Bioengineering Strategies for Developing Vaccines against Respiratory Viral Diseases

Shalini Iyer,[a] Rajesh Yadav,[a] Smriti Agarwal,[a] Shashank Tripathi,[b] Rachit Agarwal[a]

[a]Center for BioSystems Science and Engineering, Indian Institute of Science, Bengaluru, India
[b]Department of Microbiology and Cell Biology, Center for Infectious Disease Research, Indian Institute of Science, Bengaluru, India

Shalini Iyer, Rajesh Yadav, and Smriti Agarwal contributed equally to this work. The order of names was decided based on the time spent on writing.

SUMMARY Respiratory viral pathogens like influenza and coronaviruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have caused outbreaks leading to millions of deaths. Vaccinations are, to date, the best and most economical way to control such outbreaks and have been highly successful for several pathogens. Currently used vaccines for respiratory viral pathogens are primarily live attenuated or inactivated and can risk reversion to virulence or confer inadequate immunity. The recent trend of using potent biomolecules like DNA, RNA, and protein antigenic components to synthesize vaccines for diseases has shown promising results. Still, it remains challenging to translate due to their high susceptibility to degradation during storage and after delivery. Advances in bioengineering technology for vaccine design have made it possible to control the physicochemical properties of the vaccines for rapid synthesis, heightened antigen presentation, safer formulations, and more robust immunogenicity. Bioengineering techniques and materials have been used to synthesize several potent vaccines, approved or in trials, against coronavirus disease 2019 (COVID-19) and are being explored for influenza, SARS, and Middle East respiratory syndrome (MERS) vaccines as well. Here, we review bioengineering strategies such as the use of polymeric particles, liposomes, and virus-like particles in vaccine development

against influenza and coronaviruses and the feasibility of adopting these technologies for clinical use.

INTRODUCTION

Currently, all over the world, around 86% of children get their required dosage of life-saving vaccines (1). Vaccines have saved many lives and are very effective against diseases like diphtheria, tetanus, polio, hepatitis B, measles, and rubella (2). Today, vaccination is still the most effective and simplest approach used against acute respiratory diseases, especially in low- and middle-income countries, the primary hot spots for infectious diseases (3). Vaccine designs and delivery strategies should continuously evolve to enhance their activity and make them effective against old and new pathogens. Despite the high rate of success for several diseases, there is still a need for improvement of vaccines against viral respiratory diseases due to the high rate of viral mutation and rapid spread.

Viruses that show zoonotic adaptation and transmission, especially RNA viruses, which include influenza (flu) and coronaviruses, have the highest pandemic potential among emerging infectious diseases (4). As the current seasonal influenza vaccines are only 30 to 40% effective (5), there is an urgent unmet requirement for a universal influenza vaccine that is efficacious against most, if not all, strains of influenza. Similarly, diseases caused by human-infecting viruses belonging to the *Coronaviridae* family, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), have caused epidemics (6). The current coronavirus disease 2019 (COVID-19) pandemic has further emphasized the necessity of developing effective vaccines with the greatest possible speed. It is difficult to accurately estimate when and how the next pandemic will strike and how deadly and contagious it will be. Currently, the R&D Blueprint list of prioritized diseases in emergency contexts released by the World Health Organization (WHO) includes SARS, MERS, and COVID-19 (https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts). Therefore, vaccine development for influenza and coronaviruses must be enhanced so that there is preparedness to quickly tackle new virus variants with minimal effect on global health.

Global Impact of Viral Respiratory Tract Diseases

Respiratory tract diseases are prevalent worldwide and are among the primary causes of fatality, causing more than four million deaths yearly worldwide, especially in underdeveloped and developing countries (Fig. 1) (7). Acute respiratory disease-causing viruses like flu and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have caused pandemics leading to millions of deaths. Influenza pandemics by novel influenza virus strains that are antigenically unique from the already circulating virus strains have been observed every 10 to 50 years. As humans have never been exposed to such novel strains, they spread quickly and cause severe infection.

The pandemic caused by the H1N1 virus (Spanish flu) in 1918 caused severe mortality (8). According to the Centers for Disease Control and Prevention, it is estimated that it infected around 500 million people, with around 50 million deaths (https://www.cdc.gov/flu/pandemic-resources/1918-pandemic-h1n1.html). Approximately 4.5 million lives were lost during the Asian flu, Hong Kong flu, and swine flu pandemics between 1957 and 2010 (10). Since 2009, the novel influenza A virus has been circulating as a seasonal influenza virus. There are around 3 to 5 million cases of seasonal flu reported around the world every year (11).

The basic reproduction number (R_0) is a critical parameter in determining the contagiousness of an infectious disease (12). R_0 for a contagious disease is defined as the average number of people contracting the disease from an infected person. R_0 values are above 2 for coronaviruses and H1N1 1918 Spanish flu and less than that for MERS-CoV (Fig. 1) (13).

Another deadly group of viruses called coronaviruses mainly circulate among animals but have occasionally evolved and transmitted to humans, causing mild to lethal respiratory
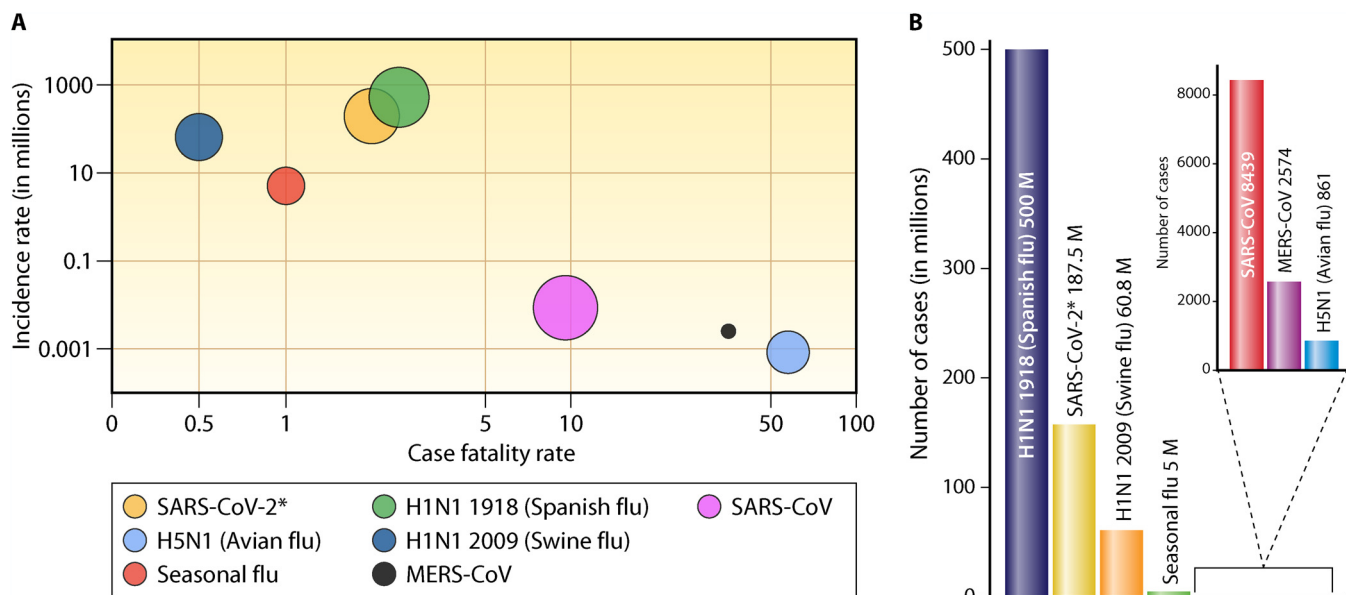
FIG 1 Statistics of viral respiratory diseases and their effect on global public health. The bubble sizes in the figure denote the R_0 value for each disease. Source: Centers for Disease Control and Prevention, WHO, and Centre for Infectious Disease Research and Policy. *, SARS-CoV-2 incidence rate until 14 July 2021.

infections. SARS-CoV caused an epidemic in 2003, with over 8,000 cases and 774 deaths in 26 different countries (Fig. 1) (14). The lung-infecting lethal MERS coronavirus (MERS-CoV) has also shown repeated zoonotic transmissions since 2012 from dromedary camels to humans after causing deadly MERS outbreaks in South Korea and Saudi Arabia in 2015 and 2018, respectively (15). By March 2021, 2,574 cases had been reported worldwide, including 885 deaths associated with the infection, bringing the case fatality rate to 34.4% (Fig. 1) (http://www.emro.who.int/health-topics/mers-cov/mers-outbreaks.html). The most recent and devastating coronavirus-mediated infection is the SARS-CoV-2-caused COVID-19 pandemic. By 14 July 2021, over 187 million people had been infected, and 4 million had died of COVID-19 globally (https://covid19.who.int/). Even more concerning is that the SARS-CoV-2 coronavirus is now known to have gathered mutations after the initial break-out, increasing its infectivity, fatality, and resistance to neutralizing antibodies (16–19). As influenza and coronaviruses have been detrimental with their massive global clinical and socioeconomic impact, we have focused on bioengineered vaccine development strategies for these viral respiratory diseases. We hope to bring forth the capability of bioengineering to improve vaccine efficacy and provide designs that can be easily adapted to develop vaccines against new strains.

Viral Structure and Pathology in Humans

The symptoms of flu disease in humans are of a wide range. For some, it may be mild respiratory discomfort associated with symptoms of the upper respiratory tract infection like fever, runny nose, throat pain, headache, cough, and muscle fatigue, while for others, it may be severe, with lethal pneumonia caused by influenza, which leads to multiple organ failure or secondary bacterial infections in the airway (20). Influenza viruses are enveloped single-stranded negative-sense RNA viruses with a seg-mented genome. Human-infecting influenza viruses have four serotypes—A, B, C, and D. Among them, A and B have pandemic-causing potential, while C and D are mild sea-sonal viruses. Both A and B type influenza viruses carry eight RNA segments, which encode viral glycoprotein subunits like hemagglutinin (HA), matrix protein (M1), neur-aminidase (NA), membrane protein (M2), nucleoprotein (Np), and nonstructural pro-teins (Fig. 2). HA and NA are surface proteins with a globular head and a stack domain and are the most antigenically variable portion in the virus. They are the primary tar-gets for antibody binding by recognition of influenza virus infection and vaccines. HA

Coronavirus   **Influenza**
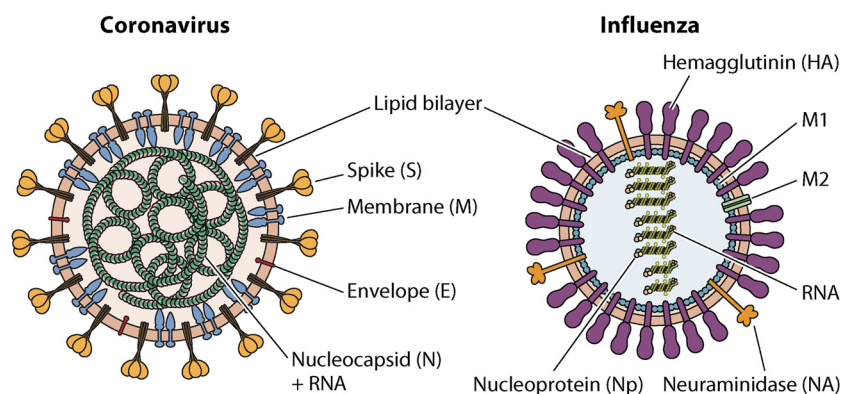
Bioengineering Technology	Parts/Sequences Used	
	Coronavirus	**Influenza**
Polymer particles	S, N	Inactivated virus, HA, Np
Inorganic nanoparticles	—	Inactivated virus
Liposomes	S	HA, Np,NA, M1, M2
Self-assembling proteins	S, E, M, N	HA, NA, M1, M2
Microneedles	S	Inactivated virus
Electroporation	S	HA

FIG 2 Structure and usage of components of coronaviruses and influenza in bioengineered vaccine development.

helps in virus entry by binding to sialic acid on the host cell surface. The primary function of NA is to release the virions from the host cell membrane (20).

Patients infected with the coronaviruses MERS-CoV, SARS-CoV, and SARS-CoV-2 typically show initial symptoms of fever, chills, cough, breathing difficulty, conjunctivitis, and myalgia, which in some cases can quickly progress to acute fatal respiratory conditions (21–23). The viral structure of coronaviruses consists of the following 5 components: membrane (M), spike (S), nucleocapsid (N), envelope (E), and a single-stranded RNA (Fig. 2) (24). The spike (S) protein expressed on the viral surface has been identified to be a component that can significantly induce the production of antigen-specific neutralizing antibodies in the serum (24). The coronavirus uses the receptor-binding domain (RBD) of S to attach to the host cell surface's angiotensin-converting enzyme 2 (ACE2) (for SARS-CoV and SARS-CoV-2) or dipeptidyl peptidase 4 (for MERS-CoV) (25, 26). The S protein undergoes structural modification and cleavage to initiate host cell-virus fusion and cytoplasmic release of the viral RNA. Due to the high immunogenicity and conservation of the S protein and its RBD, they have been in focus for vaccine development strategies. However, as mentioned previously, SARS-CoV-2 has been observed to rapidly gather multiple mutations in the S protein-encoding gene and generate new variants (17). Currently, the WHO has identified four SARS-CoV-2 variants of concern (Alpha, Beta, Gamma, and Delta) (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). Variants of concern can create a change in the disease epidemiology, transmissibility, virulence, or clinical symptoms and might be not be curbed by known public health measures or available vaccines and therapeutics. The structures of influenza and coronaviruses have been reviewed in detail elsewhere (20, 24).

