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Vaccine delivery systems and administration routes: Advanced biotechnological techniques to improve the immunization efficacy

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ABSTRACT

Since the first use of vaccine tell the last COVID-19 pandemic caused by spread of SARS-CoV-2 worldwide, the use of advanced biotechnological techniques has accelerated the development of different types and methods for immunization. The last pandemic showed that the nucleic acid-based vaccine, especially mRNA, has an advantage in terms of development time; however, it showed a very critical drawback namely, the higher costs when compared to other strategies, and its inability to protect against new variants. This showed the need of more improvement to reach a better delivery and efficacy. In this review we will describe different vaccine delivery systems including, the most used viral vector, and also variable strategies for delivering of nucleic acid-based vaccines especially lipid-based nanoparticles formulation, polymersomes, electroporation and also the new powerful tools for the delivery of mRNA, which is based on the use of cell-penetrating peptides (CPPs). Additionally, we will also discuss the main challenges associated with each system. Finlay, the efficacy and safety of the vaccines depends not only on the formulations and delivery systems, but also the dosage and route of administration are also important players, therefore we will see the different routes for the vaccine administration including traditionally routes (intramuscular, Transdermal, subcutaneous), oral inhalation or via nasal mucosa, and will describe the advantages and disadvantage of each administration route.

1. Introduction

Infection caused by microorganisms especially viruses cause serious negative effects on clinical, economic and social events [1–3]. This situation has been seen in previous cases as well as in the most recent one caused by SARS-CoV-2 virus. The COVID-19 pandemic caused serious clinical effects with more than 5 million deaths and more than 200 million infections worldwide [4]. Other chronical or seasonal diseases including AIDS (caused by HIV infection), hepatitis C or seasonal influenza could be more critical [5]. Especially for HIV infection, with more than 38 million total deaths [6] and, the influenza, which can cause up to half million deaths annually [7]. As very important strategy against infection, vaccine has been established. In our previous review, we described the different strategies used for vaccination [1]. Traditional vaccines were based on live attenuated or inactivated pathogens [8,9]. This strategies have been used as vaccine against chickenpox caused by varicella-zoster virus (varicella vaccine), influenza caused by influenza virus (FluMist), diarrhea caused by rotavirus (Rotarix) and more recently, the SARS-CoV-2 vaccines Covaxin and CoronaVac from Bharat Biotech and Sinovac use the same strategy [4]. Live attenuated vaccines causing mild infection, and the body subsequently develops a strong immune response, with immunity persisting for years. However, such traditional vaccines have very critical drawback, namely, the high infection risk due to the potential of live attenuated pathogens to become more virulent [10,11]. The inactivated vaccines are safer than live attenuated, but the induced immune responses can be weaker. Beside the classical vaccination method, we find also the new generation of vaccination methods including recombinant proteins, DNA based vaccines, viral and the most recently the mRNA based method [1]. Vaccines based on the recombinant proteins have been developed against human papillomavirus (HPV) using L1 capsid in virus-like particles [12]. The recombinant protein strategy is also used against SARS-CoV-2,

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currently, there is a study under development that uses different subunits from the recombinant spike protein for immunization [13]. Also, the injection of DNA elicits an immune reaction against viral infections [14,15]. In a previous work of Wolff et al. the author showed that intramuscular injection of nucleic acids resulted in the expression of a protein [16]. Furthermore, the vaccination with plasmid DNA can induce a strong immune response as mentioned previously [17–19]. Recently, Immunization with INO-4800, synthetic DNA-based vaccine against the SARS-CoV-2 spike protein resulted in high expression of this protein, promoting T cell responses and antibodies production, and finally the neutralization of SARS-CoV-2 infection [20]. Another strategy for vaccination uses viral vector, recently, the development of vaccines based on viral vector has yielded encouraging results, and an increasing number of studies have begun to focus on the use of different viral vectors, including *retro*-vector, Aden associated viral vector (AAV) and adenoviral vectors [21–24]. Even in the fighting against SARS-CoV-2, we find different strategies [25]. An example for vaccines used adenoviral vectors strategy were Oxford–AstraZeneca [26], Johnson & Johnson [27] and Sputnik V [28]. In the new generation vaccines, the mRNA-based strategy, is by far the most novel strategy [29–32]. Due to the instability, the negative charge, the transient expression and low delivery efficiency, naked ribonucleic acid RNA has a limited utility as vaccine [33]. Previously, the mRNA vaccine was used in cancer Immunotherapy [34] and also for Zika virus or cytomegalovirus [35, 36]. However, recently, different strategies were developed to increase the stability and the delivery of mRNA into cells, making RNA-based strategies as an efficient methods for vaccination; especial during the COVID-19 pandemic, so that different companies used this method to produce vaccines [25], such as BNT162b2 from Pfizer [32] and mRNA-1273 from Moderna [30].

During the pandemic, it was clear that the development of vaccine is very challenging, not only to produce the vaccine, but also for the establishment of the optimal doses, the reduction of costs and side effect and also to reach the mass production and increase the stability [37]. Furthermore, the choice of administration route, the dose frequency and the age of the patients are also a very important. With increasing age, the immune system ability to develop protective immunity and protect against infection declined progressively. This age-related reduction is caused by the age-related changes in adaptive and innate immune system. Which mean, that in population with increasing age the risk of pandemics is higher [38].

In this review, and based on the experience gained during the last COVID-19 pandemic, we will describe different vaccine delivery systems especially, the most used viral vector, and also the strategies for delivering of mRNA/DNA, using polymer-, lipid-based nanoparticles formulation, hydrodynamic and also using the new tools, namely, the application of cell-penetrating peptides (CPPs). Due to the fact, that the administration route has a high impact on the applied dose and development of immunity, we will also shed light on different routes for the vaccine administration including intramuscular, transdermal, subcutaneous, oral, by inhalation or via nasal mucosa, and will describe the advantages and disadvantage of each administration route.

2. Delivery systems of vaccines

In a previous review, we described the most important method used for vaccination [1]. We also mention the combination of adjuvant with next generation vaccines, especially recombinant protein and mRNA to increase the immunity. Due to the outbreak of COVID-19 pandemic, a global race to develop vaccines against SARS-CoV-2 has been started. Tell 30th Sep 2022, there is 172 vaccines in clinical and 199 in preclinical stages [4]. The technics for producing vaccines are different and start from traditional methods using live attenuated and inactivated virus, until next generation methods including the use of the full or part of the spike protein, adenoviral vectors, DNA, virus like particles (VLP) and mRNA. In this review we will limit to mentioning vaccine using delivering systems, and reaching phase 3 and 4, including strategies used in adenoviral vector (Oxford–AstraZeneca, Johnson & Johnson and Sputnik V), mRNA (BNT162b2 from Pfizer and mRNA-1273 from Moderna), and DNA (INO-4800 from Inovio Pharmaceuticals and nCov vaccine from Zydus Cadila). Furthermore, we will also mention the use of protein subunit platform (NVX-CoV2373) and VLP (CoVLP + AS03) as new promising strategy.

2.1. Replication-incompetent Adeno-vectors (RicAdV)

The idea of using viral vectors for vaccination is not recent, in the year 1980, in one of the first used viral vector vaccine, the authors used vaccina virus to express the hepatitis B antigen [39,40]. Later in the year 2019, a vaccine against Ebola virus has been approved in the USA and Europe for medical use [41,42]. Currently, there are different vectors in preclinical and clinical trials, thus including lentiviruses (HIV), adenoviruses (Modified vaccinia Ankara), poxviruses (horsepox virus), paramyxoviruses (measles virus, sendai virus, newcastle disease virus), rhabdoviruses (rabies virus, vesicular stomatitis virus), herpesviruses (cytomegalovirus) and flaviviruses (Yellow Fever virus) [43–49]. Recently, the outbreak of COVID-19 epidemic accelerated the use of viral vector in the clinical sector, especially that one based on the adenoviral vectors.

Adenoviruses are under the family of Adenoviridae, they are a doubelstranded DNA without envelop and the genome can reach up to 40 kb. There are more than 88 Adenovirus in human [50], further species are also widespread in other animals. Generally, Adenovirus are subdivided into seven HAdV species A to G. However, the species Ad5 (species C serotype 5) are the most important. Due to the large number of target receptors on the host cells, the tropism of Adenovirus is very broad. The HAd2 and HAd5 from the specie C can bind the coxsackie adenovirus receptor (CAR), localized on epithelial and endothelial cells [51]. The species HAd3 binds to CD80/CD86 receptors on the APCs [52] and HAd35 recognize CD46 expressed on different cells [53]. For vaccination, it is possible to use replicating Adenoviral vector. However, for more safety, it is possible to delete the early transcript 1A and 1B, to make the vector replication defective. Further genes could be deleted or transcribed in trans, which allowed the insertion of transgene up to 38 kb [1]. The Adenoviral vectors have also one more important property, namely, the episomal localization of the viral genome in the host cell. This prevent the appearance of other complications such as leukemia development as shown in previous study used retroviral vector for the treatment of children with severe combined immunodeficiency (SCID) [54,55]. The use of RicAdV based on Ad26 have been used previously for vaccination against Ebola [41,42], this vector allowed the production of larger quantities and induced high stimulation of T cell and B cell responses. On the other hand, adenoviral vectors have a critical drawback, caused by the partially neutralization due to the be pre-existing immunity [56].

