 1,2-distearoyl-sn-glycero-3-phosphocholine • Cholesterol	• Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate • 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 • 1,2-distearoyl-sn-glycero-3-phosphocholine • Cholesterol

C

Fig. 3. A. Different types of carrier molecules for mRNA vaccines [83]. B. Constitutes of used LNPs for delivery of COVID-19 vaccines (Retrieved from CAS; A division of the American Chemical Society). C. Delivery and mechanism of action of mRNA vaccine using LNPs [41].

adenoviruses) are a major limitation for clinical application in humans [85]. Sputnik V (Gamaleya Research Institute) is an adenovirus-based vaccine for COVID-19 that uses two adenovirus vectors, adenovirus serotype 26 (Ad26) and adenovirus serotype 5 (Ad5), for the expression of spike protein [88]. Oxford-AsteraZeneka (The University of Oxford partnered with the British-Swedish company AstraZeneca) or AZD1222 is a viral vector vaccine that comprises a non-replicating chimpanzee adenovirus vector (ChAdOx1) and is given by intramuscular injection. This vaccine contains the full-length codon-optimized coding sequence of spike protein [89].

5.5.2. Modified Vaccinia Ankara (MVA) vector
MVA is a highly attenuated non-replicating modified poxvirus vector

that is manipulated to produce exogenous antigens as a delivery vehicle with a high safety profile [90,91]. However, in rare cases, immunization by MVA vector may cause incomplete protection and side effects on the heart.

5.5.3. Live modified horsepox virus vector
In February 2020, Tonix Pharmaceuticals Co. declared collaboration with Southern Research to support the development of a live modified horsepox virus vaccine that carrying S protein for percutaneous administration [92].

5.5.4. Venezuelan equine encephalitis (VEE) virus vector
VEE vector or attenuated alphavirus replicon particles (VRPs) are

another platform that replaces the vector structural genes with genes of proteins of interest (for example, S and N proteins for SARS-CoV) SARS-CoV-2 [93]. It has shown that this type of virus vector vaccine can induce strong humoral and cellular immune responses.

5.6. Synthetic epitope peptide or fusion peptides

These technologies use immune-bioinformatics to predict immunodominant epitopes or experimental results on immunization by different immunogenic peptides. The peptides usually are prepared by chemical synthesis or in a fusion peptide construct by expression vectors. They also can be categorized into subunit vaccines. A study has predicted a set of highly conserved B and T cell epitopes of S and N proteins as peptide vaccine for SARS-CoV-2 in the pre-clinical phase [94].

Another peptide novel technology for strong immune stimulation is using a portion of the MHC class II-associated invariant chain (Ii) (so-called 'Ii-Key') to enhance MHC class II epitope presentation. Moreover, it acts by direct charging of receptor with binding to an allosteric site on MHC class II molecules for loosening their epitope-binding groove [95]. Therefore, the efficiency of cell activation will be increased. Generex Biotechnology Co. proposed Ii-Key technology that linked synthetic MHC class II virus epitopes to a 4-amino acid Ii-Key via a simple polymethylene bridge to the N-terminus to ensure robust immune system activation. Multi-epitopic or polytopic vaccines essentially for desirable immune-stimulation need to be used with potent traditional or novel adjuvants such as MF59 (an oil-in-water emulsion containing squalene), monophosphoryl lipid A, montanide ISA51, sorbitol trioleate. In comparison, the MF59 adjuvant induces the highest neutralizing antibody titer against S protein [85,96].

It is noteworthy that by using the recorded SARS-CoV-2 genomes, bioinformaticians in the immunoinformatic field are now helping to design and development of new generations of COVID-19 vaccines, especially in the area of recombinant DNA and protein/peptide vaccines in a way that *in vitro* and *in vivo* experiments can be performed more

accurately [54,97].

6. Vaccine delivery systems for COVID-19

Although the delivery of vaccines has been described to some extent in previous sections, especially in mRNA vaccines, more details will be provided in two sections separately. There are two critical steps in the vaccine development process, including 1) identification of proper antigen and 2) development of a delivery approach for that antigen to gain cellular and humoral immunity (Fig. 3). The first step has already been explained. The second step, which means developing and choosing a suitable delivery vehicle which is the biggest challenge for vaccine developers and researchers, will be discussed here.