TABLE 1 Approved COVID-19 vaccines, formulations, and developers

Vaccine name	Vaccine formulation	Developer	ClinicalTrials.gov identifier
Gam-COVID-Vac/Sputnik V	S-encoding gene carried by two different recombinant adenoviral vectors for the prime and booster doses (rAd26 and rAd5, respectively)	Gamaleya National Center	NCT04656613
EpiVacCorona	Chemically synthesized peptide antigens	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	NCT04527575
COVI-VAC	Live attenuated SARS-CoV-2 virus	Codagenix	NCT04619628
Covaxin/BBV152	Inactivated SARS-CoV-2 virus	Bharat Biotech	NCT04641481
ChAdOx1 nCoV-19/Covishield/AZD1222	Simian adenovirus vector encoding full-length S	University of Oxford	NCT04400838
mRNA-1273	Lipid NP-encapsulated mRNA encoding full-length S	Moderna	NCT04470427
BNT162/Tozinameran/COMIRNATY	Lipid NP-encapsulated mRNA encoding full-length S protein	Pfizer-BioNTech	NCT04713553
Ad26.COV2.S	Adenoviral vector (Ad26) containing S-encoding gene	Janssen Vaccines & Prevention	NCT04505722
CoronaVac	Inactivated SARS-CoV-2 virus	Sinovac Life Sciences	NCT04582344
BBIBP-CorV	Inactivated SARS-CoV-2 virus	SinoPharm	NCT04795414
Ad5-nCoV	Recombinant adenoviral vector (Ad5) containing full-length S-encoding gene	CanSino Biologics	NCT04526990
ZF2001	Tandem repeat RBD dimer	Anhui Zhifei Longcom Biopharmaceutical	NCT04646590

Existing Vaccine Development Strategies

There are no approved vaccines against SARS-CoV or MERS-CoV, but potential candidates have shown promising results (27). For SARS-CoV-2, several vaccines have been approved (Table 1), including live attenuated, inactivated, and protein antigen vaccines. Two approved bioengineered vaccines, mRNA-1273 and BNT162, are discussed later in this review. Around 272 vaccine candidates and 332 therapeutic drugs for COVID-19 are in development, with many being in clinical trials (https://covid-19tracker.milkeninstitute.org/).

Similarly, there are traditional vaccines like subunit vaccines, live attenuated vaccines, inactivated vaccines, and split virion vaccines (whole virus disrupted by a surfactant) available for influenza (28). These vaccines are generated via egg-based inoculation of the virus in chicken eggs, through animal or insect cells using cell culture technology, or manufactured as recombinant vaccines (28). These vaccines aim to elicit neutralizing antibodies against the HA protein, including its antigenically variable regions, and hence require periodic updates to suit the seasonal influenza A and B virus strains in circulation (28). Vero cell technology has also been described for rapid generation of inactivated whole-virus vaccines against emerging viral pathogens (29).

The manufacturing of vaccines for acute respiratory viral diseases is unique because processes must be changed rapidly to update the vaccine strains following the continuous evolution of the viruses (30). These process changes must occur in a compressed time frame; for example, for influenza, the strain selection is announced twice every year by the WHO after reviewing the surveillance data. It usually occurs in February for the Northern Hemisphere and in September for the Southern Hemisphere, and manufacturers typically ship the vaccine in 6 months (31). The most popular intranasal attenuated vaccine against influenza, FluMist, has been observed to have a potential risk of reverting to a virulent state (32). Employing bioengineering techniques for vaccine development can significantly help achieve clinical success while alleviating safety concerns, such as the potential risk of reverting to a virulent form in case of live attenuated or inactivated virus vaccines (32, 33).

Need for Biomaterial-Based Vaccines

The main hurdle in designing any vaccine is to maintain a balance between its safety and its efficacy. Generally, the most effective vaccines, like live attenuated or inactivated whole virus vaccines, always come with the greatest safety risk. On the other hand, safe vaccines such as subunit vaccines are often inefficient in inducing a robust immune response in the body. Another essential component in vaccine manufacturing during a pandemic is the speed with which the vaccine design can be completed so that the clinical trials can commence rapidly. The modifiability of biomaterials provides a quick solution to obtain plug-and-play systems in which the components can be easily switched to adjust to new antigens (34, 35).

Increased speed of vaccine design. During pandemics, the rapid development of suitable and effective vaccines becomes critical for preventing loss of life. The lack of such measures may lead to high death rates, as seen during many past pandemics, such as the Spanish flu pandemic.

Since coronaviruses and influenza display high antigenic variation, vaccines developed against one type of coronavirus or influenza must be designed such that they can be quickly modified to design vaccines against new types. Biomaterial platforms (such as lipid nanoparticles) allow flexibility for such alterations by virtue of their highly adaptable features, such as type of loaded drug or antigen, charge, shape, surface coating, and other such physicochemical properties. While using the same vaccine design strategy, this interchangeability of antigens has been used in designing vaccines against flu (e.g., mRNA-1440) and COVID-19 (e.g., mRNA-1273). For example, researchers working on the mRNA-1273 vaccine for COVID-19, developed by Moderna, completed *in silico* modeling and preclinical studies and started phase 1 trials just 66 days after the release of the SARS-CoV-2 viral sequence and moved to phase 2 trials 74 days later by using previously obtained data from research on SARS-CoV and MERS (36). Such preparedness enables researchers to efficiently utilize preexisting data to modulate and quickly produce vaccines to be moved to clinical trials without extensive preclinical research in such emergencies where time is of the essence.

Improved delivery of antigen. Several potent but sensitive gene-based vaccines, recombinant DNA-based vaccines, and structure-based immunogens are being developed. Such biomolecules need cold storage for stability, and even then, they tend to degrade rapidly owing to their fragility. A protein or nucleic acid antigen can be degraded by the proteases and nucleases present in the serum or extracellular medium, reducing its overall *in vivo* half-life. The use of biomaterials can offer some significant benefits in vaccine development like high stability, prevention of enzymatic degradation, control on release kinetics, high loading of immunogens, and targeted delivery to immune cells (Fig. 3) (37, 38). Biomaterials can enable the synthesis of safe vaccines that are compatible with a wide variety of biomolecules (Table 2). Controlled release facilitates long-term exposure which results in higher vaccine efficacy and reduced frequency of dosage. Such strategies can result in increased patient compliance and lower cost (39).

Biomaterials provide unique physical and chemical properties like shape, size, chemistry, and tunable degradation rate. Surface chemistry affects the immunogenicity of biomaterials, thereby providing means to alter the immune response (40). The shape of the biomaterial can change its interaction with immune cells and affect the uptake by host cells (41). Additionally, biomaterials enable the codelivery of antigens with adjuvants to cells in target, which helps activate innate and adaptive immune responses, as discussed later in this review. Overall, biomaterials improve durability, immunogenicity, increase the stability of difficult-to-use antigens, make the delivery of vaccines more efficient, increase shelf life, and enhance vaccine acceptance.

Enhanced immunogenicity of antigen. Other significant hurdles that protein and nucleic acid-based vaccines face are low internalization and lack of adequate antigen presentation (42, 43). Typically, when B cells recognize surface antigens, they differentiate into plasma cells, which release antigen-specific neutralizing antibodies called
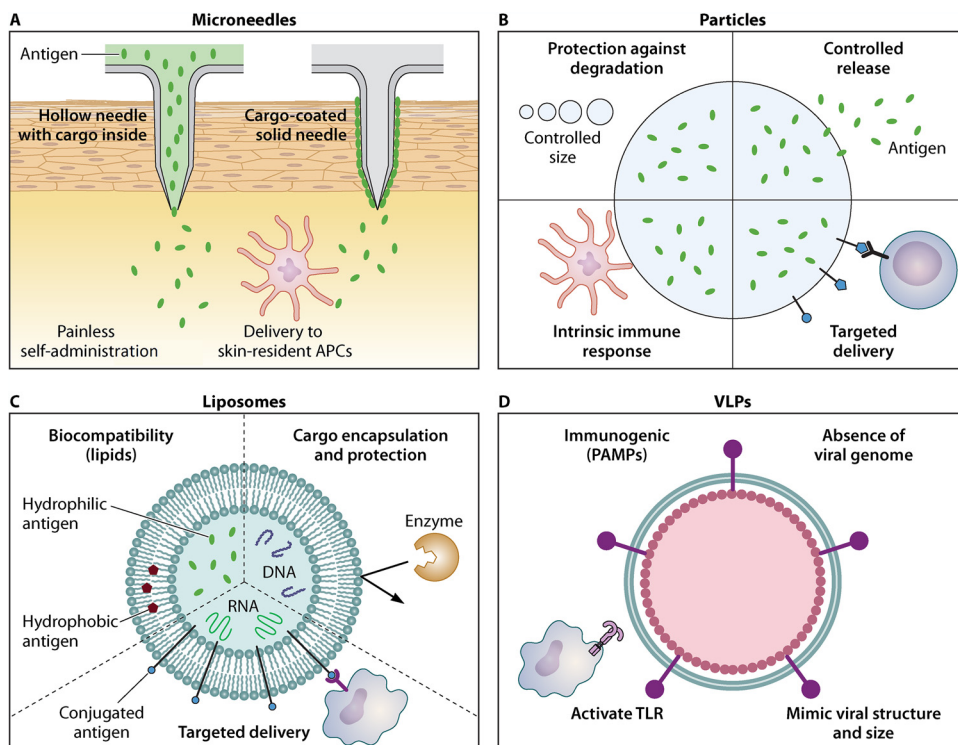
FIG 3 Advantages of using biomaterials such as microneedles (a), particles (b), liposomes (c), and virus-like particles (VLPs) (d) in vaccine development. TLR, Toll-like receptor; PAMP, pathogen-associated molecular pattern.

immunoglobulins (Ig) that can attach to the virus surface and neutralize its RBDs, thereby preventing internalization by host cells (44). Many proteins and nucleic acids have very low recognition by the surface receptors on antigen-presenting cells (APCs) or B cells, leading to a lack of internalization or neutralization. Nucleic acids might also not be efficient in triggering a robust immune response by themselves, even when used with plasmid vectors. Still, their immunogenicity can be improved using nanoparticles (NPs) for delivery (45).

Proteins that are endocytosed or phagocytosed by APCs undergo endosomal processing and are typically presented to CD4+ T cells with major histocompatibility complex (MHC) class II molecules (46). In contrast, cytosolic proteins are presented to CD8+ T cells with MHC class I molecules (Fig. 4) (46). Infected cells, including immune cells, can present foreign proteins with MHC class I via cross-presentation (47). There are three reasons for an antigen to undergo cross-presentation, as follows: (i) antigen escapes from the endosomal vesicle after internalization, (ii) antigen undergoes receptor-mediated endocytosis and is then loaded into recycling endosomes having MHC-I, or (iii) antigen directly enters the cytoplasm by pinocytosis or diffusion through the cell membrane. This cross-presentation is essential for an antigen-specific CD8+ cytotoxic T-lymphocyte (CTL) response, a significant pathway for the generation of antiviral immunity (47). Protein antigens delivered exogenously may not be able to perform endosomal escape to undergo MHC-I presentation (48). Nucleic acids and proteins can be encapsulated or coated on NPs or designed as viromimetic particles that increase their uptake by enhancing the interaction with the host cell membrane with their optimized surface chemistry and charge. They can also help in efficient intracellular delivery through pH-responsive cargo release, as described later.

The induction of a T-cell memory is essential in viral infections as some viruses frequently mutate their surface antigens to evade antigen-specific neutralizing antibodies, such as human immunodeficiency virus type 1 (HIV1) and influenza (49). Antibodies developed against SARS-CoV and MERS have been observed to have short

TABLE 2 Application and safety of biomaterials in vaccines

Biomaterial	Materials used	Biomolecules compatible	Safety prospects	Approved vaccines
Microneedles	Silicon, metals (stainless steel, titanium, etc.), polymer (polydimethylsiloxane, polyvinyl alcohol, polymethylmethacrylate, polyglycolic acid, etc.), and ceramic	Whole, inactivated virus, peptides, and nucleotides	Minimally invasive delivery systems that are safe; minor concerns like infection, local bleeding, and skin irritation may occur	MicronJet 600, MicronJet, BD Soluvia, Fluzone intradermal
Particles	Polymers (PLGA, polyanhydride, polylactic acid, chitosan, polyurethane, etc.) and inorganic metals and compounds (gold, silver, copper oxide, zinc oxide, aluminum oxide, and iron oxide)	Live attenuated viruses, subunit antigen, recombinant antigen, polysaccharides, peptides, and nucleotides	Some of the metal nanoparticles show organ accumulation and toxicity; biodegradable polymers are relatively safe	None
Liposomes	Ionizable lipidoid, PEG, structural lipids, and cholesterol	Peptides and nucleotides	Safe with low reactogenicity, biodegradable, and versatile	Inflexal V, Epaxal, mRNA-1273, Comirnaty
VLPs	Viral subunits that can self-assemble	Viral capsid proteins	Possible hypersensitivity	Engerix, Cervarix, Recombivax HB, Gardasil

lives of 24 and 34 months, respectively (50, 51). The persistence of SARS-CoV-2 specific immunity has been observed for 6 months (52, 53). Besides that, reinfection has been reported in several respiratory viruses such as influenza, SARS-CoV-2 and respiratory syncytial virus (54, 55). To effectively develop a lasting immunity in such cases, establishing a strong effector T-cell response becomes essential so that adequate memory T cells remain after the contraction phase of the antiviral T-cell response (56).