As we mentioned above, RicAdV are not able to replicate, because a part of their genome has been deleted (Fig. 1B). Early research for the generation of HIV vaccine using HAd5 demonstrated attenuation of immune responses due to pre-existing immunity [57,58]. To overcome the pre-existing immunity, the most vaccines use RicAdV which derived from animal viruses [59] or vector, which are rare in humans such as HAd26 and HAd35 [60]. Another problem for RicAdV is the vector immunity caused by the first dose due to the production of neutralizing antibodies. This is true, especially when the boosting dose is needed. To solve this problem, it is advised to use one vector for the first dose, and a different vector for the boosting dose.

During the COVID-19 pandemic, different vaccine based on adenoviral incompetent vectors were used (Table 1). Thus including ChAdOx1 and AZD2816 from AstraZeneca [59], Ad26.COV2.S from Janssen Pharmaceutical [60], Ad5-nCoV from CanSino [56,61], Sputnik V/Gam-COVID-Vac developed by Gamaleya [62] and GRAd-COV2 from ReiThera [4]. As we can see, there is different strategies used for the

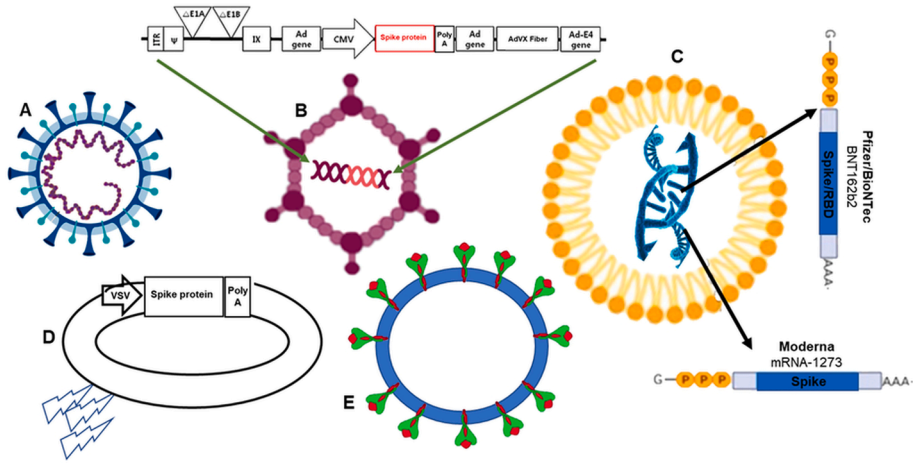

Fig. 1. New generation strategies used for SARS-CoV-2 vaccine development. A, a schematic structure of the SARS-CoV-2 virion; B, replication-incompetent vector vaccines, the genes E1, E3 are deleted, the spike CoV-2 protein or only the RBD are cloned behind CMV promoter, the AdVX fiber could be from different Ad type (5,26or35); C, mRNA vaccines (BNT162b2 and mRNA-1273) with Lipid nanoparticle (LNP); D, DNA vaccines with electroporation and E, virus; like particles (VLPs) display the spike protein on the surface but without genome.

Table 1
Vaccines using replication-incompetent Adeno-vectors in phase 3 and phase 4.

ID	Vaccine platform acronym	Vaccine platform description	Type of candidate vaccine	Number of doses	Schedule	Route of administration	Developers	Phase
1	VVnr	Viral vector (Non-replicating)	ChAdOx1-S – (AZD1222) Covishield Vaxzevria	1–2	Day 0 + 28	IM	AstraZeneca + University of Oxford	4
2	VVnr	Viral vector (Non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector) Ad5-nCoV	1	Day 0	IM	CanSino Biological Inc./Beijing Institute of Biotechnology	4
3	VVnr	Viral vector (Non-replicating)	Recombinant COVID-19 vaccine (adenovirus type 5 vector) for Inhalation (Ad5-nCoV-IH)	1	Day 0	IH	CanSino Biological Inc./Beijing Institute of Biotechnology	4
4	VVnr	Viral vector (Non-replicating)	Gam-COVID-Vac Adeno-based (rAd26-S + rAd5-S) – Sputnik V COVID-19 vaccine – Sputnik-Light Vector Vaccine (rAd26 platform)	2	Day 0 + 21	IM	Gamaleya Research Institute; Health Ministry of the Russian Federation	3
5	VVnr	Viral vector (Non-replicating)	Ad26.COV2.S	1–2	Day 0 or Day 0 + 56	IM	Janssen Pharmaceutical Johnson & Johnson	4
6	VVnr	Viral vector (Non-replicating)	GRAd-COV2 (Replication defective Simian Adenovirus (GRAd) encoding S)	1	Day 0	IM	ReiThera + Leukocare + Univercells	2/3
7	VVr	Viral vector (Replicating)	DelNS1-2019-nCoV-RBD-OPT1 (Intranasal flu-based-RBD)	2	Day 0 + 28	IN	University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy	3
8	VVr	Viral vector (Replicating)	rVSV-SARS-CoV-2-S Vaccine (IIBR-100) with antigens from SARS-CoV-2, with or without GM-CSF	1	Day 0	IM IN	Israel Institute for Biological Research National Institute of Health Research and Development; Ministry of Health Republic of Indonesia	2/3
9	VVnr	Viral vector (Non-replicating)	BBV154, Adenoviral vector COVID-19 vaccine	1	Day 0	IN	Bharat Biotech International Limited	3
10	VVnr	Viral vector (Non-replicating)	AZD2816; adenoviral vector ChAdOx platform and based on the Beta (B.1.351) variant	2	Day 0 + 28	IM	AstraZeneca + University of Oxford	2/3
11	VVr	Viral vector (Replicating)	NDV-HXP-S; A Live Recombinant Newcastle Disease Virus-vectored COVID-19 Vaccine	1	Day 0	IN	Sean Liu, Icahn School of Medicine at Mount Sinai	2/3
12	VVnr	Viral vector (Non-replicating)	Convidecia Vaccine (Ad5-nCoV). Bivalent Recombinant COVID-19 Vaccine (Adenovirus Type 5 Vector)	2	Day 0 + 21	IM	CanSino Biologics Inc.	3

production of vaccine against COVID-19. However, A better understanding of vector-induced immunity by human or animal vectors is needed to could develop new viral vaccines and to evaluate their use in repeated booster doses.

2.1.1. ChAdOx1 nCov-19

ChAdOx1 nCoV-19 AZD1222 and AZD2816 vaccines are based on replication-deficient chimpanzee adenovirus serotype Y25 [59,63,64] and it encoding the spike (S) protein of SARS-CoV-2, based on the first published full-length sequence (AZD1222) and the Beta (B.1.351) variant (AZD2816). Both vaccines were developed by AstraZeneca, the University of Oxford, and the Serum Institute of India (Table 1). The vaccine ChAdOx1 nCoV-19, expresses the spike protein in full-length [63] and it was administered 2 times intramuscularly with a dose of $2.5x10^{10}$-$5x10^{10}$ viral particles and an interval of 28 days between the prime and boost dose. The vaccine showed protection from disease and viral replication in the lung [25]. The efficacy of the vaccine reached 62 % when patients received two standard doses of $5x10^{10}$ viral particles. Interestingly, the efficacy was higher and reached 90 % when a low dose ($2.5x10^{10}$) was applied followed by a standard dose [65]. In previous study, immune cell activation against SARS-CoV-2 and neutralizing antibodies were measured [66,67]. After vaccination, the characterization of $CD4^+$ and $CD8^+$ T cells in patients between 18 and 85 years showed that both $CD4^+$ and $CD8^+$ cells were increased after receiving the booster dose [66,67]. Also, the neutralizing antibodies including natural killer cell activation, complement activation and antibody-dependent neutrophil/monocyte phagocytosis were increased after second dose [67].