Progress in nanomedicine and vaccine delivery technologies results in non-viral vaccines based on DNA or mRNA. Therefore, delivery vehicles or electroporation devices are needed because of limited cellular uptake and instability of naked mRNA and DNA. Many studies have been performed to evaluate the use of nanoparticles as delivery systems following intranasal administration. It should be noted that the majority of these studies is based on pre-clinical data from small animal but cannot generalized to humans. Generally, nanoparticles are divided into three broad categories: virus-like or self-assembling protein (mentioned above), organic and inorganic nanoparticles (Table 2) [98].

6.1. Organic nanoparticles

6.1.1. Lipid nanoparticles (LNP)

LNPs are one of the delivery systems suitable for encapsulating mRNA using a solid lipid structure. This structure is generally composed of four components, including cationic lipids for mRNA complexity, cholesterol for stabilization of LNP, helper phospholipids that facilitates the formation and intracellular release of mRNA, and PEGylated lipids that decrease unspecific interactions. LNP as non-viral vectors have many advantages as follows: 1) They can efficiently encapsulate and

Table 2
Some system deliveries used for COVID-19 vaccines.

Vaccine form Developer	Vaccine form Developer	Platform	Delivery system	Stage of development
RNA	mRNA-1273, Moderna/NIAID	Perfusion stabilized S protein mRNA encapsulated in LNP	Lipid nanoparticle	Emergency use authorization by the FDA
	BNT-162, BioNTech/ Fosun Pharma/ Pfizer	3 LNP-formulation encapsulated mRNA	Lipid nanoparticle	Emergency use authorization by the FDA
	LNP-n CoVsaRNA, Imperial College London	Self-amplifying RNA packaged into tiny droplets of fat	Lipid nanoparticle	Phase 1 ISRCTN17072692
	LUNAR-COV19, Arcturus/Duke-NUS	Lipid mediated delivery system for self-amplifying mRNA		Phase 1/2 NCT04480957
	CVnCOV/ Curevac	Lipid nanoparticle encapsulated mRNA	Lipid nanoparticle	Phase 2 NCT04515147
DNA	INO-4800, Inovio Pharmaceuticals	DNA plasmid vaccine with electroporation	electroporation	
Protein subunit	Novavax	Full length recombinant SARS-CoV-2 glycoprotein nanoparticles adjuvanted with Matrix M	nanoparticle	Phase 2b 2020–004123-16 NCT04533399
Non-replicating viral vector	AZD1222 (ChAdOx1 nCoV-19)/ University of Oxford/ AstraZeneca	Chimpanzee adenovirus vector displaying Spike protein on its surface	Adenovirus vector	Phase 3 ISRCTN89951424 NCT04516746 NCT04540393 CTRI/ 2020/08/027170
	Ad5-nCoV, CanSino Biological Inc	Adenovirus serotype 5 expressing Spike protein	Adenovirus serotype 5	Phase 3 NCT04526990 NCT04540419
	Ad26 Cov S1, Janssen Pharmaceutical	Adenovirus serotype 26 expressing Spike protein	Adenovirus serotype 26	Phase 3 NCT04505722 NCT04614948
	Gam-COVID-Vac Lyo/ Gamaleya Research Institute	rAd26 + rAd5 expressing Spike protein	Adenovirus serotype 5 and Adenovirus serotype 26	Phase 3 NCT04530396
	GRAd-S ReiThera/ LEUKOCARE	Replicative defective Simian Adenovirus (GRAd) encoding S	Simian Adenovirus	Phase 1 NCT04528641
	Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Non-replicating viral vector Ad5	Adenovirus serotype 5	Phase 1 NCT04552366
Replicating viral vector	Institute Pasteur/Themis/Univ. of Pittsburgh	Measles vector based	Measles	Phase 1 NCT04497298
	Xiamen University	Intranasal flu based RBD	Influenza	Phase 1 ChiCTR2000037782

compress mRNA, 2) LNPs helps intracellular delivery of mRNA by increasing cellular uptake and triggering endosomal escape, 3) The stability of mRNA will be increased by protecting it from degrading in extracellular environments, 4) LNP components are suitable and approved for human usage, and 5) Large scale synthesis of GMP-grade LNP is possible. There are two essential factors in developing an LNP to be an effective delivery carrier for COVID-19 vaccine mRNA. The first one is selecting a suitable cationic or ionizable lipid that could efficiently complex COVID-19 mRNA. DLin-KC2-DMA, DLin-MC3-DMA, L319, TT3, ssPalmE, Acuitas (A9), and Moderna (L5) are representative of the most suitable ionizable lipids [99]. Optimization of cholesterol, helper phospholipids and combination of lipid-PEG long with their relative ratio in LNPs is the second critical factor. Both factors significantly affect on efficacy and implementation of the mRNA vaccine. Based on the fact that "increasing ionizable lipid: mRNA ratio could enhance the efficacy of mRNA delivery," C12-200-containing LNP was optimized for erythropoietin mRNA delivery in which 7-fold improvement in the potential of the vaccine was observed. Similar methods may also be proposed for the optimization of LNP for COVID-19 vaccine delivery.