An ideal vaccine should generate a strong immune response that mounts rapidly and lasts for the lifetime of the individual (57). It must enable development of powerful and broad B and CTL responses against conserved epitopes so that there are enough memory T cells and neutralizing antibodies to prevent reinfection from any viral variants. It should have high efficacy and safety and must impart protection in all vaccinated individuals, including the more vulnerable population, while transferring the protection from mother to fetus. It should require minimum doses (ideally single administration), allow administration via least painful method, be easily manufactured industrially, and have high stability during synthesis and storage for efficient distribution (57). Use of bioengineering can facilitate an increase in the vaccine immune response (e.g., liposomes, electroporation), improve stability of vaccines (e.g., polymers, pH-responsive particles), ease the manufacturing process (e.g., self-assembling particles, inorganic particles), and provide painless delivery (e.g., microneedles).

BIOENGINEERING STRATEGIES USED IN DEVELOPING VACCINES AGAINST INFLUENZA AND CORONAVIRUSES

Bioengineering-based vaccine development approaches that can be used for respiratory viral diseases are classified based on the biomaterial used in the synthesis and delivery of the vaccine (Table 3).

Polymer-Based Vaccines

Polymers are large biomolecules made from repetitive monomer or oligomer subunits arranged in linear, branched, or dendrimer structures (58). Polymeric NPs are typically prepared by solvent evaporation, spontaneous emulsification, solvent diffusion, or polymerization (58). With the help of bioengineering techniques, one can tune the NPs to suit the pharmacokinetics of antigens or enhance their targeted delivery (59). A wide variety of physicochemical properties of polymers can be modified, such as charge, hydrophobicity,
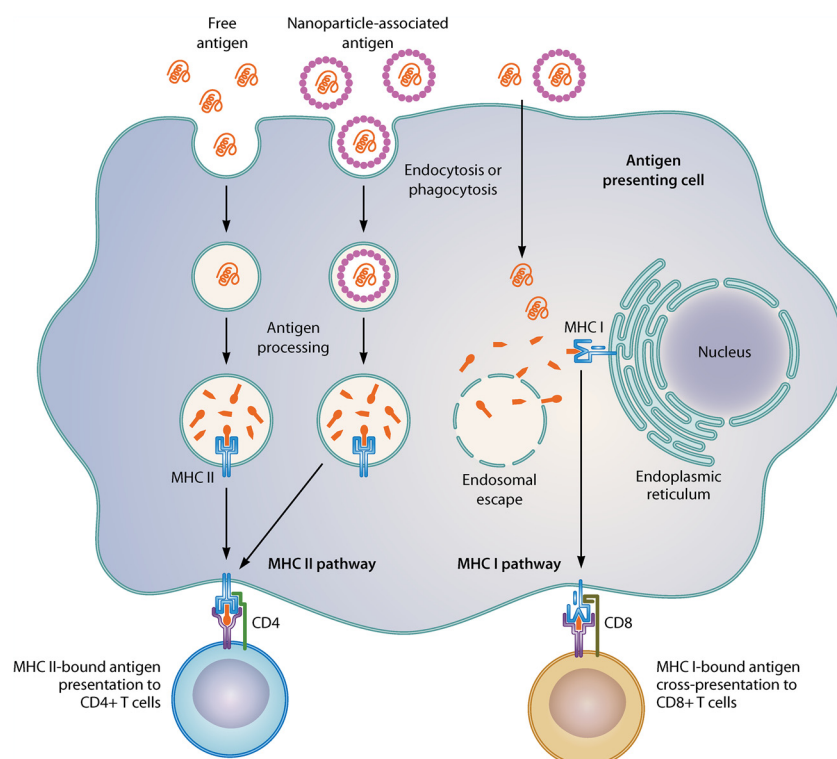
FIG 4 Processing and presentation of free and nanoparticle-associated antigen by antigen-presenting cells (APCs).

size, shape, retention, solubility, and strength of the NP-antigen complex, among many others (59). This adaptability provides a platform to increase the delivered antigen's efficacy in generating a robust immune response. Particles have been shown to protect the antigen from proteolytic degradation, increase antigen delivery to APCs, prolong the antigen activity, and act as adjuvants (60, 61). Polymers can be used to encapsulate nucleic acid and protein antigens or can be synthesized as particles that can conjugate or adsorb DNA and protein antigens on the surface (59).

Synthetic polymers. Poly(lactic-*co*-glycolic acid) (PLGA) is a widely used synthetic polymer in biomedical applications due to its controlled and sustained release of the cargo, low cytotoxicity, biocompatibility with tissues and cells, and prolonged residence time (62). PLGA is used in several commercially available drug delivery products, including Lupron Depot (Abbott Laboratories, USA) and Ozurdex (Allergan, Inc., USA) (63). To induce a strong CTL response and an efficient antigen cross-presentation, biodegradable poly(lactic-*co*-glycolic acid) nanoparticle (PLGA-NP)-based vaccine delivery systems need to be accurately designed and fabricated. This involves optimizing the method of preparation, characterization technique, surface modification, and drug release mechanism to increase vaccine efficacy (62). Encapsulation of antigens in PLGA-NPs can induce cross-presentation at much lower concentrations, prolonged antigen release and T-cell stimulation, and potent immune responses (62, 64, 65).

Dhakal et al. observed a robust T cell-based immune response in pigs vaccinated intranasally with 200- to 300-nm-sized spherical PLGA-NPs encapsulating the swine influenza virus H1N2 antigenic protein KAg (PLGA-KAg) (66). *In vitro* PLGA-KAg treatment increased APC maturation by 40% in monocyte-derived dendritic cells of pigs than KAg alone or empty PLGA-NP treatment. On subsequent challenge with heterologous swine influenza virus H1N1, PLGA-KAg vaccinated pigs were protected from clinical symptoms and lung pathology. In contrast, the mock-vaccinated pigs had a fever for 4 days with macroscopically visible lung lesions (66). Alkie et al. generated chitosan-coated PLGA-NPs with encapsulated cytosine-phosphate-guanine oligodeoxynucleotide (CpG ODN) and inactivated

TABLE 3 Bioengineering approaches utilized in vaccine development

Bioengineering approach	Targeted virus	Material	Antigen	Reference(s)
Polymer particles	Influenza	PLGA-NPs	H1N2 antigenic protein KAg	66
			CpG ODN and inactivated avian flu H4N6	67
		Polyanhydride NPs	Swine influenza a virus H1N2 antigens	76
		Block copolymer of pyridyl disulfide ethyl methacrylate and dimethylaminoethyl methacrylate plus propyl acrylic acid, butyl methacrylate, and dimethylaminoethyl methacrylate	H1N1 HA antigen	79
	SARS-CoV	Polyethylenimine NPs	S-encoding DNA in plasmid	82
	SARS-CoV-2	PLGA and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol)–maleimide	S RBD linked on the surface and encapsulated STING agonist	70
	Influenza	N-trimethyl chitosan NPs	Monovalent influenza A H3N2 subunits	98
		Acetalated dextran microparticles	H1N1 M2 protein ectodomain	105
Inorganic nanoparticles	SARS-CoV	Biotinylated chitosan NPs	N protein-encoding plasmid DNA	95, 97
	Influenza	Silver NPs	H3N2 inactivated flu virus	110
			Plasmid DNA vaccines for the H5N1 influenza virus	111
Liposomes	Influenza	Lecithin-phospholipid liposome (Inflexal V)	HA surface molecules of H1N1, H3N2, and B/Massachusetts/2/2012 virus	119, 120
		Oleoyl liposome	H3N2 Np	121
		Multilamellar negatively charged liposome vesicles	HA and NA	122
	SARS-CoV	Liposomes	N-protein epitope	123, 124
			Polyprotein 1a	121
	Influenza	Synthetic lipid NPs	Universal influenza mRNA vaccine encoding wild-type H1N1 NA and Np and shortened HA and M2	129
		Liposomes	mRNA encoding viral proteins like HA, NA, M1, and M2 (products: mRNA-1851 for H7N9 and mRNA-1440 for H10N8)	137, 138
		Liposomes	Modified nucleoside-containing mRNA for synthesizing H1N1 HA-encoding mRNA	131, 232
	SARS-COV-2	Liposomes	mRNA encoding full-length S protein (mRNA-1273)	133–136
			mRNA for full-length S protein (Comirnaty)	139–142
			LNP-encapsulated mRNA for full-length S protein (CVnCoV)	144, 145
			LNP-encapsulated mRNA for full-length S protein of D614G variant	146

TABLE 3 (Continued)

Bioengineering approach	Targeted virus	Material	Antigen	Reference(s)
		SAM	Full-length SARS-CoV-2 S-encoding mRNA	152
		Liposomes	Full-length SARS-CoV-2 S-encoding mRNA using Lunar technology (Lunar-CoV/ARCT-021)	153
		Lipid inorganic NPs	SARS-CoV-2 S-encoding sequence	155
	Influenza	Liposomes	H1N1 HA-encoding plasmid DNA	159
			M1 protein-encoding plasmid DNA of H1N1	160
Self-assembling proteins	Influenza	VLPs	HA and NA	166
	SARS-CoV, influenza	VLPs	M, N, and S glycoproteins in an expression vector	169
	MERS-CoV, SARS-CoV, SARS-CoV-2	VLPs	Recombinant baculovirus having genes encoding S, E, and M proteins	167, 168
	Influenza	OMVs with modified lipopolysaccharides at lipid A moiety	NA	173–180
	Influenza, MERS-CoV	OMVs	Recombinant HA from influenza A H1N1 and the RBD from MERS-CoV S	179
	Influenza	Ferritin NPs	H1N1 HA protein	184
	SARS-CoV-2	Self-assembling ferritin	S or its RBD	185, 186
	SARS-CoV-2	Polysorbate-20 micelles NPs	SARS-CoV-2 S proteins	189, 192–195, 233, 234
	Influenza	Icosahedral I53_dn5 protein NP	HA ectodomain of H1N1, H3N2 and two B-lineage viruses	188
Microneedles	Influenza	High-density microarray patch	Split inactivated influenza vaccine against H1N1 (Vaxxas)	203, 204
	MERS-CoV	Dissolvable carboxymethyl cellulose microneedle array	MERS S	205
	SARS-CoV-2	Dissolvable carboxymethyl cellulose microneedle array	SARS-CoV-2 S	205
Electroporation	Influenza	Injection method specifically to deliver electric constant-current pulses along with injecting antigen	H1N1 HA synthetic mRNA for influenza, S-encoding synthetic DNA for MERS-CoV and SARS-CoV-2	208
	MERS-CoV			212
	SARS-CoV-2			213–216

avian flu H4N6, where the encapsulation was performed by adding CpG ODN and H4N6 during PLGA-NP preparation (67). The outer surface of PLGA-NPs was coated with chitosan via adsorption. As chitosan is mucoadhesive, it enhances the interaction between NPs and mucus, which leads to an increase in the retention time of vaccines on mucosal membranes. At 4 weeks after vaccination, it was observed that mucosal (ocular and nasal) vaccination of chickens with the chitosan-coated PLGA-NPs induced 2-fold higher antigen-specific IgG in sera and 1.5 and 2-fold higher IgG and IgA titers, respectively, in lachrymal secretions compared to those induced by free inactivated viral antigens. Chitosan-coated PLGA-NPs showed the induction of similar hemagglutination inhibition titer as free inactivated viral antigens (67).

Stimulator of interferon genes (STING) generates a potent antiviral innate immune response via activation of the cytosolic DNA-induced type I interferon (IFN) by preferentially promoting the generation of antigen-specific antibodies, reinforcement of CTL, and impairing the suppressive activity of regulatory T cells (68). SARS-CoV-2 S protein adjuvanted with modified STING has been observed to induce nearly 30-fold higher antigen-specific IgG levels than free S protein (69). Lin et al. constructed hollow 114-nm viromimetic PLGA-NPs made using the water-in-oil-in-water double emulsion method, wherein the STING agonist cyclic di-GMP was encapsulated as an adjuvant (70). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) was added for surface functionalizing the outer shell. After these NPs were prepared, recombinant S-protein RBD of MERS-CoV was conjugated to the 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) via thiol-maleimide linkage. The structural and functional antigenicity of these PLGA-NPs protected the mice from the lethal infection challenge. High neutralizing antibody titers (~150-fold compared to that of the control group injected with only STING agonist-encapsulated PLGA-NP), no detectable infectious viral load (control having an ~2.6 $\log_{10}$ median tissue culture infectious dose), and 100% survival (0% survival in control) demonstrated the efficacy as well as the safety of PLGA formulations. Moreover, the construct was stable during storage and showed sustained release of the STING agonist for at least 100 h at 37°C during dialysis at pH 7.4. The release rate was much faster at pH 5, as expected due to the acid-catalyzed hydrolysis of PLGA particles (70). Such systems allow the PLGA-NPs to release the bulk of the drug once they reach the endosomal vesicle, improving delivery and immune response efficacy (71).