The observed side effects were increased temperature, fatigue and headache. The booster dose showed less side effects and was better tolerated. In a phase 3 study, the overall estimated efficacy reached 74.0 %, with low appearance of serious adverse events [68]. Measurement of neutralizing antibodies showed increment after the first dose and increased further when measured 4 weeks after the second dose [68]. The relatively low efficacy and the better toleration after second dose could be caused by the pre-existing adenovirus immunity after the first immunization. In this context, in a recent study about immunogenicity, and reactogenicity to the ChAdOx1 nCoV-19 vaccine, the author showed that, persons without preexisting adenovirus immunity presented a higher frequency of reactogenicity and increased immune response to ChAdOx1 nCoV-19 [69]. Later, and due to the appearance of new virus strains, AstraZeneca developed another vaccine (AZD2816) using the same ChAdOx1 viral vector and based on the Beta (B.1.351) variant (Table1).

2.1.2. Ad26.COV2.S

This is another RicAdV vaccine from Janssen Pharmaceutical (Johnson & Johnson), The Ad26.COV2.S vaccine is based on replication-incompetent human adenovirus type 26 vector (Table 1) encoding full-length SARS-CoV-2 spike protein in a prefusion-stabilized conformation [60,70]. The virus particles were administered 1 time intramuscularly with a dose of $5x10^{10}$ or $1x10^{11}$ viral particles [70]. The study showed that the Ad26.COV2.S vaccine protect against moderate to severe–critical COVID-19, the efficacy reached 66.9 % and 66.1 % with onset at 14 and 28 days after administration respectively. The Ad26.COV2.S vaccine induced robust neutralizing antibody responses [60] and induction of T cell responses against different variants of SARS-CoV-2 [71–73]. In the same study [60], the author showed that the other seven constructs tested were less effective, however, they all induced part protection with no enhanced disease.

2.1.3. Ad5-nCoV

The company CanSino Biological Inc. and Beijing Institute of Biotechnology developed a Ad5-nCoV vaccine based on replication-incompetent human adenovirus type 5 vector (Table 1) and encoding full-length SARS-CoV-2 spike protein [56,61]. This vaccine was licensed at the beginning for the use in the Chinese military. The virus particles were administered 1 time intramuscularly with a dose of $5x10^{10}$ or $1x10^{11}$ viral particles. T cell responses using interferon-γ ELISA and neutralizing antibody levels were tested 28 days after vaccination. The antibody responses were 1:18.3 and 1:19.5. The T cell responses reached 11 and 10 spot-forming units (SFU) per 10^5 peripheral blood mononuclear cells (PBMCs) for the $5x10^{10}$ and $1x10^{11}$ dose respectively. Interestingly, it was found that elderly person and patients with pre-existing immunity to AdV5 have lower immune responses after vaccination. This attenuation of immune responses due to pre-existing neutralizing antibodies, have been demonstrated previously in Ad5 vectored HIV vaccine trials [57,58]. Concerning the side effects after vaccination, 50 % of the patients have fatigue, fever and headache especially the higher dose. Moreover, 1 % of individuals in the low-dose group and 9 % of individuals in the high-dose group have grade 3 adverse reaction.

2.1.4. rAd26-S + rAd5-S

Other vaccine which are also developed on the basis of Replication-incompetent Adenoviral vectors is Sputnik V also known as Gam-COVID-Vac (Table 1). The vaccine is developed by Gamaleya Research Institute/Health Ministry of the Russian Federation and is based on rAd26-S/rAd5-S as vectors for the expression of the SARS-CoV-2 spike protein [74]. This vaccine takes the vector immunity caused by the production of neutralizing antibodies in consideration and try to solve this problem using one vector for the first dose, and a different vector for the boosting dose. Only this vaccine uses the two varying serotypes strategy and the two varying serotypes are given 21 days apar with a dose of $1x10^{10}$ and $1x10^{11}$. The interim results from a phase 3 shows a strong protection across all participant from age 18–60 years [75]. The published data showed also a promising safety results and indicates that the immune response was consistent with the protection. The vaccine efficacy was more than 91.6 % even in participants older than 60 years [75]. The efficacy against severe or moderate COVID-19 was 100 %. For the analysis of humoral immune response, 42 days after vaccination, the serum samples were analyzed for the presence of antibodies specific to the receptor-binding domain of SARS-CoV-2S, the study found the RBD-specific IgG in 98 % of the vaccinated persons. Serious adverse events were recorded in 68 participants, however, none of them were considered associated with vaccination.

All vaccines we described above use replication incompetent adenoviral vector systems (Fig. 1). However, there are also other viral vectors in use including human parainfluenza virus vectors, modified vaccinia ankara (MVA), sendai virus and adeno-associated virus [4,56,59–61,63,76]. Other vaccines used replication competent vector are also in development (Table 1), however these vaccines are less advanced compared to replication incompetent adenoviral vectors.

2.2. Non-viral vectors

Duo to the negative charge, instability, the transient expression and the low transfection efficiency naked ribonucleic acid (RNA) has a limited ability as system for vaccination, [33]. At the beginning, the mRNA vaccination has been used in cancer Immunotherapy [34] and also for Zika virus or cytomegalovirus [35,36]. However, recently, different strategies were developed to increase the stability and the delivery of mRNA into cells using different methods including Lipid-Based Systems and Polymer- Based Systems, making RNA-based platform the most efficient method for vaccination, and conducting to development of different vaccines such as BNT162b1 from Pfizer [32] and mRNA-1273 from Moderna [30] (Fig. 1C) (Table 2). The other delivery system is based on DNA (Fig. 1D), this system has high stability, and long expression. However, the strategies based on DNA as cargo need an additionally step namely, the translocation into the nucleus. There are few research groups used DNA strategy for vaccination, recently, immunization with INO-4800, synthetic DNA-based vaccine

Table 2
Vaccines using mrna and lnp in phase 3 and phase 4.

ID	Vaccine platform acronym	Vaccine platform description	Type of candidate vaccine	Number of doses	Schedule	Route of adminis tration	Developers	Phase
1	RNA	RNA based vaccine	mRNA-1273 (Spikevax)	2	Day 0 + 28	IM	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	4
2	RNA	RNA based vaccine	BNT162b2 (3 LNP-mRNAs), also known as "Comirnaty"	2	Day 0 + 21	IM	Pfizer/BioNTech + Fosun Pharma	4
3	RNA	RNA based vaccine	CVnCoV Vaccine	2	Day 0 + 28	IM	CureVac AG	3
4	RNA	RNA based vaccine	SARS-CoV-2 mRNA vaccine (ARCoV)	2	Day 0 + 14 or Day 0 + 28	IM	WestVac Biopharma Co., Ltd. Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	3
5	RNA	RNA based vaccine	PTX-COVID19-B, mRNA vaccine	2	Day 0 + 28	IM	Providence Therapeutics	3
6	RNA	RNA based vaccine	mRNA-1273.351. A lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of the SARS-CoV-2B.1.351 variant.	3	Day 0 or Day 0 + 28 or Day 56	IM	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	4
7	RNA	RNA based vaccine	DS-5670a, coronavirus-modified uridine RNA vaccine (SARS-CoV-2)	2	NR	IM	Daiichi Sankyo Co., Ltd.	2/3
8	RNA	RNA based vaccine	HDT-301: Self-replicating mRNA vaccine formulated as a lipid nanoparticle. NA MCTI CIMATEC HDT	2	Day 0 + 28	IM	SENAI CIMATEC	2/3
9	RNA	RNA based vaccine	mRNA-1273.211. A multivalent booster candidate combining mRNA-1273 plus mRNA-1273.351.	1	Day 0	IM	ModernaTX, Inc.	2/3
10	RNA	RNA based vaccine	ARCT-154 mRNA Vaccine	2	Day 0 + 28	IM	Arcturus Therapeutics, Inc.	3
11	RNA	RNA based vaccine	Coronavirus mRNA vaccine (LVRNA009)	2	Day 0 + 28	IM	AIM Vaccine and Liverna Therapeutics	3
12	RNA	RNA based vaccine	mRNA GEMCOVAC-19 (COVID-19 vaccine)	2	Day 0 + 28	IM	Gennova Biopharmaceuticals Limited	2/3
13	RNA	RNA based vaccine	mRNA-1273.214 (Booster)	2	Day 0 + 55	IM	ModernaTX	3

against the SARS-CoV-2 spike protein resulted in high expression of this protein, promoting T cell responses and antibodies production, and the neutralization of SARS-CoV-2 infection [20]. Finally, we want to describe the last strategy, which use VLP for vaccination, such as the CoVLP. This is an enveloped, coronavirus-like particle produced in plants and displayed SARS-CoV-2S (ancestral Wuhan strain) and is adjuvanted with AS03 [77] and ABNCoV2 [78]. The DNA and VLP vaccines were the last to arrive in the corona vaccine race, same of them reached now the phase 3 in clinical trial.