Among various LNPs, liposomes are one of the lipid-based formulations adapted for intranasal delivery. Liposomes have numerous advantages as follows: 1) Efficacy in encapsulating conjugate constructs, and 2) Feasibility of simple modification that increases liposomes' mucosal and cellular uptake and promotes their biocompatibility. The surface charge of the liposomes plays a critical role in their pharmacokinetic properties [98]. Cationic liposomes are made up of positively charged lipids that are progressively used in gene therapy because of their desirable interactions with negatively charged DNA and cell membrane. As a delivery system, cationic liposomes can be applied for mRNA–based vaccine usage. Studies on cationic liposomes following intranasal administration revealed higher absorption and enhanced bioaccessibility than their negatively charged counterparts [98]. Liu *et al.* used cationic liposomes as a delivery vehicle to develop an adjuvanted nanovaccine with recombinant S1 subunit from SARS-CoV-2 [100].

6.1.2. Polymer-based nanoparticles

Polymeric nanoparticles (NPs) are an attractive delivery system that has unique properties. Synthesis of these nanoparticles is carried out by adding several monomers into different configurations as linear, branched, and 3D networks so that their shape, size, and surface charge could be simply modified. Among various formulations, chitosan attracts a lot of attention due to its non-toxic nature, biocompatibility, and biodegradability in non-toxic products [101]. Since a variety of antigens can be placed in the surface or encapsulated into the core of these polymeric nanoparticles or both, the design and usage of polymeric structures may be a thought-provoking subject to control infectious diseases especially the emerging diseases caused by viruses (e.g., Zika virus, Ebola virus, and the Middle East Respiratory Syndrome Coronavirus) [101].

6.1.3. Dendrimer nanoparticles.

These nanoparticles are symmetric molecules with homogenous structures that are synthesized in highly branched 3D networks and have the potential of encapsulating non-water-soluble agents in their core. Due to strong interaction with viruses, dendrimers showed to have antiviral activity and prevent viral infections. Consequently, they can be an important tool in treating viral infections such as Ebola, HIV, and Influenza virus [98].

6.1.4. Micelles and emulsion.

These are other delivery systems used for mRNA delivery applications. For instance, a micelle system on branched PEI-stearic acid conjugates has been applied to deliver HIV mRNA-based vaccine [102].

6.2. Inorganic nanoparticles

Engineered inorganic nanoparticles are ideal tools because of their ability to act as conventional delivery vehicles. Among these nanoparticles, Gold nanoparticles (AuNPs) have shown a significant role in vaccine development to induce the immune system. They can be adopted and optimized for intranasal delivery [98]. Other inorganic nanoparticles such as carbon nanotubes have also been applied for vaccination [103].

7. COVID-19 vaccine delivery strategies

The vaccination route is so important as it will determine the strength of viral antigens immunogenically and the vaccine's protective immunity and durability [104]. For the respiratory mucosal pathogens like "SARS-CoV-2", the vaccination route needs to adapt innate immunity and neutralizing antibodies [105]. The COVID-19 vaccine is passed through the muscles like current vaccines (parenteral or injectable route of vaccination) used for human immunization [106]. The optimal time for this route is the COVID-19 asymptomatic period which is 2 to 12 days since most of the immune protective factors are in the respiratory mucosa before injection [107,108]. The injectable route of vaccination induces IgG antibodies as the principal vaccine mechanism to protect humans. This route of vaccination is not much effective on T cells and IgA antibodies in the lungs [109].

A respiratory mucosal vaccine strategy is another route that induces the respiratory mucosa directly, and it is very effective in control and clearance of this harmful virus. It induces T cells and antibodies, especially IgG, in respiratory mucosa and macrophages [104]. The recombinant viral vaccines, especially with Ad5, are effective and safe for the respiratory mucosal vaccine [110]. Using nasal delivery in the nanovaccine approach, which mimics the virosome, can prevent virus infection in the respiratory tract. Being needle-free and needing smaller doses than the injectable route are some of the advantages of this strategy. Still, it requires safer platforms for respiratory vaccination and inhalational equipment, limiting factors in public applications [108].