One of the drawbacks of PLGA is that antigen degradation can occur during vaccine preparation, storage, or after delivery (72). Unfolding, chemical instability, and aggregation are all examples of protein instability that occur when encapsulated in PLGA, resulting in a loss of antigenicity. PLGA particles may aggregate, and maintaining sterility during and after the PLGA particles are synthesized is challenging, as PLGA cannot be sterile filtered (72). The stability of the antigen can be improved by using new techniques, such as self-healing PLGA-NPs with an interconnected network of pores that trap the protein within the pores using a protein-trapping agent (65). This prevents the proteins from exposure to the harsh microenvironment and the resulting degradation (65).

Polyanhydride NPs are used to deliver several vaccines as they stimulate the innate immune response by engaging Toll-like receptors (TLRs) and activating APCs, thereby acting as an adjuvant (73, 74). These are inert surface-eroding polymers that protect the antigen from degradation and retain its biological and structural activity (75). Dhakal et al. also used polyanhydride-based NPs to encapsulate swine influenza A virus H1N2 (KAg), which presented a 6- to 8-fold reduction of nasal shedding compared to sham control (mock or without NPs) at 4 days postchallenge in pigs (76). These NPs were made of a copolymer containing 1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane and 1,6-bis(p-carboxyphenoxy)hexane monomers combined in an 80:20 ratio. There was an increase in interferon gamma (IFN-γ)-secreting T lymphocyte in the KAg-NP group compared to levels with the antigen alone (76). Other similar studies indicate that the polyanhydride sphere makes a good potential vaccine delivery carrier (77, 78).

Knight et al. demonstrated a polymer-based pH-responsive NP that generates antigen-specific CD8$^+$ tissue-resident memory T cells in the lungs (79). The NP comprises

of two functional blocks, a hydrophilic copolymer made up of pyridyl disulfide ethyl methacrylate and dimethylaminoethyl methacrylate and a hydrophobic pH-responsive block composed of propyl acrylic acid, butyl methacrylate, and dimethylaminoethyl methacrylate. Mice immunized with an H1N1 antigen-carrying NP formulation were given a lethal viral challenge after 30 and 60 days. The survival rate at day 60 was 83% for CpG-adjuvanted NP group, compared to 0% for free antigen and sham groups (79).

Polyethylenimine is a cationic polymer that finds extensive use in nucleic acid delivery owing to its high transfection efficiency and buffering capacity (80). Particle-DNA complexes are also known to induce vigorous mucosal and systemic immune responses (81). Shim et al. demonstrated the intranasal delivery of S-encoding plasmid DNA encapsulated in polyethylenimine NPs for immunization against SARS-CoV (82). There were 15- and 4-fold increases in the production of S-specific IgG1 in sera and IgA in lung mucosal wash, respectively, compared to those immunized with naked S DNA plasmid (82). One of the main translational obstacles of polyethylenimine is the safety issue. It is known for its high cytotoxicity, as the high positive charge on the surface of these NPs can cause necrotic cell death and apoptosis (83). However, some modifications, such as using linear and lower-molecular-weight polyethylenimine and shielding the surface charge, can reduce the cytotoxicity (84).

Other synthetic polymeric materials have also been explored for vaccine delivery applications like biodegradable pluronic-stabilized polypropylene sulfide as an antigen nanocarrier system (85). Among the synthetic polymers, only PLGA-based vaccines have been widely studied and shown to have high safety. Several other biomaterials are still in the initial stages of research, and more evidence is therefore required to test their safety.

Natural polymers. Chitosan, a natural cationic polymer of *N*-acetyl-D-glucosamine and D-glucosamine, is an ideal nucleic acid delivery vehicle (86). It is highly biocompatible, nontoxic, and biodegradable (86). It demonstrates a strong binding affinity with nucleic acids, cell surfaces, and mucous membranes due to the presence of several amines that impart a positive charge on it (87, 88). Studies have shown that chitosan can function as an efficient adjuvant by inducing a type I IFN response by activating STING (89). Chitosan particles can be engineered to improve selectivity and protect the antigen (90). Chitosan NPs can be utilized for several other delivery applications, such as for adjuvants, drugs, genes, and proteins; preventing wound infections; enhancing healing; and as a skin regeneration material in anti-aging skincare products, among numerous other uses (86, 91–93). Moreover, it is a suitable nasal vaccine delivery system, as it enhances the antigen absorption by mucosal lymphoid tissues and causes robust immune responses against respiratory viral diseases (94). Raghuwanshi et al. demonstrated a plasmid DNA (pDNA) vaccine encoding SARS-CoV N protein (pVAXN) loaded in biotinylated chitosan NPs with a surface-functionalized bifunctional fusion protein (bfFp) for dendritic cell-targeted immunization (Fig. 5) (95). The bfFp had a streptavidin site to bind to the NP, as well as an anti-DEC-205 antibody, which binds specifically to the dendritic cells. At the same time, the pVAXN encapsulation was done during chitosan NP preparation using a complex coacervation method. The addition of the bfFp allowed these particles to perform selective targeting to dendritic cells. It hence reduced the dosage of the antigen by approximately 500-fold compared to the nontargeted antigen to get a similar immune response to that previously established (96). The mice were intranasally immunized with the NPs mixed with free anti-CD40 for dendritic cell maturation. It was observed that this experimental group had significantly higher N protein-specific serum IgG and mucosal IgA and IFN-γ levels compared to only chitosan NP, only pVAXN, or NP plus bfFp groups (95, 97).

Amidi et al. prepared an intranasal delivery system using the electrostatic interaction between the positive surface charge on *N*-trimethyl chitosan NPs of 800-nm size and the negatively charged monovalent influenza A H3N2 subunit at room temperature and pH 7.4 (98). After administering antigen-conjugated NPs, the authors observed 10-fold higher anti-influenza antigen-specific serum IgG titers than the free antigen (98). This vaccination technique is a potential noninvasive method of targeted antigen delivery at low doses and can be employed for other antigens as well. Similar
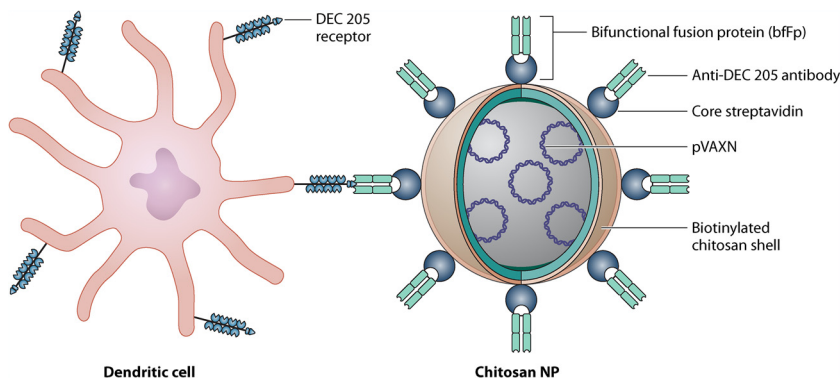
FIG 5 Design of a chitosan-based nanoparticle having encapsulated plasmid DNA (pDNA) vaccine encoding SARS-CoV N protein (pVAXN) and surface-functionalized bifunctional fusion protein for enhanced dendritic cell targeting.

studies have been carried out using chitosan as the delivery vehicle carrying different influenza proteins (HA, NA, M, and/or Np) alone or in combination (99–102).

Natural polymers, such as polysaccharides like pullulan, alginate, inulin, dextran, and cellulose, have been used to make NP vaccines for infectious diseases (103, 104). Researchers have been trying to use natural polymers to develop respiratory virus vaccines; e.g., Chen et al. investigated the coadministration of cyclic GMP–AMP and the M2 ectodomain protein of H1N1, both encapsulated in acetalated dextran (hydroxyl groups of dextran modified with acetal moieties) NPs, to enhance IgG antibody titer by nearly 5-fold and mice survival rate to 80% compared to 20 to 30% survival with M2 protein alone (105). Most of these natural polymers are explored as adjuvants in vaccine formulation (106).

Inorganic Nanoparticles

Metal NPs, especially gold, have been used extensively in vaccine research against various infectious diseases due to their adjuvant properties and ease of synthesis and functionalization (107). Gold and silver NPs are known to be effective platforms for the bioconjugation of protein and nucleic acid antigens or can be used as adjuvants (107–109).

Researchers have used inorganic NPs chemically conjugated or coated with the antigen to develop subunit flu vaccines. Inactivated H3N2 influenza A virus (IAV) adjuvanted with silver NPs (AgNPs) have been shown to reduce viral loads by 100-fold compared to only IAV alone (110). Pulmonary immunization with IAV and AgNPs increased the IgA antibody titer in bronchoalveolar lavage by 75-fold compared to free IAV. A 50-fold increase in virus-specific IgA-secreting plasma cell number compared to IAV plus polyinosinic:poly(C) (a TLR-3 ligand) and AddaVax (an MF59-like adjuvant) was also observed in mice (110). Jazayeri et al. demonstrated the oral delivery of a plasmid DNA vaccine for avian influenza virus H5N1 using AgNPs in chickens (111). The AgNPs and H5 hemagglutinin-containing plasmid (pcDNA3.1) self-assembled into nanocomplexes (AgNP/H5) via electrostatic interactions and enhanced the T_H1-like proinflammatory response in chickens. On day 7, the AgNP/H5 plasmid nanocomplexes produced 2-, 5-, 12-, and 10-fold changes in cytokine expressions for interleukin 1 beta (IL-1β), TNFSF13B, IL-15, and IL-12β, respectively. Also, about 2- to 5-fold higher CD8$^+$ and CD4$^+$ T-cell proliferation and 18-fold higher IL-18 levels were seen in AgNP/H5 plasmid-injected chickens than AgNP plus empty vector group on day 14 (111).

Although some studies have shown that the use of inorganic NPs enhances the delivery of DNA vaccines, there are concerns that inorganic NPs accumulate in organs such as the liver and spleen for a long time, leading to organ damage if multiple doses of vaccines are given (112). Compared to the well-established field of degradable and organic NPs, the clinical translation of inorganic NPs is still under debate, mainly due to their nondegradability and lack of long-term toxicity assessment (113).
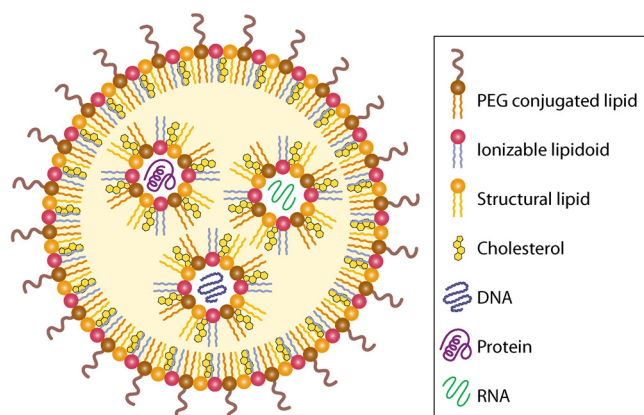
FIG 6 Schematic representation of lipid nanoparticle encapsulating cargo such as DNA, RNA, or protein antigens.

Liposome-Based Vaccines

Liposomes have widely been studied and used to generate vaccines (114). Liposome-based vaccine systems are composed of micro- or nanosized vesicles made up of nontoxic and biodegradable phospholipid bilayers. These layers usually comprise an ionizable lipidoid, polyethylene glycol (PEG), structural lipids, and cholesterol. They can be used to encapsulate various chemicals, such as drugs, nucleic acids, and proteins (Fig. 6). Liposomes can be modified to the required size, charge, surface chemistry, and entrapping capacity for the antigen or adjuvant (114–116).

Researchers have also found liposomes useful for site-specific delivery by conjugating them with ligands specific for the targeted sites (117). Many liposomal vaccine formulations have already been approved for clinical use, such as Infexal for influenza and Epaxal for hepatitis A infection (118, 119). Based on the type of cargo, we have defined them in the following categories.

Protein–liposome-based nanoparticles. Liposomes find varied use in the delivery of peptides (116). Inflexal V is a clinically approved virosomal influenza vaccine that Crucell Berna Biotech developed. It has HA surface molecules of H1N1, H3N2, and B/Massachusetts/2/2012 virus, fused with a lecithin-phospholipid liposome double membrane (119). The vaccine comprises 70% lecithin, 20% cephalin, and 10% viral envelope phospholipids, which form 150-nm-diameter unilamellar spherical vesicles (119). Conne et al. demonstrated that 68.4% of subjects injected with a single-dose intramuscular injection of Inflexal developed protective levels of antibody titer (anti-HA titer $\geq$ 40), whereas only 38% of subjects injected with the subunit vaccine (Influvac) attained protective antibody titer values (120).

In another study, Nagata et al. conjugated H3N2 influenza Np protein on the surface of oleoyl liposomes using the cross-linking reagent disuccinimidyl suberate and adjuvanted it with CpG ODN (121). It provided a protective response in mice and demonstrated almost 25-fold higher viral inhibition than that of saline control. The study also demonstrated that surface-conjugated ovalbumin was 6-fold more effective in inducing CTL activity *in vivo* than multilamellar liposome with encapsulated ovalbumin (121).