2.2.1. *RNA vaccines*

2.2.1.1. mRNA-1273. RNA vaccines are the newest vaccination strategies, which deliver the genetic information for the target antigen instead of delivering the antigen itself (Fig. 1 C). The mRNA-1273 was the first candidate vaccine, it needs only 86 days after identification of the virus. This was possible because the mRNA vaccine needs only the genome sequence compared to other methods [79]. The vaccine mRNA-1273 was developed by Moderna and the Vaccine Research Center (VRC) at the National Institutes of Health (Table 2). The mRNA codes for the full-length spike protein with two stabilizing mutations, has been formulated and delivered in lipid nanoparticles (LNPs) [31]. In the study, three doses with 25 μg, 100 μg and 250 μg were used for vaccination and the interval between prime and boost regime was 4 weeks [80]. The participant's age was between 18–55 years and later expanded until 71 years and more. The phase 1 study found increasing titer of neutralizing antibodies after the second dose. Measurement of T cell population showed high $CD4^{+}$ responses in the groups with 25 and 100 μg doses. After vaccination minor side effects have been registered such as headaches, chills, fatigue and localized pain but there is no major adverse effect [80]. Interestingly, after the second dose, there is more adverse events, more than 21 % of the participant received the 250 μg dose reported severe adverse events, therefore, this dose was not used in the further trials [80,81]. In the Phase 3 trial, after analysis of 30351, there were 11COVID-19 cases in mRNA-1273 group (n = 15181) versus 185 COVID-19 cases in the placebo group (n = 15170), representing a vaccine efficacy of 94.1 % [82]. Immunogenicity data up to 3 months after the booster vaccine for the 100 μg dose has been made and it showed, that the vaccine produced high levels of binding and neutralizing antibodies, which maintained high [83].

2.2.1.2. BNT162b1 and BNT162b2. Both vaccines are from Pfizer, in collaboration with BioNTech. As like the vaccine from Moderna, this vaccine is based on mRNA technology which is delivered in LNPs (Fig. 1C) (Table 2). In the case of the BNT162b1 and in order to increase the immunogenicity, the SARSCOV-2 RBD, was trimerized by adding a T4 fibritin foldon domain. For the BNT162b2, the mRNA encoding the full-length SARS-CoV-2S with to 2 proline mutation as modification [84]. In preclinical study, 7 days after the intramuscular (IM) application of the second dose, the vaccine caused dose-dependent antibody response sufficient for inhibition of the virus entry, the vaccination showed lung protection and low tissue inflammation [84],especially for BNT162b2 which showed a more favorable safety profile. In clinical trial, the BNT162b2 vaccine was applied in three doses started from 10 μg, 30 μg and 100 μg with a 21-days interval. The most commune adverse effect after the first dose was fever, however, other systemic adverse effects were dose-dependent, especially in the group with 100 μg for which headache, fatigue and chills were seen in 50 % of

individuals. For this reason, this group don't receive the boosting dose. In the Phase 3 trial the participants aged 16 and older received 2 doses of 30 μg IM with 21 days interval. Participants had a median of safety data for at last 2 months after boosting dose. After analysis of 43448, there were 8 COVID-19 cases in BNT162b2 group (n = 21720) versus 162 COVID-19 cases in the placebo group (n = 21728), representing a vaccine efficacy of 95 % [32]. The efficacy level was observed in all subgroups, Including, gender, age, ethnicity, and comorbidities. For the systemic side effects such as fatigue, localized pain and headache, all events were for short time and mild. There were low serious adverse effects which were similar as in the placebo groups [32].

2.2.2. DNA vaccines

The next type is the DNA vaccines, these are based on plasmid DNA, the advantage of this technology is the stability of DNA, the long expression of the gene and the most important advantage is the possibility of large-scale and production in *Escherichia coli*. However, the DNA based vaccines have also a serious drawback, due to the negative charge of DNA, it is not possible for DNA to enter the cells, furthermore, the translocation of the DNA in the nucleus is needed. For the administration of the DNA, delivery devices such as electroporators are very important (Fig. 1D). In previous works, there are different researches used DNA vaccines and showed significant immune response [85–89]. Recently, there are different DNA vaccine candidates against SARS-CoV-2 (Table 3), same of them are in different clinical trial phases [4]. The most advanced DNA vaccine is INO-4800, this vaccine targeting the full-length Spike antigen of SARS-CoV-2. INO-4800 was tested in 401 participants who received 2 doses of 1 mg or 2 mg intradermally (ID) followed by electroporation (EP) using electroporators (CELLECTRA® 2000), the interval between the two doses was 28 days. INO-4800 vaccine induced a balanced immune response including both T cells and B cells response [20,90,91]. Both doses (1and2mg) regimen appeared to be well-tolerated and safe in all adult patients. The adverse event registered in the participant were Grade 1 and 2 and there was no difference between the two doses, furthermore, the boosting dose did not increase the adverse event. However, the level of produced neutralizing antibody and the T cell immune responses were significantly higher in the 2 mg dose group versus the 1 mg dose group. Therefore the 2 mg dose have been sectioned for further study [91].

2.2.3. VLP vaccines

According to the World Health Organization report, until 30th. Sep. 2022, there were 371 candidates vaccines, with 172 vaccines in different clinical phases and 199 vaccines in the preclinical stage [4]. For vaccines in the clinical phases, eleven platforms were used including 55 vaccines (32 %) with protein subunit platform, 40 vaccines (23 %) with RNA platform, 23 vaccines (13 %) with a viral vector non-replicating (VVnr) platform, 22 vaccines (13 %) with inactivated virus platform, 16 vaccines (9 %) with DNA platform and 6 vaccines (4 %) with VLP platform. Other platform were less presented such as live attenuated, viral vector replicating (VVr), VVr plus antigen presenting cell, VVnr plus antigen presenting cell and bacterial antigen-spore expression vector with around 1 % for each platform [4]. In this part, we will describe the VLP as vaccine delivery systems.

The VLP could have different structures [92–94], the reason for using the VLP platform comes from its susceptibility to cause excellent immune response including the induction of specific and non-specific responses [92,95]. The VLP platform is similar to the traditional vaccines, but they don't have the ability to replicate due to the absences of viral genome, which make the platform safer as vaccine (Fig. 1E). Another important advantage of the VLP platform is the possibility of producing it in large quantities.

The CoVLP + AS03 is a plant-based vaccine and is developed by Medicago and GlaxoSmithKline (GSK). This vaccine, has been described previously [96] and it displayed the full-length, prestabilized S glycoprotein trimers from SARS-CoV-2 and is expressed in *Nicotiana benthamiana* [97]. In the plant leaves, the expression of the SARS-CoV-2 S protein leads to the formation of VLP with 100 nm-150 nm. After harvesting and purification, the particles are stable for more than 6 months at 2–8 °C.

In the phase 3 clinical trial, 24,141 persons participated in the trial have a median age of 29 years. The vaccine group received 3.75 μg of CoVLP combined with AS03 in a final volume of 0.5 ml. The placebo group received 0.5 ml of phosphate-buffered saline with polysorbate-80. Both the placebo and vaccine were injected in two doses with an interval of 21 days. Analysis of the efficacy showed 69.5 % (95 % confidence interval [CI], 56.7 to 78.8) against any symptomatic COVID-19. In a post hoc analysis, the efficacy reached 78.8 % (95 % CI, 55.8 to 90.8) against moderate-to-severe disease, and no severe cases of COVID-19 occurred in the vaccine group. The registered adverse events were transient and mostly mild or moderate and were more frequent in the vaccine group versus the placebo group; also the local and systemic adverse events were higher in the vaccine group versus control group and reached 92.3 % and 45.5 % of participants for the local and 87.3 % and 65.0 % for systemic, respectively [97].

2.2.4. Protein subunit vaccines

With the spread of COVID-19 pandemic, the scientific community was exposed to several challenges, the most important of which was developing an effective vaccine in record time. The technology used for vaccine production starting from the inactivated virus vaccines, protein subunit, non-replicating viral vector, DNA, VLP, to the latest more

Table 3
Vaccines using dna and vlp and are in phase 3.