Another developed vaccination technique is the vaccines based on DNA and mRNA used to encode the antigen proteins in the cells, as spike protein in COVID-19. This kind of vaccine induces the DNA or RNA into the cells to produce protein in the cell surface to immunize the body [105]. The biggest challenge of these vaccines is that the target cells should accept the genetic materials. Two common routes apply to deliver the DNA and mRNA to the immune cells: using a carrier of DNA/mRNA to the cells and promoting protein production by using viral vectors [107]. Another delivery system that can be used in the DNA-based COVID-19 vaccine is electroporation, which uses high-voltage electrical pulses to make the cell membrane more permeable for DNA. Inconvenience and pain are the mentioned disadvantages of this method [109]. Also, the required devices and the trained medical staff will make this method less desirable among other vaccination methods [105]. One of the clinical candidates for the COVID-19 delivery system is an experimental DNA vaccine that utilizes the electroporation method. An initial fault of this method is pain and inconvenience at the application site and involuntary muscle contraction, and mild to severe asymptomatic increase in CPK (creatine phosphokinase) level blood of participants. Moreover, needing special equipment along with the necessity of training medical staff will preclude the widespread usage of this technique as a mass vaccination [102,105]. During the IN00-4800 DNA vaccine development, a device named CALLECTRA is used for the delivery of DNA directly into the skin (targeting SARS-CoV-2 S protein).

The mRNA vaccines as a rapid platform to quickly respond to the COVID-19 pandemic are along with some challenges like instability and degradability by RNases both *in vivo* and *in vitro*; also, the mRNA can activate the immune system unfavorably, therefore, cause toxicity and inflammation. The mRNA vaccine needs a severe cold distribution and storage condition, making it very difficult to be widely used [99].

Oral vaccine delivery has become a favorable delivery system due to its safety, patient admission, and reduced cost. The bac-TRL-Spike vaccine candidate developed by Symvivo is a lyophilized gel capsule containing genetically modified probiotic bacteria (Bifidobacteria). This oral vaccine is highly suitable for mass vaccination, and its delivery system is one of two administered COVID-19 DNA vaccines currently evaluating in human clinical trials.

PharmaJet's jet injector is another delivery strategy used for GX-19 DNA vaccine candidates. In this technique, a needle is replaced with a high-pressure, narrow stream of fluid to penetrate the skin. Pharmjet technology has proved to be safe and effective compared to commercial vaccines, including influenza, HPV, and polio vaccines.

Microneedles (MN) is an alternative method for using DNA vaccine transcutaneously, allowing deposition of the vaccine candidate in immune-cell-rich epidermis and dermis and induce humoral and cellular immunity much more than soluble DNA vaccine [111].

It is believed that nanoparticle delivery may help the DNA/RNA vaccines to be more effective; for example, a recent silica nanoparticle, Nuvec®, is one of these types of delivery systems. However, some factors should be considered such as high loading capacity, controlled release with an extended half-life, no interference with the stability of the therapeutic module, no leakage, no toxicity, being biocompatible, having no interference with other therapeutic modules, being easy and, comfortable in the delivery system [105,107]. Both delivery systems, nanoparticles, and electroporation have a big limitation in mass-scale distribution and storage (1). The mentioned problems make DNA/RNA-based vaccines less desirable.

8. Conclusion and future challenges

There are growing efforts to develop an effective vaccine against the recent large COVID-19 outbreak. Due to the conditions created by this pandemic and the request of many governments, many preclinical studies have been conducted on the design of different vaccines. Their number is increasing every day, which has led to the introduction of several vaccines with desirable efficacy in the market. There are various vaccines available today, and fortunately, the introduction of next-generation and high-tech vaccines has provided better conditions for the production of successful vaccines. The most important advantage of these new vaccines is their development based on sequence information. This also can eliminate the need for virus culture. Several vaccines based on nucleic acid and viral vectors have been developed for COVID-19 or are in the pre-clinical stages. Some of these vaccines are more effective than conventional vaccines. Some of these vaccines seem to be able to cover the problems associated with new mutations as well [112]. However, the developments that are now being made for different vaccine platforms will pay off when they can show an even faster and stronger response to a new virus.

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.

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