Joseph et al. utilized the adjuvant activity of CpG ODNs for generating vaccines with HN (HA and NA proteins of influenza) antigens encapsulated in multilamellar negatively charged liposome vesicles (diameter of 1.3 to 1.5 μm) (122). Liposomes were made up of dimyristoyl-phosphatidylglycerol and dimyristoyl-phosphatidylcholine in a ratio of 1:9. Mice coimmunized with HN+Lip(ODNs) showed 10- and 6-fold higher IgG2a levels in the serum and the lungs of mice, respectively, compared to those in the mice coimmunized with HN+free ODNs. The vaccine formulations containing [Lip−ODNs]+HN, Lip[ODNs+HN], and Lip[ODNs]+Lip[HN] were around 30-fold more effective than free ODNs or HN antigens in producing antigen-specific antibodies, IFN-γ production, proliferative response, and protection against virus challenge (122).

Ohno et al. also designed an oleoyl liposome vaccine with a SARS-CoV N-protein epitope surface linked using disuccinimidyl suberate and adjuvanted with CpG ODN (123). The virus titers in mice vaccinated with the liposomal formulation were 1,000-fold lower than those in mice vaccinated with empty liposomes (123). In their subsequent study, Ohno et al. used similarly produced oleoyl liposome with surface-conjugated SARS-CoV nonstructural protein polyprotein 1a for vaccine production and showed the induction of high titers of CD8$^+$ T cells, indicating an increase in cross-presentation (124). The overall strategy used is fast and effective, but it is yet to be seen whether the results in humans are similar, as there are differences in antigen processing and presentation.

mRNA–liposome-based nanoparticles. RNA-based vaccines are of two types, traditional mRNA and self-amplifying mRNA (SAM) vaccines. The basic principle behind such vaccines is to use the host cell's translation system for the generation of peptide antigens and, therefore, trigger an adaptive immune response leading to the generation of antibodies and memory T cells (125). mRNA-based vaccines provide many benefits, such as easy large-scale production, safety, and capacity to be presentation. [functionally modified to selectively express antigenic structures, reduce immunogenicity and increase stability (126)]. mRNA can be formulated with nanocarriers to increase their uptake and cytoplasmic expression and protect them from premature degradation (127). Liposomal delivery of mRNA for increasing cellular uptake has been extensively explored in vaccinology (128).

Freyn et al. developed a universal influenza mRNA vaccine encoding wild-type H1N1 NA and Np and shortened HA and M2, or only these individual components encapsulated in synthetic lipid 80 nm particles using a self-assembly process in which the mRNA was added to an acidic solution containing the cationic lipid, cholesterol, PEG, and phosphatidylcholine under rapid stirring (129). The encapsulated combination mRNA and NA mRNA vaccines provided the highest neutralizing antibody titers in mice, i.e., nearly 15- and 30-fold that provided by the approved influenza vaccine, respectively (129).

Injected viral mRNA can activate the cytoplasmic RNA sensors; hence, modified mRNA nucleotides are used to reduce the activation of RNA-sensing TLRs and their downstream innate immune signaling, which can hinder the translation of the antigen-encoding mRNA (130). A similar study performed by Pardi et al. showed that using 1-methylpseudouridine instead of uridine to develop modified nucleoside-containing mRNA for synthesizing H1N1 HA-encoding mRNA increased the survival rate in mice (100% compared to 0% of the unmodified monovalent vaccine without liposomes) and provides complete protection against H1N1 and H5N1 (131). Previous studies performed by them have shown that this modification increases the expression and decreases the immunogenicity of the mRNA even at low doses, especially when encapsulated with liposomes (132). Such enhancements have a high potential to add to the efficacy of recently successful mRNA vaccines.

Keeping in mind the urgent need for a vaccine against SARS-CoV-2, the mRNA-1273 vaccine designed by Moderna consists of prefusion-stabilized SARS-CoV-2 S-protein trimer sequence mRNA (two consecutive proline substitutions introduced at the loop between the first heptad repeat and the central helix to maintain the stability and homogeneity of the prefusion spike morphology) encapsulated in liposomes constituted using cholesterol, an ionizable lipid, PEG, and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (36, 133, 134). It was well tolerated and induced reliable seroconversion in healthy adults, with neutralizing antibody induction equivalent to that of convalescent patient sera and the capacity to neutralize at least 80% of SARS-CoV-2 viruses on infection, just 15 days after the prime-boost regime of 100 μg mRNA was delivered intramuscularly (134). Similar results were observed in the phase 1 study in older adults with efficient induction of a T_H1 response (135). mRNA-1273 showed 94.1% vaccine efficacy in phase 3 trials and is now approved for clinical administration (136).

A similar formulation previously designed by Moderna encodes HA of H10N8 (mRNA-1851) or H7N9 (mRNA-1440) encapsulated in the same liposome composition,

which provided complete protection to mice against a viral challenge and high hemagglutination inhibition titers in ferrets and nonhuman primates (137). Their phase 1 trials showed that the vaccines were well tolerated and had seroconversion rates of 87% for 100 μg H10N8 formulation and 100% for 50 μg H7N9 formulation, both delivered intramuscularly (138).

Another vaccine approved for SARS-CoV-2, developed by Pfizer-BioNTech, is Comirnaty, also known as BNT162b2, a liposomal NP encapsulating similarly prefusion-stabilized RBD-encoding mRNA that showed 95% efficacy in human trials (139–142). It has been observed to be well tolerated in human trials with S1-specific IgG, virus-neutralizing titers, and IFN-γ-secreting CD4$^+$ and CD8$^+$ titers to be about 10^4-, 30-, and 2,500-fold prevaccination values, respectively, at 30-μg intramuscularly delivered prime-booster doses (140). However, Chang et al. observed that although BNT162b2 also developed a broad cross-reactivity against mutated SARS-CoV-2 strains, the recognition potential was 2.5- to 10-fold lower for variants compared to that for the original virus (143).

CureVac also synthesized SARS-CoV-2 S-encoding mRNA with similar stabilizing sequence modifications entrapped in liposomes, called CVnCoV, and found that vaccinated hamsters had nearly 6-fold lower lung viral load compared to that of hamsters treated with S protein plus alum (144). Their phase 1 study showed that it was safe for human inoculation and induced strong immune responses and complete seroconversion in all participants at just 12-μg prime-booster doses (145). It is interesting to note that CVnCoV could induce a significant immune response at such low doses (compared to 100 μg of mRNA-1273 or 2 mg of INO-4800), making it advantageous for quick large-scale production and distribution. The study is currently in phase 2/3 clinical trials (ClinicalTrials.gov identifiers NCT04652102 and NCT04674189). A similar vaccine was made by Hong et al. using full-length SARS-CoV-2 S-encoding mRNA of the more infectious and fatal variant D614G (146). The uniqueness of this vaccine is that it can be lyophilized without loss of immunogenicity and is, therefore, highly advantageous for storage and distribution (146).

Self-amplifying mRNAs (SAMs) are another type of mRNA vaccine strategy made from engineered RNA viruses such as the *Alphavirus* genus (147). The structural genes are replaced with the gene of interest (148, 149). As they still possess the genes coding for the viral RNA replicase, when the SAM enters the cells, such as APCs, the replicon is expressed at a higher frequency, leading to the generation of multiple copies of the antigen protein for enhanced immune responses at doses much lower than those for traditional mRNA (142, 150, 151). McKay et al. designed a liposome-encapsulated SAM construct encoding the same modified SARS-CoV-2 S-encoding sequence used in mRNA-1273 (152). It generated SARS-CoV-2 neutralizing antibody titers 100-fold higher than those generated by convalescent patient sera and electroporated plasmid DNA, as well as a remarkable T$_H$1-biased cellular immune response. Here, the ionizable cationic lipid dilinoleylmethyl-4-dimethylaminobutyrate electrostatically interacts with the SAM for quick encapsulation and helps in endosomal escape after endocytosis (152). Lunar-COV19/ARCT-021 is another lipid-encapsulated SAM vaccine encoding full-length S mRNA of SARS-CoV-2 using the lipid-enabled and unlocked nucleic acid modified RNA (Lunar) technology (153). The liposomes include the proprietary lipid ATX (Arcturus Therapeutics), which is pH sensitive and has ester bonds that are chemically stable during storage but easily degraded by esterases present in tissues and intracellular environment after delivery (154). In mouse studies, the vaccine was observed to be safe, and SARS-CoV-2 neutralization titers were observed to be nearly 15- and 3-fold higher than those for free mRNA and convalescent patient sera, respectively (153). It is currently in phase 1/2 clinical trials (ClinicalTrials.gov identifiers NCT04668339 and NCT04480957).

Erasmus et al. also constructed a SAM vaccine encoding the full-length S mRNA sequence of SARS-CoV-2 encapsulated in lipid inorganic NPs, which provided reliable protection in preclinical studies in mice and nonhuman primates (155). Lipid inorganic NPs are a nanoemulsion composed of cationic squalene emulsified with superparamagnetic iron

oxide, and this mixture is embedded in the commonly used immunogenic cationic lipid 1,2-di-oleoyl-3-trimethylammonium propane (155). The presence of 1,2-di-oleoyl-3-trimethylammonium propane enables complexing with the mRNA molecules through electrostatic interactions and acts as a beneficial delivery vehicle for SAM vaccines to protect them from premature degradation and enhance host cell uptake (156, 157). From the studies using SAM for vaccination, it is evident that its significantly higher expression level provides an advantage with heightened immunogenicity (158).

DNA–liposome-based nanoparticles. Similarly to proteins and RNA, DNA can also be delivered through liposomes. Wang et al. demonstrated that intranasal administration of H1N1 HA-encoding plasmid DNA encapsulated in liposomes synthesized with 1,2-dioleoyl-3-dimethylammonium chloride, 1,2-dioleoyl-sn-glycero-3-phosphoenthanolamine, and PEG C8 enhances humoral immunity. Mice immunized with this formulation showed 4- and 10-fold increases in serum IgG and IgA levels, respectively, compared to those for naked DNA and saline controls on PR8 viral challenge and ensured 100% survival (compared to 0% in controls) (159). In a study by Liu et al., mice were shown to be protected from influenza infection after administration of an oral vaccine containing the liposome-encapsulated M1 gene of H1N1 influenza (160). The human gastrointestinal tract has gut-associated lymphoid tissue comprising more than 300 m^2 of immunosensitive mucosal surface, and hence the oral route is appealing for vaccination (161). After day 7 of liposome-DNA immunization of mice, the authors observed a 2-fold increase in IFN-γ and IL-4 cytokine levels in bronchoalveolar lavage fluid and IgG in sera compared to those with naked DNA. Also, the CTL activity induced by the liposome-DNA complex was 50% higher than that induced by naked DNA (160).

Overall, liposomes have proved to be an efficient carrier for proteins and nucleic acid vaccines. They are easy to synthesize on an industrial scale and can be adapted for delivering many types of biomolecules or drugs and can even be modified to control the release using pH-responsive additives, as described above. Protein-liposome complexes have shown promising *in vivo* results due to reduced extracellular degradation and better delivery; however, protein synthesis for large-scale immunization is time consuming. Liposome-mRNA complexes, on the other hand, have been translatable and have successfully been deployed for quick human immunization during the COVID-19 pandemic. mRNA vaccines have no requirement for integrating with host DNA for their expression and can simply use the host translation machinery for *in vivo* antigen production; hence, they do not pose the risk of causing mutations in the host genome. The mRNA vaccines discussed above have also not reported adverse effects, such as lung pathology in mice, and have shown efficiency at low doses. With the use of liposomes to deliver mRNA vaccines, targeted delivery and vaccine stability have also improved. Although DNA-liposome complexes have shown promising results and can result in a prolonged antigen expression compared to that produced by mRNA vaccines, they pose some potential risks, including integration with the host genome and vertical transmission (162). Therefore, protein and mRNA liposome vaccines can be considered to be safer than DNA-liposome complexes.

Self-Assembling Nanoparticles

Virus-like particles (VLPs) are viral subunits that can self-assemble to form 20- to 200-nm NPs that bud from the infected host cell (163). They have a 3-dimensional conformation similar in structure to that of the virus of origin but are deficient in nuclear material, hence removing the hazard of reverting to a virulent state while preserving the structural antigenicity of the virus (164). Several VLP-based influenza vaccine formulations have been tested on mouse and ferret models (165). These VLPs generally contain the HA and NA components in the membrane (166). MERS-CoV, SARS-CoV, and SARS-CoV-2 VLPs are typically created by expressing the native or modified S, E, and M proteins (167, 168). Other modifications that augment VLPs are the addition of N protein to enhance packing, encapsulating adjuvants for enhanced immune response, or inserting the structural genes of interest into viruses infecting other species, such as canine parvovirus or mouse hepatitis virus to generate chimeric VLPs (169). While VLP

vaccines impart an increased neutralizing antibody titer and systemic response, they have been observed to cause lung pathology in mice on viral challenge due to their high immunogenicity, with substantial eosinophil infiltration suggestive of T_H2 hypersensitivity, despite having no viral load (169, 170). This could lead to safety concerns regarding VLP vaccines and needs further testing.

Outer membrane vesicles (OMVs) are spherical membrane-bound vesicles naturally generated by Gram-negative bacteria via budding and play a crucial part in bacterium-host interactions like delivering pathogenic cargo and altering the host immune response (171). Their ease of production, modifiability, and self-adjuvant properties make them an excellent choice for vaccine manufacture (172). One OMV vaccine against meningitis has been approved (4CMenB/Bexsero) (173), and several are in clinical trials (174–178). Shehata et al. engineered a dual OMV vaccine containing recombinant HA from influenza A H1N1 and RBD from MERS-CoV S by transforming *Escherichia coli* to secrete OMVs containing the additional protein subunits together (179). After prime-boost vaccination, mice showed hemagglutinin inhibition antibody titers against H1N1 and neutralizing antibody titers against MERS-CoV to be almost 2- and 3.5-fold higher, respectively, than those for the control groups (empty OMV or phosphate-buffered saline [PBS]), hence showing its effectiveness in eliciting specific immune responses against both pathogens, while demonstrating complete protection on H1N1 challenge in mice (179).