ID	Vaccine platform acronym	Vaccine platform description	Type of candidate vaccine	Number of doses	Schedule	Route of administration	Developers	Phase
1	DNA	DNA based vaccine	INO-4800 + electroporation	2	Day 0 + 28	ID	Inovio Pharmaceuticals + International Vaccine Institute + Advaccine (Suzhou) Biopharmaceutical Co., Ltd	3
2	DNA	DNA based vaccine	AG0301-COVID19	2	Day 0 + 14	IM	AnGes + Takara Bio + Osaka University	2/3
3	DNA	DNA based vaccine	nCov vaccine	3	Day 0 + 28 + 56	ID	Zydus Cadila	3
4	DNA	DNA based vaccine	GX-19N	2	Day 0 + 28	IM	Genexine Consortium	2/3
5	VLP	Virus like particle	Coronavirus-Like Particle COVID-19 (CoVLP)	2	Day 0 + 21	IM	Medicago Inc.	3
6	VLP	Virus like particle	ABNCoV2 capsid virus-like particle (cVLP) +/- adjuvant MF59	2	Day 0 + 28	IM	Radboud University	3

recent RNA vaccines. The most of the vaccines use the SARS-CoV-2 spike (S) protein, usually based on the ancestral Wuhan strain, as an antigen [30,32,62,65]. Which has a critical drawback represented by development of variants which impact virulence or susceptibility to vaccine-induced immunity. There are different vaccines in the clinical phases, which are based on protein subunit platform (Table 4) such as NVX-CoV2373. This COVID-19 vaccine uses a trimeric full-length SARS-CoV-2 spike glycoprotein assembled into nanoparticles and co-formulated with a saponin-based adjuvant (Matrix-M™) [98]. The adjuvant increases the activated B cell, T cell and APC populations. As well as recruit and enhance the frequency of $CD8^+$ and $CD4^+$ neutralizing antibodies [99]. The NVX-CoV2373 vaccine was co-developed by Novavax and the Coalition for Epidemic Preparedness Innovations foundation and is stable at 2–8 °C [98,100]. In phase 1–2 trial, participants were randomly assigned and each one received one or two intramuscular doses of 5 μg (low dose) or 25 μg (high dose) NVX-CoV2373 and/or placebo in a one-dose, 21 days apart. The 5-μg NVX-CoV2373 induced robust geometric mean titer (GMT) and wild-type virus neutralizing antibody (981 and 2201 titers) responses in older and younger participants respectively [100]. Vaccination with 5 μg and 25-μg NVX-CoV2373, caused local adverse events after the first dose but only for short period (median of 2 days) across both age groups. Further side effects were fever in less than 2 % of vaccine recipients and around 20 % muscle pain of short duration. After the second vaccination with 5-μg and 25-μg dose, the side effects of short duration were fatigue (36 % and 43 %), muscle pain (31 % and 41 %), headache (30 % and 34 %), and malaise (26 % and 30 %) [100]. In phase III clinical trial a post hoc analysis identified non-Alpha variant in 29 individuals and the Alpha variant in 66 participants. The vaccine efficacy reached 96.4 % against non-B.1.1.7 variants and 86.3 % against the B.1.1.7 variant [101].

2.3. Non-viral vectors as delivery systems

During the COVID-19 pandemic, different methods were used, thus include traditional method such as inactivated or live attenuated virus, as well as the use of replicating or non-replicating pseudo-virus. For the new strategies such as DNA and RNA, and duo to low transfection efficiency of naked ribonucleic acid, there is a need for delivery system. Recently, different strategies were developed to increase the stability and the delivery of mRNA and DNA into cells using different methods including lipid-based systems like lipid nanoparticles, polymer-based systems like polymeric nanoparticles and also the new powerful tools which is based on the use of cell-penetrating peptides (CPPs) [102]. Altogether, making RNA-based strategies one of the most efficient methods for vaccination. There are few advantages to use non-viral vector for vaccine delivery, this includes the efficacy, safety and protection from degradation [103]. After the penetration of the non-viral vector loaded with the antigen material thorough the plasma membrane, the carrier DNA, RNA or peptide could be trapped in the endosome and degraded bevor the cargos exert their effects. There are different strategies for the penetration of non-viral vector including CPP [104] and also different strategies for endosomal escape [105]. In previous works, several system have been developed [104], one system for the endosomal escape is based on the lower pH of the endosome compared to the cytoplasm (pH 5 versus pH 7). In previous work of Plaza-GA et al., the authors used Listeriolysin O toxin-coated gold nanoparticles in which they substitute an alanine with histidine at position 311 in the listeriolysin O peptide. This change caused pore formation at $pH < 6$, which take place only in the endosome [106]. Beside the pore formation strategy [106], there are other strategies including membrane budding, membrane disruption and Proton sponge effect [107].

2.3.1. Lipid-based systems

For the applications of mRNA, delivery carriers are used to protect the mRNA from degradation by RNases and to increase the immunogenicity [108]. In this regard, systems lipids-based system and polymers-based system have been previously reviewed [109–112]. There are different types of lipids with variable functions, for in vivo delivery and endosomal escape, the ionizable lipids are the favorite, however, for the mRNA complexation and transport, the cationic lipids are the first choice. To improve the LNPs stability, biodistribution and tolerability, other components could be added such as cholesterol, phospholipids and PEGylated [111,113,114]. During the recent COVID-19 pandemic, there are two vaccines used this type of NPs, Pfizer-BioNTech BNT162b2 and Moderna's mRNA-1273 [30–32,115] (Fig. 1C). For the delivery of both vaccines. The NPs used for the complexation and transport of mRNA are composed of ionizable lipids, furthermore, other component have been added such as cholesterol to increased NPs stability, PEGylated lipid to increase NPs blood circulation time and phospholipids for structural support. As we mentioned above, the NPs encapsulate modified mRNA encoding for the spike protein of SARS-CoV-2 virus [116,117], which allowed interaction with ACE2 receptor and the penetration of the host cells causing infection [118]. Clinical study demonstrated the safety without significant systemic or local toxicity after two doses application [30,32]. In previous study, LNPs were used in mRNA vaccines against Ebola [119], Dengue [120],Powassan [121] and Zika [122] and shown promising results. Another type of Lipid-Based Systems are Liposomes and Lipoplexes. Liposomes were the most explored and the first system for drug and antigen delivery [116,123,124]. NPs, liposomes are biodegradable, biocompatible and have the ability to package hydrophobic agents [125, 126]. Which make them the optimal vehicles for peptides, proteins and drugs. Liposomes could be divided into anionic, cationic and neutral based on the surface charge. For sustained antigen release, cationic liposomes are better than anionic and neutral liposomes due to the long persisting interaction with the cellular membranes [127].

Liposomes could be used as adjuvants, a new formulation with saponin QS-21 and monophosphoryl lipid A (MPLA) was approved in vaccine [128]. In other study, synthetic liposomes were covalently coupled with BG505 MD39 and gp140 trimer to study the humoral responses [129]. Lipoplex belongs also to the lipid-based systems, cationic lipid like 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and 1,2-di-O-octadecenyl-3 trimethylammonium-propane (DOTMA) were used for the delivery of mRNA vaccine.

2.3.2. Polymersomes as non-viral carriers

The efficacy of a vaccine can be influenced by various factors, including the delivery system and administration route. This is where advanced biotechnological techniques come into play, specifically the use of polymersomes as non-viral carriers. Polymersomes are synthetic structures that can encapsulate drugs and also protein or nucleic acid for the use in vaccination. They are made up of amphiphilic block copolymers, which can self-assemble into hollow spheres. This structure allows them to encapsulate and protect the vaccine from degradation while providing a controlled release mechanism [130].

One of the advantages of using polymersomes as vaccine carriers is their ability to target specific cells or tissues. This is achieved by modifying the surface of the polymersome with ligands that can selectively bind to receptors on the target cells. This targeted delivery approach can increase the vaccine's efficacy and reduce side effects [131]. Moreover, polymersomes can also enhance the immune response by acting as adjuvants. This are substances that can stimulate the immune system and improve the vaccine's immunogenicity. Polymersomes can act as adjuvants by activating the dendritic cells responsible for presenting the vaccine antigen to the immune system [132].

Polymersomes have radii ranging from 50 nm to 5 μm or more and are manufactured with amphiphilic synthetic block copolymers to construct the vesicle membrane. The majority of known polymersomes include an aqueous solution in their center and are beneficial for encapsulating and safeguarding delicate molecules including medicines, enzymes, various proteins and peptides, and DNA and RNA fragments

Table 4
Vaccines using protein subunit in phase 3 and phase 4.

ID	Vaccine platform acronym	Vaccine platform description	Type of candidate vaccine	Number of doses	Schedule	Route of adminis tration	Developers	Phase
1	PS	Protein subunit	SARS-CoV-2 rS/Matrix M1-Adjuvant (Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M) NVX-CoV2373 CovovaxTM NVX-CoV2515 (booster)	2	Day 0 + 21	IM	Novavax	3
2	PS	Protein subunit	Recombinant SARS-CoV-2 vaccine (CHO Cell) Zifivax (ZF2001)	2–3	Day 0 + 28 or Day 0 + 28 + 56	IM	Anhui Zhifei Longcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences Zhongyianke Biotech Liaoning Maokangyuan Biotech Academy of Military Medical Sciences	3
3	PS	Protein subunit	VAT00008: SARS-CoV-2 S protein with adjuvant (1) CoV2 preS dTM monovalent D614 antigen (2) Bivalent (2-antigen) vaccine comprising spike protein of D614 and spike protein of the SARS-CoV-2 Beta variant (B.1.351)	2	Day 0 + 21	IM	Sanofi Pasteur + GSK	3
4	PS	Protein subunit	CpG 1018/Alum-adjuvanted Recombinant SARS-CoV-2 Trimeric S-protein Subunit Vaccine (SCB-2019)	2	Day 0 + 21	IM	Clover Biopharmaceuticals Inc./ Dynavax	3
5	PS	Protein subunit	COVAX-19® Recombinant spike protein + adjuvant SPIKOGEN®	2	Day 0 + 21	IM	Vaxine Pty Ltd./CinnaGen Co.	3
6	PS	Protein subunit	MF59 adjuvanted SARS-CoV-2 Sclamp vaccine	2	Day 0 + 28	IM	CSL Ltd. + Seqirus + University of Queensland	2/3
7	PS	Protein subunit	MVC-COV1901 (Spike-2P protein + adjuvant CpG 1018) MVC-COV1901(Beta)	2	Day 0 + 28	IM	Medigen Vaccine Biologics + Dynavax + National Institute of Allergy and Infectious Diseases (NIAID)	4
8	PS	Protein subunit	FINLAY-FR-2 anti-SARS-CoV-2 Vaccine (RBD chemically conjugated to tetanus toxoid plus adjuvant) Soberana 02 (The vaccine is known as PastoCovac in Iran)	2	Day 0 + 28	IM	Instituto Finlay de Vacunas	3
9	PS	Protein subunit	EpiVacCorona (EpiVacCorona vaccine based on peptide antigens for the prevention of COVID-19)	2	Day 0 + 21	IM	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology "Vector"	3
10	PS	Protein subunit	RBD (baculovirus production expressed in Sf9 cells) Recombinant SARS-CoV-2 vaccine (Sf9 Cell)	2	Day 0 + 28	IM	West China Hospital + Sichuan University WestVac Biopharma Co., Ltd.	3
11	PS	Protein subunit	UB-612 (Multitope peptide based S1-RBD-protein based vaccine)	2	Day 0 + 28	IM	Vaxxinity	3
12	PS	Protein subunit	CIGB-66 (RBD + aluminium hydroxide)	3	Day 0 + 14 + 28 or Day 0 + 28 + 56	IM	Center for Genetic Engineering and Biotechnology (CIGB)	3
13	PS	Protein subunit	BECOV2 (Corbevax)	2	Day 0 + 28	IM	Biological E. Limited	3
14	PS	Protein subunit	Recombinant Sars-CoV-2 Spike protein, Aluminum adjuvanted (Nanocovax)	2	Day 0 + 21	IM	Nanogen Pharmaceutical Biotechnology	3
15	PS	Protein subunit	Recombinant protein vaccine S-268019 (using Baculovirus expression vector system)	2	Day 0 + 21	IM	Shionogi	3
16	PS	Protein subunit	GBP510, a recombinant surface protein vaccine with adjuvant AS03 (aluminium hydroxide)	2	Day 0 + 28	IM	SK Bioscience Co., Ltd. and CEPI	3
17	PS	Protein subunit	Razi Cov Pars, recombinant spike protein	3	Day 0 + 21 + 51	IM IN	Razi Vaccine and Serum Research Institute	3
18	PS	Protein subunit	ReCOV: Recombinant two-component spike and RBD protein COVID-19 vaccine (CHO cell).	2	Day 0 + 21	IM	Jiangsu Rec-Biotechnology	3
19	PS	Protein subunit	Recombinant SARS-CoV-2 Fusion Protein Vaccine (V-01)	2	Day 0 + 21	IM	Livzon Pharmaceutical	3

(*continued on next page*)

Table 4 (*continued*)

ID	Vaccine platform acronym	Vaccine platform description	Type of candidate vaccine	Number of doses	Schedule	Route of administration	Developers	Phase
20	PS	Protein subunit	RBD protein recombinant SARS-CoV-2 vaccine (Noora Vaccine)	3	Day 0 + 21 + 35	IM	Bagheiat-allah University of Medical Sciences/AmitisGen	3
21	PS	Protein subunit	Recombinant protein RBD fusion dimer adjuvanted vaccine (COVID-19 Vaccine Hipra) PHH-1 V	2	Day 0 + 21	IM	Laboratorios Hipra, S.A.	3
22	PS	Protein subunit	SCTV01C. A Bivalent Recombinant Trimeric S Protein vaccine against SARS-CoV-2 Variants	1	Day 0	IM	Sinocelltech Ltd. Research and Development of Immune-and-Biological Products	3
23	PS	Protein subunit	SARS-CoV-2 Protein Subunit Recombinant Vaccine adjuvanted With Alum + CpG 1018	2	Day 0 + 28	IM	PT Bio Farma	3

[133]. Polymersomes are comparable to liposomes, which are vesicles generated from naturally occurring lipids. While polymersomes have many of the same features as natural liposomes, they are more stable and have lower permeability. Furthermore, the use of synthetic polymers allows designers to adjust the features of the membrane, allowing them to regulate permeability, release rates, stability, and other attributes of the polymersome [131] (Fig. 2).

In terms of administration routes, polymersomes can be delivered through various methods, including intramuscular, subcutaneous, and intranasal routes. The choice of administration route depends on the vaccine's properties and the targeted immune response. For example, intranasal delivery is an attractive approach for vaccines that target respiratory infections, as it can induce both mucosal and systemic immune responses [132,134]. One of the challenges in vaccine delivery is the development of stable formulations that can withstand storage and transportation. Polymersomes offer a solution to this problem, as they are highly stable and can be easily lyophilized (freeze-dried) for long-term storage. This makes them ideal for global vaccination programs, especially in low-income countries with limited cold chain storage [132]. One of the advantages of polymersomes over viral vectors is their safety profile. Unlike viruses, which can trigger immune responses and have the potential to integrate into the host genome, polymersomes are non-immunogenic and non-genotoxic. This makes them an attractive option for delivering genetic therapies and other sensitive drugs [130,135,136].

The shape and structure of polymersomes play an important role in determining their properties and performance as drug carriers. Several factors influence the shape of polymersomes, including the type of polymer used, the method of preparation, and the conditions under which they are formed [132,134]. One common method of preparing polymersomes is through the use of block copolymers, which consist of two or more different types of polymer chains. These copolymers can self-assemble into a variety of shapes, including spheres, cylinders, and vesicles. The shape of the polymersome can affect its stability, permeability, and ability to interact with target cells. For example, spherical polymersomes tend to be more stable than cylindrical or vesicular polymersomes, which can be prone to buckling or collapsing under stress. On the other hand, vesicular polymersomes have a larger surface area and can hold more cargo than spherical polymersomes, making them potentially more efficient drug carriers [137,138].

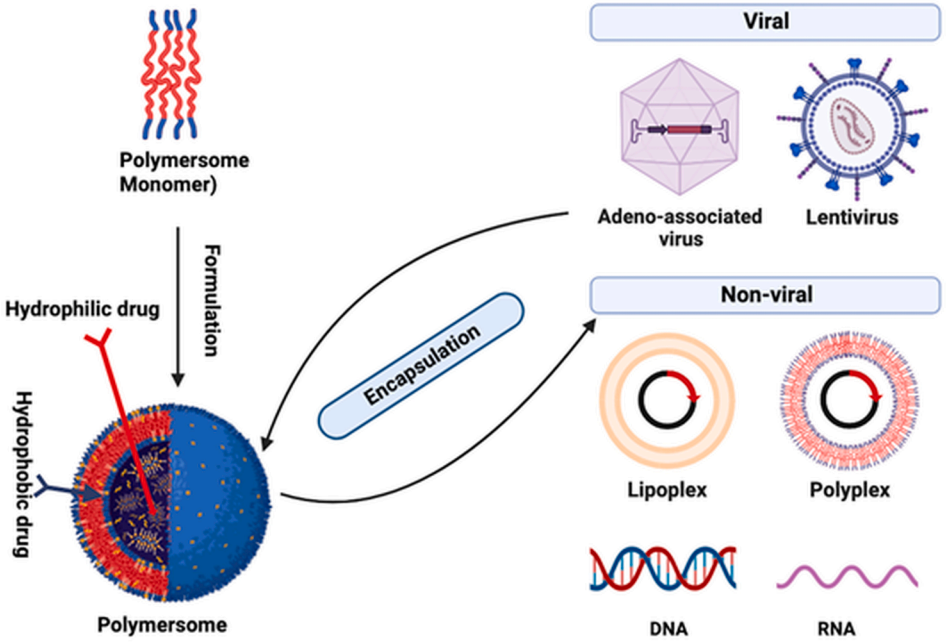

Fig. 2. Schematic diagram representing the shape and structure of polymersome. The polymersome can encapsulate viral carriers such as adeno-associated viruses and Lentivirus, and non-viral carriers such as DNA and RNA. The encapsulated vectors could be hydrophilic (inside the vesicle) or hydrophobic drugs (in the membrane).