In another study, Bae et al. demonstrated that administering an attenuated bacterial OMV with modified lipopolysaccharides at lipid A moiety provides better protection against diverse influenza A viruses (H1N1, PR8, H5N2, and H5N1) compared to that provided by other TLR ligands (180). The modified OMV-injected mice showed an ~80 to 100% survival rate when challenged with PR8, H5N2, and H5N1 viruses at lethal doses, which was significantly higher than that with TLR ligands (180). However, OMV vaccines are highly immunogenic due to lipopolysaccharides and other bacterial pathogen-associated molecular patterns (PAMPs) on their surface (181). They might need modification of their lipopolysaccharide composition to make them safe for human use. The stability of the formulation needs to be evaluated along with the assessment of its effects in higher animal models.

Another example of a self-assembled protein NP is ferritin, an iron storage protein consisting of 24 subunits (mass, 450 to 500 kDa), naturally forming hollow spheres of 12-nm outer diameter (182). Ferritin has been reported widely for its application in biomedical engineering because of its unique features, such as the formation of monodisperse particles due to natural polymerization, genetically modifiable surface groups that allow the insertion of antigens for displaying on its cage-like surface, and the potential to encapsulate drugs, vaccines, or adjuvants in its hollow core (183). Kanekiyo et al. found that ferritin NPs that displayed H1N1 HA protein could elicit 10-fold higher HA inhibition titers in mice than the licensed trivalent inactivated vaccine (2006–2007 and 2011–2012 Fluzone from Sanofi Pasteur containing HA from H1N1, H3N2, and influenza B viruses) (184). Yao et al. designed complexes made from human L-ferritin conjugated with SARS-CoV-2 S RBD that self-assembled to form 15-nm particles and showed a 1.5-fold higher affinity of binding to the angiotensin-converting enzyme 2 receptor than the native RBD (185). Another study incorporating full-length S-protein trimer in ferritin NPs showed that it is safe and immunogenic to induce S-specific neutralizing antibodies 1,000-fold higher than free RBD in a single dose in mice (186).

Self-assembling particles can also be designed using synthetic proteins; one such example is the self-assembling icosahedral I53_dn5 protein NP (187). The I53_dn5 particle is a two-component system made up of a 12-pentameric protein, I53_dnA, and a 20-trimeric protein, I53_dnB (187). The authors used this platform to develop a universal influenza vaccine in which the N terminus of trimeric component of the protein (I53_dnB) is genetically fused to the influenza hemagglutinin ectodomain (H1N1, H3N2, and two B lineage influenza virus) of four viruses circulating during the 2017 to 2018 seasonal flu (188). This construct was tested for the immune response elicited by

both the mosaic (HA proteins of all four viruses on a single particle) and the cocktail NP vaccine (carrying each of the four HA proteins on separate particles). The authors observed similar or higher antibody responses, HA inhibition titers, and microneutralization titers for both mosaic and cocktail NPs against vaccine-matched and mismatched strains of influenza compared with the approved quadrivalent vaccine in mice, ferret, and macaques. Heterosubtypic virus challenge (challenge with influenza A virus of subtypes other than those included in vaccine) showed >95% survival (until day 14) in mice vaccinated with both mosaic and cocktail particles along with AddaVax adjuvant, while the quadrivalent vaccine showed <25% survival of mice. The geometric assembly of the conserved ectodomain of influenza on the I53_dn particle enhances the interaction of stem region-directed antibodies. Hence, the immune response given by the I53_dn5 particle is cross-reactive to different types of influenza A viruses (188). The cytotoxic effect of I53_dn particle alone has not been studied yet and more research is required to understand the safety of this self-assembling protein strategy.

Another example of a self-assembling particle is Novavax's NVX-CoV2373, which was synthesized using full-length stabilized SARS-CoV-2 S proteins self-assembled on polysorbate-20 micelles. It forms 27.2-nm NPs and is adjuvanted with Matrix-M, a plant saponin-based immune-stimulating complex (189, 190). It was designed based on mouse studies using SARS-CoV and MERS-CoV S-protein micellular NPs, which demonstrated neutralizing antibody generation 36- to 39-fold higher in SARS-CoV S NP plus Matrix M and 27- to 68-fold higher in MERS-CoV S NP plus Matrix-M than their respective S-NPs only (191). Studies on nonhuman primates showed postvaccination neutralizing antibody titers about 300% higher than those of the placebo group and 37% higher than those for convalescent patient sera, anti-S IgG nearly 37% higher than that for convalescent-phase sera, and complete protection on infection (189, 192). Clinical trials showed that NVX-CoV2373 was safe and highly immunogenic in all populations, including older adults, and generated neutralizing antibodies 4- to 6-fold higher than those generated by convalescent patient sera (193–195). It showed an efficacy of 89.7% against the original SARS-CoV-2 virus, 86.3% against the Alpha variant, and 60.1% against the Beta variant (194, 196). It was also found that coadministering NVX-CoV2373 with seasonal influenza vaccines did not affect its efficacy or safety (197). Coadministration of multiple vaccines with no effect on the efficacy of any and no additional safety risks can be a valuable asset for efficient and faster global immunization.

One significant benefit of using NPs that can self-assemble is that the antigens displayed by these NPs can be designed to retain their native configuration, mimicking their presence on viral membranes, hence providing the benefit of structural antigenicity. Besides, unlike the synthetic particles described in the last section, antigen presentation with self-assembling polymers such as ferritin is uniform across the particle, and there is no need to perform separate chemical reactions to add the antigen to the particles. Self-assembling NPs can encapsulate nucleic acid and protein antigens and can also be complexed with proteins for surface display.

Microneedle Arrays for Vaccine Delivery

Microneedles array patches are an array of micron-scale needles that can efficiently pierce the skin's stratum corneum without affecting underlying pain receptors for painless delivery of the drug or vaccine. The drug can either be coated over the solid metallic surface or encapsulated inside the hollow needle matrix (198, 199). These vaccines can have a long shelf life (>1 year) at room temperature and can be self-administered, making them suitable for mass production and distribution (200–202). Microneedles can deliver vaccines consisting of inactivated viruses, proteins, virus particles, and nucleic acids (198).

A high-density microarray patch developed by Vaxxas and coated with a split inactivated influenza vaccine against H1N1 has been shown to be safe and resulted in an enhanced immune response (203, 204). The researchers stored vaccine-coated microneedles at 40°C for 12 months and observed that the vaccine was antigenically stable. This approach could be cost effective, as it reduces the complication of continuous refrigeration. The Vaxxas patch showed similar immune responses at one-sixth of a dose

(dose sparing) compared to those induced by intramuscular injections of the same antigen formulation in humans (203, 204). A dissolvable microarray patch-based vaccine was created by Kim et al. for COVID-19 (205). In their study, the S-protein trimer of SARS-CoV-2 or S-protein subunits of MERS-CoV was loaded into polydimethylsiloxane molds, followed by overlaying of carboxymethyl cellulose to create hydrogel microneedles. Use of microneedle arrays to deliver S proteins of MERS and SARS-CoV-2 in mice showed approximately 1.5- and 16-fold higher IgG and virus-neutralizing titer levels, respectively, than intramuscular delivery of the same antigenic formulation, in several different formulations. Mice immunized with microarray patch vaccines had 15- to 30-fold higher S-specific IgG in sera. It was shown to induce nearly 10-fold higher neutralizing antibody titers than those induced by intravenous vaccine delivery. The IgG titers were sustained through 55 weeks after single-dose vaccination and increased by about 2.5-fold after the booster dose (205).

The use of microneedles has some advantages over other forms of delivery techniques. It delivers the antigen across the skin's stratum corneum, which has a high quantity of antigen-presenting cells and other mediators of the innate immune response, improving the immunogenicity and pharmacokinetics of the vaccines (206). There are many ways by which transdermal delivery can benefit vaccination. Besides being painless and increasing patient compliance, microneedles can improve the vaccine's thermostability, be self-administered, reduce medical waste (in the case of dissolvable patches), do not require reconstitution, and provide ease of storage (206). Transdermal patches can be designed to release a vaccine or therapeutic drug for a longer duration through sustained release (207).

Electroporation for Vaccine Delivery

Another technique to enhance uptake of the delivered vaccine is electroporation at the site of injection. Inovio Pharmaceutical's Cellectra 2000 is a delivery device that has a needle for intramuscular or intradermal injection and 2 to 4 needles around the injection needle to precisely deliver electric pulses for constant-current electroporation (208). The electroporation temporarily destabilizes cell membranes of the local cells and allows the vaccine components to directly enter cells without the need for phagocytosis or any other mode of internalization. Hence, it increases the cellular uptake of the delivered vaccine up to 1,000-fold more than that without electroporation, especially in the epidermis and muscle cells, promoting immune cell infiltration and establishing a proinflammatory response (208, 209). The increased uptake allows more effective antigen presentation through MHC class I and II pathways and can generate both T-cell responses and strong B cell-mediated antibody generation. It is safe, well tolerated, and does not cause any adverse effects by itself (210, 211). Electroporation can be used to deliver protein and nucleic acid vaccines (198).

Amante et al. used Cellectra to deliver H1N1 HA synthetic mRNA (208). Their nonhuman primate study of the vaccine suggested that intramuscular and intradermal electroporation vaccine delivery produced hemagglutination inhibition titers of 1:190 and 1:280, respectively, far above what is generally required for protection (1:40) (208). Cellectra was also used to deliver a spike sequence synthetic DNA vaccine for MERS-CoV (GLS-5300/INO-4700), which completed phase 1 trials but did not proceed further (ClinicalTrials.gov identifier NCT02670187) (212). It is noteworthy that GLS-5300 induced the generation of neutralizing antibodies and cellular and humoral responses in only 50%, 64%, and 77% of the study population, respectively. Eighty-five percent of participants had an immune response at 1 year of follow-up after two doses of vaccine (212). Currently, it is in trials to deliver a SARS-CoV-2 synthetic DNA vaccine encoding the S (INO-4800). INO-4800 testing in guinea pigs showed effective neutralizing antibodies against SARS-CoV-2 with an ND_{50} titer (serum dilution required for neutralization of 50% live virus) of >320, while the ND_{50} titer was <25 without electroporation (213). It has been observed to be well tolerated, safe, and immunogenic, with complete seroconversion in phase 1 trials, and it is currently in phase 2/3 trials (ClinicalTrials.gov identifiers NCT04447781, NCT04642638, and NCT04336410) (214). The tolerability and efficacy of INO-4800 seem to make it a good candidate for a pandemic

vaccination. INO-4800 provides adequate protection against most other variants as well, as seen from serum immune responses of phase 1 trial candidates and studies on ferrets (215, 216).

The use of electroporation for vaccination comes with the risk of disturbing the host DNA due to the electrical voltage imparted (217). Its distribution also remains limited to the site of injection. However, uptake by muscle cells can be enhanced by increasing their permeability by pretreatment with hyaluronidase before electroporation. Muscle tissue necrosis and skin edema at the injection site are also risk factors associated with this technique (217). Therefore, although electroporation seems to be successful in preclinical trials, its possible local adverse effects might keep it a long way from moving to widespread clinical use.

POTENTIAL TECHNIQUES FOR VACCINE DEVELOPMENT AND FUTURE PERSPECTIVES

As bioengineered products and techniques are being explored in almost every branch of biomedical sciences, several lessons can be taken from fields such as cancer therapy or drug delivery to develop better technologies in vaccine manufacture for respiratory diseases (38). For example, Cheng et al. demonstrated that altering the percentage of cationic lipid in the NP formulation results in selective targeting of various organs in a mouse model after intravenous delivery (218). This allowed selective targeting to the lung, spleen, and liver (218). Such targeted organ delivery of RNA using lipid NPs can selectively target the lungs and respiratory mucosa to develop robust mucosal immunity against respiratory viral diseases (219). The commonly used intramuscular delivery mode often elicits weak mucosal immunity, targeting only systemic immunity. On the other hand, direct mucosal delivery of vaccines ensures strong mucosal and systemic immune responses, which can be achieved using NPs (220).

It was shown that injectable mesoporous silica rods that are capable of self-assembly *in vivo* via nonspecific particle assembly could be used to create macroporous 3-dimensional structures that resulted in sustained release of ovalbumin for at least 10 days, leading to efficient recruitment of dendritic cells and their sequential infiltration into the lymph nodes and hence serving as an effective antigen to provoke host adaptive immunity and modulate immune cell functioning (221). These silica rods accumulate at the injection site and undergo sustained antigen release for at least 2 weeks (221). This prolonged release period increases the duration of immune response mounted against the vaccine antigen and can subsequently boost the repertoire of memory cells for potent protection. Such sustained release strategies can be very effective for large-scale vaccination during a pandemic, as they have the potential of being designed as a prime-only vaccine and may not need booster doses.