Another factor that affects the shape and structure of polymersomes is the presence of additives or functional groups. By adding chemical groups to the surface of the polymersome, scientists can modify its properties and improve its ability to target specific cells or tissues. For example, researchers have developed polymersomes that can respond to changes in pH or temperature, allowing them to release their cargo in response to specific environmental cues. Other groups have attached targeting ligands or antibodies to the surface of polymersomes, which can help guide them to specific cells or tissues [139,140].

Overall, the shape and structure of polymersomes play an important role in determining their properties and performance as drug carriers. By understanding the factors that influence their formation and stability, scientists can design polymersomes that are optimized for specific drug delivery applications. Polymersomes are a promising class of non-viral carriers that offer several advantages over viral vectors for delivering drugs to patients. Their shape and structure are important factors that influence their properties and performance, and scientists are continuing to explore new ways to optimize these parameters for specific drug delivery applications. As research in this field continues to progress, we can expect to see more innovative drug delivery solutions based on polymersomes in the years to come [141].

2.3.3. Cell penetrating peptides (CPPs)

As in the last COVID-19 Pandemic, mRNA shown to have great potential to prevent infection and also as therapeutic treatment against cancer and for genetic disease, there are different delivery system used as carrier such as lipid-based system to improving mRNA bioavailability and stability with success. After the discovering of the Tat peptide of HIV-1 a new power full tool named cell-penetrating peptides (CPPs) has been emerged [142,143]. CPPs are peptide with 4–40 amino acids and are normally developed to deliver membrane-imperviable substrates such as proteins, peptides, drugs and nucleic acids in cells and tissue [144–148]. The CPPs could be combined with lipid-based nanoparticles (LNP) or with poly(ethylene glycol) (PEG)-GALA, and polycation (Fig. 3) to build a complex for the mRNA transfer [102]. Furthermore, the functionalities of CPPs could be modified by changing the sequence and composition of non-natural and natural amino acid. Some of the improved functionalities are the modulation of endosomal pathways, selective targeting of dendritic cells, improved endosomal escape efficiencies and finally a prolonged protein expression via mRNA stabilization. CPPs could be easily designed to exhibit variable function. The synthesis follows different methods like the solid phase peptide synthesis. Furthermore, addition of amino acid with specific characters such as low pKa, non-natural amino acids, could give the CPPs specific properties like easy endosomal escape and resistance to enzymatic degradation [149–152]. Some modification like the addition of guanidino groups of Arg residues plays a key role in cellular internalization via direct penetration and/or endocytosis [153–156]. Cationic CPPs, which are Arg-rich, interact with nucleic acids (DNA, siRNA, antisense oligonucleotide and RNA); negatively charged nucleic acids interact spontaneously with cationic CPPs to build a non-covalent conjugation [144,157,158]. In previous study, CPPs were used for the delivery of antisense oligonucleotide, plasmid DNA and siRNA [159].

Considering the potential of CPPs we described above, it is clear that CPPs could be promising carriers for mRNA delivery, in previous study, the author described the potential of cationic peptides for mRNA delivery [160]. In another work, the research group used hydrophobic alanine (Ala) and leucine (Leu) residues, and cationic arginine to develop mRNA vaccines [161]. The effect of the RALA/mRNA complex was higher than that of a standard liposomal mRNA formulation composed of DOTAP and DOPE. Based on these achievements made in mRNA delivery using CPPs, as well as previous successes for the delivery of siRNA and pDNA using CPPs, we believe that CPPs will have an important contribution in the mRNA vaccines development.

3. Other advanced vaccine delivery systems

Beside the Electroporation, different nanoparticles formulation and CPPs, there is other new developed methods for the vaccine delivery such as hydrogels and microneedles. Hydrogel are soft biomaterials with great potential in vaccine delivery as well as in variety of biomedical and pharmaceutical applications. Hydrogel presents a three-dimensional connection of natural or synthetic polymers with more than 90 % content of water [162,163] and are stabilized by hydrogen bonding, hydrophobic, aromatic and electrostatic interactions [126].

3.1. Hydrogels

There are different types of hydrogel, such as peptide and polymeric hydrogels. Due to their important properties like the biodegradability, biocompatibility, viscoelasticity and mucoadhesion, hydrogels became more interesse for the use as vehicles for vaccines [164]. The administration can be by spraying for mucosal immunization or by injection for parenteral immunization [165–167]. In previous study, Tian et al. used short aromatic peptide to build nanofibrous hydrogels capable for the packaging and delivery of DNA sequence coding for the HIV gp145 glycoprotein [168]. The author found that the formulation proved a strong protection of DNA against degradation, high transfection and a strong gene expression which resulted in increased cellular and humoral immune responses [168]. In other work, the author found that the use of peptide-based hydrogel for vaccination caused high immune response without the need for any other adjuvants [169]. This is the case, hence the structure of the nanofibrous hydrogel works as reservoir for prolonged and sustained delivery caused by the slowly release of the antigen from hydrogel. Which cause strong activation of APCs with improved cellular and humoral immune responses and prolonged

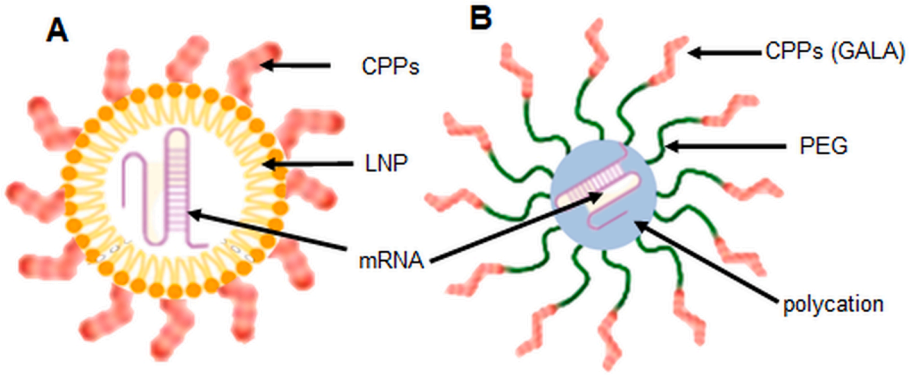

Fig. 3. Combination of CPPs with LNP and Polyplex for mRNA delivery. A) Combinations of CPPs with lipid-based nanoparticles and; **B**) CPPs with poly (ethylene glycol) (PEG)-GALA, and polycation to build a complex for mRNA transfer [102].

immunogenicity [169]. In other work, peptide hydrogels were subcutaneously applied for vaccination, this strategy caused increased antibody response and high protection against West Nile Virus (WNV) [170].

Polymeric hydrogels are another type of hydrogels which are also used for vaccine delivery. In previous work, the polymer-nanoparticle (PNP) hydrogels were used for long release and introduction of antigens to the immune system [171]. The long-lasting release of antigens from the hydrogel resulted in high immunity and enhanced antibody affinity [171]. Recently, hydrogels synthesized from a combination of dodecyl-modified hydroxypropylmethylcellulose (HPMC-C12) and poly (ethylene glycol)-b-poly(lactic acid) (PEG-PLA) PNP were used for the long-lasting delivery of receptor-binding domain (RBD) of SARS-CoV-2 spike protein, CpG and alum as vaccine against SARS-CoV-2 [172]. This method has increased the RBD-specific antibody significantly when compared to the bolus administration. Finally, the development of hydrogel as a reservoir for controlled release, has been emerged as brilliant alternative to the repetitive conventional vaccination strategy [173]. In this context, the group of Wu et al. developed thermo-sensitive hydrogel for intranasal (IN) delivery of H5N1 antigen [174]. This system resulted in long availability of the antigen in the nasal cavity and caused high immune responses. Similar hydrogel was also used for IN vaccination against Ebola virus, hence the antigen maintains for long time in the nasal cavity, it caused high mucosal IgA (Fig. 4) antibodies as well as increased IgG2a, IgG1 and IgG in the serum [175].

3.2. Microneedles

As alternative to the conventional parenteral routes and the local IN administration for vaccines, the skin has been emerged as an interesting new alternative route for vaccination. This is true, due to the advantages for use of skin for vaccination such as the extensiveness of the skin, the abundant immune cells in the dermis and the ease accessibility. Taken all together, this well increased the efficient and systemic immune reaction [178]. To reach the dermis where the immune cells persist, it needs the development of methods such as electroporation and microneedle (MN) to disrupt the superficial layer of the skin [179]. The last method has shown promising results as vaccine delivery system. Microneedle could be synthesized from polymers, ceramics, glass or metals, they are usually less than 1 mm in long, very fine and have different arrangements. The production of MN could be through different methods like laser micromachining, solvent casting and three-dimensional printing [179,180]. The solid MN were the first developed type which allowing the permeation of the substrates by pores created in the skin [181]. Later, other type such as hollow and coated MN were developed to improve the efficiency [182]. Recently, it follows the combination of the hydrogel technology we described above with the MN strategy to develop hydrogel-forming polymer-based MN [183,184].