One challenge with particle-based vaccines is the premature release of antigens and adjuvants in serum, leading to their degradation by serum proteases and DNAses. A strategy to overcome this challenge is to use pH-responsive materials such as poly(N-vinylformamide). Such nanogels have minimal release in serum but dissolve faster or even burst-release at lower pH, thus protecting their cargo until it reaches an acidic environment such as the endosomal vesicle, ensuring better loading on MHC molecules (222, 223). A pH-responsive polymer micelle particle has been shown to increase cytosolic delivery and reduced exocytosis, which causes enhanced CD8$^+$ T-cell response in mice (224). The mice show a 30-fold increase in antigen uptake by dendritic cells relative to free protein 24 h postvaccination with the ovalbumin polymer conjugate (224). Hence, the use of pH-responsive biomaterials can be used to significantly enhance CD8$^+$ responses and has the potential to amplify antiviral immunity for better protection.

Microneedles can be coated with a polyelectrolyte multilayer assembly of the pH-responsive copolymer oligo(sulfamethazine)-*b*-poly(ethylene glycol)-*b*-poly(amino urethane), and heparin (225). This copolymer has a charge reversal property, i.e., it exhibits a positive charge at pH below 4.03, allowing easy electrostatic assembly. At a physiological pH of 7.4, it takes up a negative charge, leading to disassembly of the multilayer

and rapid release of DNA vaccines due to repulsion with the heparin layer. This methodology can effectively deliver DNA vaccines to the interior layers of the skin, such as the dermis and the epidermis, for an effective immune response (225).

Hajj et al. have shown potent delivery of luciferase mRNA in liver cells (mRNA taken up by >80% of three major types of liver cells after delivery in mice) by using $306O_{i10}$ lipid NPs (branched tail, ionizable lipid) (226). $306O_{i10}$ belongs to a class of lipid-like materials called lipidoids with 10 carbon tails and a single carbon branch at the end (227). Systemic injection of 0.5 mg mRNA/kg encapsulated in $306O_{i10}$ NPs in mice model showed 3-fold and 20-fold higher protein expression in liver cells compared to that shown with two approved gold-standard lipids, Dlin-MC3-DMA and C12-200, respectively (226, 227). This high delivery rate of mRNA by $306O_{i10}$ NPs is primarily due to its potential to be strongly ionized and attain a positive charge in the acidic environment of late endosomes, which facilitates endosomal escape and allows mRNA to be translated in the cytoplasm (226). Further research on developing such ionizable and pH-sensitive nanoliposomes to deliver nucleic acid vaccines can boost delivery and increase immunostimulation for better protection.

Moon et al. synthesized stable interbilayer cross-linked multilamellar vesicles carrying ovalbumin, which, when mixed with immunostimulatory molecules, elicited antibody titers nearly 1,000-fold that of free ovalbumin and ovalbumin encapsulated in a monolayered liposomal formulation (228). It also provided additional advantages such as antigen loading nearly 2-fold higher than that with monolayered liposomes and increased stability by adding short covalent cross-links (sustained release for up to 30 days in serum while rapidly degrading on exposure to endolysosomal phospholipase A) (228). These particles provide multiple advantageous features, like biodegradability for easy metabolism of products, high encapsulation efficiency of both hydrophilic and hydrophobic cargos, sustained release, and potent T- and B-cell responses, thereby making them suitable to adopt for viral vaccine formulations.

We would like to highlight the main areas where further research might support vaccine development against viral respiratory diseases. First, it is vital to obtain a better understanding of engineered vaccines, such as trafficking, toxicity, and the generation and persistence of immunoprotection. There should be more detailed preclinical animal research to confirm the designed vaccine's translatability. The lack of such studies on large animals, such as for MERS vaccines, might be detrimental to adopting them for similar viral respiratory diseases in the future.

Second, observing the high rate of antigenic shifts and drifts in respiratory viruses and the ever-increasing population of humans to be vaccinated, it is an unmet clinical need to develop a single-dose, broad-spectrum vaccine against different strains of respiratory viruses to develop immunity swiftly at an affordable cost. Vaccines developed using bioengineering strategies can contribute to achieving this goal. Bioengineered particles such as VLPs and hollow polymeric microspheres and nanospheres allow insertion of multiple and diverse antigens in the same particle, encapsulated or surface displayed, hence enabling the development of a broader immunity. Antigens from different viruses can also be used, such as in Inflexal and the dual vaccine against MERS and influenza mentioned previously (119, 179). Many engineered products also allow encapsulation of adjuvants, sustained antigen release, or higher antigen encapsulation capability, all of which are highly advantageous for developing single-dose vaccines. The demonstrable potency of bioengineered NP vaccines, along with their high biocompatibility, provides the opportunity to tackle persistent viral respiratory infections, and such provisions must be used to the greatest advantage.

Third, among the essential factors to be considered for vaccine development against respiratory viruses are reproducibility of synthesis, rapid scale-up potential, and vaccine sterilization. Major changes in the manufacturing process, including new equipment, facilities, and regulatory requirements, are required to accommodate new techniques and products (229). When new processes are introduced, the cost of manufacturing also increases, such as personnel training costs and costs on raw materials and licensing (229).

While initially setting up facilities for biomaterial-based vaccines would be costly, in the long run, this cost can be vastly reduced because of their adaptability to new antigens without needing extensive changes in the synthesis process. Vaccines based on VLPs and OMVs can show batch-to-batch variation because of the high dependability on the host cell behavior and lack of absolute control over the yield. Self-assembling, inorganic, and polymeric particles, on the other hand, do not have this problem because of their highly controlled production process. The last roadblock is the sterility of the vaccine formulation. There are several methods for sterilizing NPs, depending on their formulation, including sterile filtration, autoclaving, and radiation (230). However, it is challenging to identify suitable sterilization processes when new materials are used for research. For example, radiation-based sterilization can alter the quality of the polymeric particles (231). More research is required to optimize the industrial sterilization of bioengineered vaccines.

CONCLUSION

Vaccines are the most effective way of controlling an outbreak, given that correct and timely strategies are employed for their development. Despite their success for many diseases, vaccines for respiratory viral infections such as flu and SARS are still suboptimal and do not offer broad-spectrum protection. Bioengineered vaccine formulations have shown their potential as efficient antigen and adjuvant delivery vehicles. They can enhance antigen stability and provide controlled release, site-specific targeted delivery, and increased immunogenicity. So far, self-assembling proteins (e.g., Bexsero for meningococcal group B), liposomes (e.g., Comirnaty for SARS-CoV-2), and VLPs (e.g., Gardasil for human papillomavirus, Engerix for hepatitis B virus, Recombivax HB for hepatitis B virus, and Cervarix for human papillomavirus) have been the only components of bioengineering to enter the vaccine market. Several of these platforms, such as liposomes, polymeric particles, and self-assembling particles, have shown promise in clinics. More research is required into understanding the interactions of such bioengineered materials with the antigens so that the vaccine development timeline is shortened and the vaccines produced are better and safer. In the future, we hope to see interdisciplinary study between immunologists, material scientists, computational biologists, and clinicians aimed toward the designing of effective vaccines against respiratory diseases. The rapidity of designing and industrial synthesis is an advantage that biomaterials can provide to deliver potent DNA, RNA, and recombinant protein vaccines, especially for curbing respiratory viral pandemics.

REFERENCES

1. Peck M, Gacic-Dobo M, Diallo MS, Nedelec Y, Sodha SV, Wallace AS. 2019. Global routine vaccination coverage, 2018. MMWR Morb Mortal Wkly Rep 68:937–942. https://doi.org/10.15585/mmwr.mm 6842a1.

2. WHO Emergency Preparedness ADGO. 2019. Chapter 6—Vaccine-preventable diseases and vaccines. In International travel and health. WHO, Geneva, Switzerland.

3. Lewnard JA, Lo NC, Arinaminpathy N, Frost I, Laxminarayan R. 2020. Childhood vaccines and antibiotic use in low- and middle-income countries. Nature 581: 94–99. https://doi.org/10.1038/s41586-020-2238-4.

4. Morse SS, Mazet JA, Woolhouse M, Parrish CR, Carroll D, Karesh WB, Zambrana-Torrelio C, Lipkin WI, Daszak P. 2012. Prediction and prevention of the next pandemic zoonosis. Lancet 380:1956–1965. https://doi .org/10.1016/S0140-6736(12)61684-5.

5. Osterholm MT, Kelley NS, Sommer A, Belongia EA. 2012. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis 12:36–44. https://doi.org/10.1016/S1473-3099(11)70295-X.

6. Guarner J. 2020. Three emerging coronaviruses in two decades: the story of SARS, MERS, and now COVID-19. Am J Clin Pathol 153:420–421. https://doi.org/10.1093/ajcp/aqaa029.

7. Soriano JB, Kendrick PJ, Paulson KR, Gupta V, Abrams EM, Adedoyin RA, Adhikari TB, Advani SM, Agrawal A, Ahmadian E, Alahdab F, Aljunid SM, Altirkawi KA, Alvis-Guzman N, Anber NH, Andrei CL, Anjomshoa M, Ansari F, Antó JM, Arabloo J, Athari SM, Athari SS, Awoke N, Badawi A, Banoub JAM, Bennett DA, Bensenor IM, Berfield KSS, Bernstein RS, Bhattacharyya K, Bijani A, Brauer M, Bukhman G, Butt ZA, Cámera LA, Car J, Carrero JJ, Carvalho F, Castañeda-Orjuela CA, Choi J-YJ, Christopher DJ, Cohen AJ, Dandona L, Dandona R, Dang AK, Daryani A, de Courten B,

Demeke FM, Demoz GT, De Neve J-W, et al. 2020. Prevalence and attributable health burden of chronic respiratory diseases, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med 8:585–596. https://doi.org/10.1016/S2213-2600(20)30105-3.

8. Taubenberger JK, Morens DM. 2006. 1918 Influenza: the mother of all pandemics. Emerg Infect Dis 12:15–22. https://doi.org/10.3201/eid1201.050979.

9. Reference deleted.

10. Saunders-Hastings PR, Krewski D. 2016. Reviewing the history of pandemic influenza: understanding patterns of emergence and transmission. Pathogens 5:66. https://doi.org/10.3390/pathogens5040066.

11. Petrova VN, Russell CA. 2018. The evolution of seasonal influenza viruses. Nat Rev Microbiol 16:60. https://doi.org/10.1038/nrmicro.2017.146.

12. Dietz K. 1993. The estimation of the basic reproduction number for infectious diseases. Stat Methods Med Res 2:23–41. https://doi.org/10.1177/096228029300200103.

13. Callaway E, Cyranoski D, Mallapaty S, Stoye E, Tollefson J. 2020. The coronavirus pandemic in five powerful charts. Nature 579:482–483.https://doi.org/10.1038/d41586-020-00758-2.

14. Peiris JS, Guan Y, Yuen KY. 2004. Severe acute respiratory syndrome. Nat Med 10:S88–97. https://doi.org/10.1038/nm1143.

15. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus A, Fouchier RAMM. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367:1814–1820. https://doi.org/10.1056/NEJMoa1211721.

16. Becerra-Flores M, Cardozo T. 2020. SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate. Int J Clin Pr 74:e13525. https://doi.org/10.1111/ijcp.13525.

17. Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, Zhao C, Zhang Q, Liu H, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Zhang L, Li X, Huang W, Wang Y. 2020. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell 182:1284–1294.e9. https://doi.org/10.1016/j.cell.2020.07.012.

18. Weisblum Y, Schmidt F, Zhang F, DaSilva J, Poston D, Lorenzi JC, Muecksch F, Rutkowska M, Hoffmann HH, Michailidis E, Gaebler C, Agudelo M, Cho A, Wang Z, Gazumyan A, Cipolla M, Luchsinger L, Hillyer CD, Caskey M, Robbiani DF, Rice CM, Nussenzweig MC, Hatziioannou T, Bieniasz PD. 2020. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. Elife 9:e61312. https://doi.org/10.7554/eLife.61312.

19. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. 2020. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun 11:6013. https://doi.org/10.1038/s41467-020-19808-4.

20. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, Palese P, Shaw ML, Treanor J, Webster RG, García-Sastre A. 2018. Influenza. Nat Rev Dis Primers 4:3. https://doi.org/10.1038/s41572-018-0002-y.

21. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, Flemban H, Al-Nassir WN, Balkhy HH, Al-Hakeem RF, Makhdoom HQ, Zumla AI, Memish ZA. 2013. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis 13:752–761. https://doi.org/10.1016/S1473-3099(13)70204-4.

22. Rothan HA, Byrareddy SN. 2020. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 109:102433. https://doi.org/10.1016/j.jaut.2020.102433.

23. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, Tang BS, Hon TY, Chan CS, Chan KH, Ng JS, Zheng BJ, Ng WL, Lai RW, Guan Y, Yuen KY, Group HUSS. 2003. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 361:1767–1772. https://doi.org/10.1016/S0140-6736(03)13412-5.

24. Masters PS. 2006. The molecular biology of coronaviruses. Adv Virus Res 66:193–292. https://doi.org/10.1016/S0065-3527(06)66005-3.

25. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181:271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052.

26. Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, Guo D, Fu L, Cui Y, Liu X, Arledge KC, Chen YH, Zhang L, Wang X. 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res 23:986–993. https://doi.org/10.1038/cr.2013.92.

27. Roper RL, Rehm KE. 2009. SARS vaccines: where are we? Expert Rev Vaccines 8:887–898. https://doi.org/10.1586/erv.09.43.

28. Wong SS, Webby RJ. 2013. Traditional and new influenza vaccines. Clin Microbiol Rev 26:476–492. https://doi.org/10.1128/CMR.00097-12.