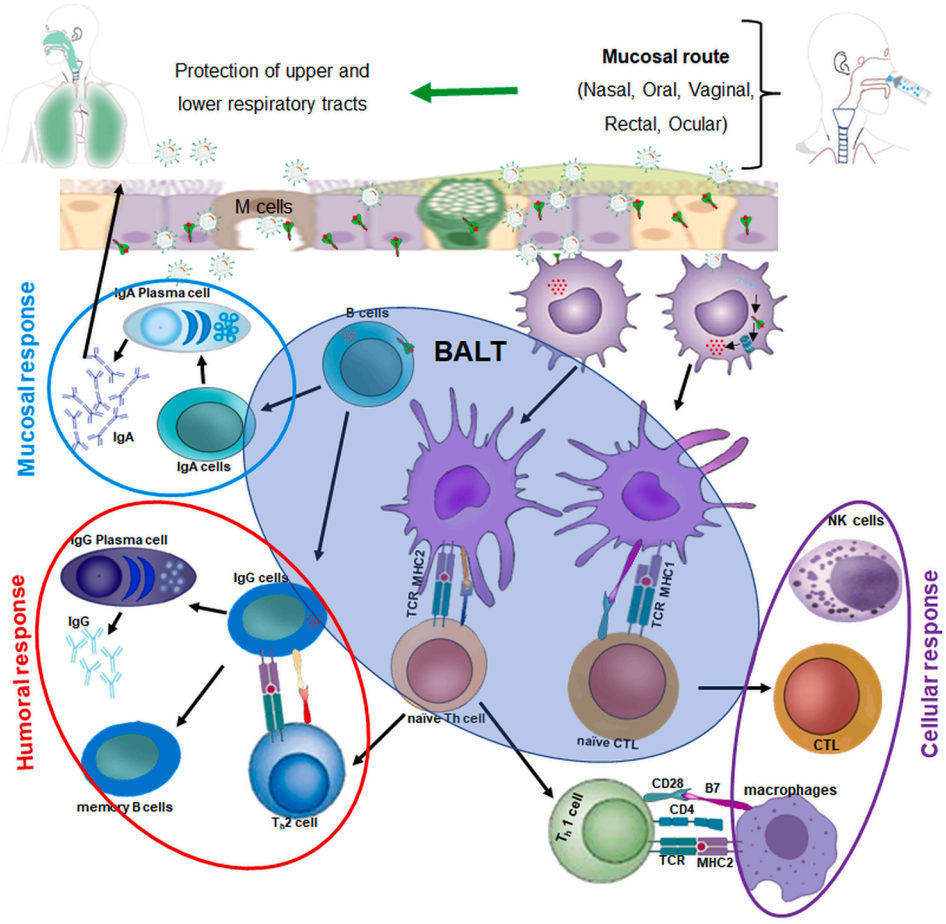

Fig. 4. vaccines administration via Nasal route using ChAdV, Adenoviral vector passe into the epithelial tissues by microfold cells (M cells) or passively through epithelial cell junctions or internalized by B cells or dendritic cells (DCs). B cells convert themselves to IgA plasma cells that produce IgA antibodies for neutralizing the pathogens at the mucosal surfaces (mucosal response) and conducted to the protection of upper and lower respiratory tracts Dendritic cells (DCs) with antigen migrate to mucosal-associated lymphoid tissues (MALTs) and present the antigen via major histocompatibility complex (MHC) class I and class II molecules to naïve CTL and naïve Th cells. The activation pathway produces cytotoxic T lymphocytes (CTL) and the Th1 cells and Th2 cells from naïve Th cells. Th1 cells activated macrophages to kill intracellular pathogens and stimulate NK cells (cellular response). The Th2 cells activated B cells to transform to IgG plasma cell that secret antibodies for neutralization of extracellular pathogens (humoral response) [176,177].

This strategy allows the delivery of high doses of antigens/drugs, long availability of the antigen and finally high immune responses.

4. Routes of administration

Traditionally, vaccines are applied via parenteral routes such as transdermal (TD), subcutaneous (SC) and intramuscular (IM) routes. These application forms build a local depot, from which antigen can passively pass to the local lymph node (LN). Alternatively, the immune cells can capture and transport the antigen to the LN. Due to the superiority of transdermal (TD) and subcutaneous (SC) routes in terms of vaccine drainage to the LN and the strong immunogenicity [185], it is considered that SC and TD route are very interesting alternative to the classical IM method. However, the IM route showed less side effects when compared to TD and SC which make the IM the most preferred method until now [186]. Meanwhile, the IM route has other disadvantages including risks associated with needle-stick, need of qualified human resources, waste management and others. Which makes finding other administration routes very necessary. One important alternative is the mucosal routes including nasal and oral routes (Fig. 4), this method elicits local mucosal immunity by the mucosal-associated lymphatic tissue (MALT) [187]. After administration of the antigen, this will be presented by dendritic cells (DC) and macrophages to the resident B and T cells, followed by the secretion of antigen-specific immunoglobulin A (IgA) antibodies (Fig. 4A) capable to bind to the pathogen at the entry site and therefore inhibit further infection [188,189]. This advanced defense against the pathogen does not exist in the case of parenteral immunization strategies, making mucosal routes very particular and represent a reason to give more effort to develop formulations for mucosal vaccine. One of the first mucosal vaccines is the oral polio vaccine (OPV), this was very successful in reducing transmission and infection of polio virus [190]. The other alternative in mucosal routes is the intranasal (IN) route which is less invasive and has more other advantages like rapid absorption of antigens, high vascularization and the normal pH level, which allowed the omission of the antigen protection against low pH as in the oral route [191]. One of the most important factors for developing IN vaccine is the fact, that the most pathogens enter though this route and causing critical respiratory disease such as the COVID-19 pandemic, which make this method more attractive and very important to develop vaccines against pathogens. In this context, research group showed that IN delivery of vaccine against SARS-CoV-2 (Table 1) provide high immunity in the lung and nose which prohibited the entry of the virus and offering advanced protection [192]. Recently, and in the racing to develop vaccine against COVID-19 pandemic, A. O. Hassan et al. found that the application of a single intranasal dose of ChAd-SARS-CoV-2-S enhanced the levels of neutralizing antibodies, stimulates T cell responses and induces systemic and mucosal immunoglobulin A (IgA), and prevents significantly the SARS-CoV-2 infection in both the lower and upper respiratory tracts [193] (Fig. 4B). Although, the IN method has also one drawback presented in the rapid clearance of the antigens from the nasal mucosa. Which is challenging and showing the necessity to take different condition in consideration to develop IN vaccines.

Another interesting administration rout is the inhalation, At the end of the year, 2022, the authorities in China started the registrations for booster immunizations with the inhaled Convidecia^{TM} vaccine [194]. The vaccine has been developed by CanSinoBIO and it is based on Ad5 to deliver DNA material encoding the S protein of SARS-CoV-2 into the body. The inhaled particles reach the lung tissue to stimulate mucosal, cellular, and humoral immune responses. A clinical trial of booster immunizations with this vaccine was carried out in healthy adults. The results showed, that booster dose with Convidecia^{TM} vaccine and an inactivated virus vaccine increased the immune response significantly. The study found that the neutralizing antibodies against Omicron variant was 14 times higher when compared to a booster dose with the inactivated virus vaccine. Moreover, the booster dose with Convidecia^{TM} vaccine protects against new infection for at least 6 months, suggesting the superiority of the inhaled Convidecia^{TM} vaccine as booster.

5. Conclusion

The development of vaccine against COVID-19 pandemic has shown, that on one hand, each of the vaccines has to face specific challenges. Example, the adenoviral vaccines have problem with neutralization antibodies after the first dose, the mRNA and DNA vaccine need high concentration and specific delivery systems. One the other hand, all vaccines have to master general challenges such as storage, logistics and mass production and vaccination. Also, the different routes for vaccine administration still need to solve critical problems to reach the ideal vaccination method. Another serious problem for all vaccine strategies is the clearly impaired immune response in elderly. Taken all together, and despite the amazing progress in the vaccine development, we can conclude that the race to develop vaccine is not over yet.

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Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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Not applicable.

CRediT authorship contribution statement

Abdellatif Bouazzaoui: Conceptualization, Data curation, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Ahmed A.H. Abdellatif:** Conceptualization, Data curation, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

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

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