29. Barrett PN, Terpening SJ, Snow D, Cobb RR, Kistner O. 2017. Vero cell technology for rapid development of inactivated whole virus vaccines for emerging viral diseases. Expert Rev Vaccines 16:883–894. https://doi.org/10.1080/14760584.2017.1357471.

30. Kanekiyo M, Ellis D, King NP. 2019. New vaccine design and delivery technologies. J Infect Dis 219:S88–S96. https://doi.org/10.1093/infdis/jiy745.

31. Herold S, Sander LE. 2020. Toward a universal flu vaccine. Science 367:852–853. https://doi.org/10.1126/science.aba2754.

32. Zhou B, Meliopoulos VA, Wang W, Lin X, Stucker KM, Halpin RA, Stockwell TB, Schultz-Cherry S, Wentworth DE. 2016. Reversion of cold-adapted live attenuated influenza vaccine into a pathogenic virus. J Virol 90:8454–8463. https://doi.org/10.1128/JVI.00163-16.

33. Jimenez-Guardeno JM, Regla-Nava JA, Nieto-Torres JL, DeDiego ML, Castano-Rodriguez C, Fernandez-Delgado R, Perlman S, Enjuanes L. 2015. Identification of the mechanisms causing reversion to virulence in an attenuated SARS-CoV for the design of a genetically stable vaccine. PLoS Pathog 11:e1005215. https://doi.org/10.1371/journal.ppat.1005215.

34. Vartak A, Sucheck SJ. 2016. Recent advances in subunit vaccine carriers. Vaccines 4:12. https://doi.org/10.3390/vaccines4020012.

35. Huebsch N, Mooney DJ. 2009. Inspiration and application in the evolution of biomaterials. Nature 462:426–432. https://doi.org/10.1038/nature08601.

36. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, Himansu S, Schäfer A, Ziwawo CT, DiPiazza AT, Dinnon KH, Elbashir SM, Shaw CA, Woods A, Fritch EJ, Martinez DR, Bock KW, Minai M, Nagata BM, Hutchinson GB, Wu K, Henry C, Bahl K, Garcia-Dominguez D, Ma LZhi, Renzi I, Kong W-P, Schmidt SD, Wang L, Zhang Y, Phung E, Chang LA, Loomis RJ, Altaras NE, Narayanan E, Metkar M, Presnyak V, Liu C, Louder MK, Shi W, Leung K, Yang ES, West A, Gully KL, Stevens LJ, Wang N, Wrapp D, Doria-Rose NA, Stewart-Jones G, Bennett H, et al. 2020. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 586:567–571. https://doi.org/10.1038/s41586-020-2622-0.

37. Bookstaver ML, Tsai SJ, Bromberg JS, Jewell CM. 2018. Improving vaccine and immunotherapy design using biomaterials. Trends Immunol 39:135–150. https://doi.org/10.1016/j.it.2017.10.002.

38. Fenton OS, Olafson KN, Pillai PS, Mitchell MJ, Langer R. 2018. Advances in biomaterials for drug delivery. Adv Mater 30:e1705328. https://doi.org/10.1002/adma.201705328.

39. Kamaly N, Yameen B, Wu J, Farokhzad OC. 2016. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chem Rev 116:2602–2663. https://doi.org/10.1021/acs.chemrev.5b00346.

40. Shahbazi M-A, Fernández TD, Mäkilä EM, Le Guével X, Mayorga C, Kaasalainen MH, Salonen JJ, Hirvonen JT, Santos HA. 2014. Surface chemistry dependent immunostimulative potential of porous silicon nanoplatforms. Biomaterials 35:9224–9235. https://doi.org/10.1016/j.biomaterials.2014.07.050.

41. Champion JA, Mitragotri S. 2006. Role of target geometry in phagocytosis. Proc Natl Acad Sci U S A 103:4930–4934. https://doi.org/10.1073/pnas.0600997103.

42. Bottger R, Hoffmann R, Knappe D. 2017. Differential stability of therapeutic peptides with different proteolytic cleavage sites in blood, plasma and serum. PLoS One 12:e0178943. https://doi.org/10.1371/journal.pone.0178943.

43. Amoscato AA, Prenovitz DA, Lotze MT. 1998. Rapid extracellular degradation of synthetic class I peptides by human dendritic cells. J Immunol 161:4023–4032.

44. Janeway C, Janeway CA, Jr, Travers P, Walport M, Shlomchik MJ. 2001. Chapter 3. Antigen recognition by B-cell and T-cell receptors. In Immunobiology: the immune system in health and disease. Garland Science, New York, NY.

45. Ho W, Gao M, Li F, Li Z, Zhang X-Q, Xu X. 2021. Next-generation vaccines: nanoparticle-mediated DNA and mRNA delivery. Adv Heal Mater 10:2001812. https://doi.org/10.1002/adhm.202001812.

46. Rock KL, Reits E, Neefjes J. 2016. Present yourself! By MHC class I and MHC class II molecules. Trends Immunol 37:724–737. https://doi.org/10.1016/j.it.2016.08.010.

47. Carbone FR, Bevan MJ. 1990. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. J Exp Med 171:377–387. https://doi.org/10.1084/jem.171.2.377.

48. Fehres CM, Unger WW, Garcia-Vallejo JJ, van Kooyk Y. 2014. Understanding the biology of antigen cross-presentation for the design of vaccines against cancer. Front Immunol 5:149. https://doi.org/10.3389/fimmu.2014.00149.

49. Iwasaki A, Omer SB. 2020. Why and how vaccines work. Cell 183:290–295. https://doi.org/10.1016/j.cell.2020.09.040.

50. Wu LP, Wang NC, Chang YH, Tian XY, Na DY, Zhang LY, Zheng L, Lan T, Wang LF, Liang GD. 2007. Duration of antibody responses after severe

acute respiratory syndrome. Emerg Infect Dis 13:1562–1564. https://doi.org/10.3201/eid1310.070576.

51. Payne DC, Iblan I, Rha B, Alqasrawi S, Haddadin A, Al Nsour M, Alsanouri T, Ali SS, Harcourt J, Miao C, Tamin A, Gerber SI, Haynes LM, Al Abdallat MM. 2016. Persistence of antibodies against Middle East respiratory syndrome coronavirus. Emerg Infect Dis 22:1824–1826. https://doi.org/10.3201/eid2210.160706.

52. Breton G, Mendoza P, Hagglof T, Oliveira TY, Schaefer-Babajew D, Gaebler C, Turroja M, Hurley A, Caskey M, Nussenzweig MC. 2021. Persistent cellular immunity to SARS-CoV-2 infection. J Exp Med 218: e20202515. https://doi.org/10.1084/jem.20202515.

53. L'Huillier AG, Meyer B, Andrey DO, Arm-Vernez I, Baggio S, Didierlaurent A, Eberhardt CS, Eckerle I, Grasset-Salomon C, Huttner A, Posfay-Barbe KM, Royo IS, Pralong JA, Vuilleumier N, Yerly S, Siegrist C-A, Kaiser L, Geneva Centre for Emerging Viral Diseases. 2021. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: a prospective longitudinal study. Clin Microbiol Infect 27:784.e1–784.e8. https://doi.org/10.1016/j.cmi.2021.01.005.

54. Cohen JI, Burbelo PD. 2020. Reinfection with SARS-CoV-2: implications for vaccines. Clin Infect Dis https://doi.org/10.1093/cid/ciaa1866.

55. Openshaw PJM, Chiu C, Culley FJ, Johansson C. 2017. Protective and harmful immunity to RSV infection. Annu Rev Immunol 35:501–532. https://doi.org/10.1146/annurev-immunol-051116-052206.

56. Kaech SM, Wherry EJ, Ahmed R. 2002. Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immunol 2: 251–262. https://doi.org/10.1038/nri778.

57. Beverley PCL. 2002. Immunology of vaccination. Br Med Bull 62:15–28. https://doi.org/10.1093/bmb/62.1.15.

58. Young RJ, Lovell PA. 2011. Introduction to polymers. CRC Press, Boca Raton, FL.

59. Ulery BD, Nair LS, Laurencin CT. 2011. Biomedical applications of biodegradable polymers. J Polym Sci B Polym Phys 49:832–864. https://doi.org/10.1002/polb.22259.

60. Mamo T, Poland GA. 2012. Nanovaccinology: the next generation of vaccines meets 21st century materials science and engineering. Vaccine 30: 6609–6611. https://doi.org/10.1016/j.vaccine.2012.08.023.

61. Pachioni-Vasconcelos JA, Lopes AM, Apolinario AC, Valenzuela-Oses JK, Costa JS, Nascimento LO, Pessoa A, Barbosa LR, Rangel-Yagui CO. 2016. Nanostructures for protein drug delivery. Biomater Sci 4:205–218. https://doi.org/10.1039/c5bm00360a.

62. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V. 2012. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release 161:505–522. https://doi.org/10.1016/j.jconrel.2012.01.043.

63. Wang Y, Choi SH, Qu W. 16 June 2016. FDA's regulatory science program for generic PLA/PLGA-based drug products. American Pharmaceutical Review. https://www.americanpharmaceuticalreview.com/Featured-Articles/188841-FDA-s-Regulatory-Science-Program-for-Generic-PLA-PLGA-Based-Drug-Products/.

64. Shen H, Ackerman AL, Cody V, Giodini A, Hinson ER, Cresswell P, Edelson RL, Saltzman WM, Hanlon DJ. 2006. Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. Immunology 117:78–88. https://doi.org/10.1111/j.1365-2567.2005.02268.x.

65. Bailey BA, Desai K-GH, Ochyl LJ, Ciotti SM, Moon JJ, Schwendeman SP. 2017. Self-encapsulating poly(lactic-co-glycolic acid) (PLGA) microspheres for intranasal vaccine delivery. Mol Pharm 14:3228–3237. https://doi.org/10.1021/acs.molpharmaceut.7b00586.

66. Dhakal S, Hiremath J, Bondra K, Lakshmanappa YS, Shyu DL, Ouyang K, Kang KI, Binjawadagi B, Goodman J, Tabynov K, Krakowka S, Narasimhan B, Lee CW, Renukaradhya GJ. 2017. Biodegradable nanoparticle delivery of inactivated swine influenza virus vaccine provides heterologous cell-mediated immune response in pigs. J Control Release 247:194–205. https://doi.org/10.1016/j.jconrel.2016.12.039.

67. Alkie TN, Yitbarek A, Taha-Abdelaziz K, Astill J, Sharif S. 2018. Characterization of immunogenicity of avian influenza antigens encapsulated in PLGA nanoparticles following mucosal and subcutaneous delivery in chickens. PLoS One 13:e0206324. https://doi.org/10.1371/journal.pone.0206324.

68. Ishikawa H, Ma Z, Barber GN. 2009. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 461: 788–792. https://doi.org/10.1038/nature08476.

69. Wu J-J, Zhao L, Han B-B, Hu H-G, Zhang B-D, Li W-H, Chen Y-X, Li Y-M. 2021. A novel STING agonist for cancer immunotherapy and a SARS-CoV-2 vaccine adjuvant. Chem Commun (Camb) (Camb) 57:504–507. https://doi.org/10.1039/d0cc06959k.

70. Lin LC, Huang CY, Yao BY, Lin JC, Agrawal A, Algaissi A, Peng BH, Liu YH, Huang PH, Juang RH, Chang YC, Tseng CT, Chen HW, Hu CJ. 2019. Viromimetic STING agonist-loaded hollow polymeric nanoparticles for safe and effective vaccination against Middle East respiratory syndrome coronavirus. Adv Funct Mater 29:1807616. https://doi.org/10.1002/adfm.201807616.

71. Liu Q, Chen X, Jia J, Zhang W, Yang T, Wang L, Ma G. 2015. pH-Responsive poly(D,L-lactic-co-glycolic acid) nanoparticles with rapid antigen release behavior promote immune response. ACS Nano 9:4925–4938. https://doi.org/10.1021/nn5066793.

72. Silva AL, Soema PC, Slütter B, Ossendorp F, Jiskoot W. 2016. PLGA particulate delivery systems for subunit vaccines: linking particle properties to immunogenicity. Hum Vaccin Immunother 12:1056–1069. https://doi.org/10.1080/21645515.2015.1117714.

73. Tamayo I, Irache JM, Mansilla C, Ochoa-Repáraz J, Lasarte JJ, Gamazo C. 2010. Poly(anhydride) nanoparticles act as active Th1 adjuvants through Toll-like receptor exploitation. Clin Vaccine Immunol 17:1356–1362. https://doi.org/10.1128/CVI.00164-10.

74. Camacho AI, Da Costa Martins R, Tamayo I, de Souza J, Lasarte JJ, Mansilla C, Esparza I, Irache JM, Gamazo C. 2011. Poly(methyl vinyl ether-co-maleic anhydride) nanoparticles as innate immune system activators. Vaccine 29:7130–7135. https://doi.org/10.1016/j.vaccine.2011.05.072.

75. Göpferich A, Tessmar J. 2002. Polyanhydride degradation and erosion. Adv Drug Deliv Rev 54:911–931. https://doi.org/10.1016/s0169-409x(02)00051-0.

76. Dhakal S, Goodman J, Bondra K, Lakshmanappa YS, Hiremath J, Shyu D-L, Ouyang K, Kang K, Krakowka S, Wannemuehler MJ, Won Lee C, Narasimhan B, Renukaradhya GJ. 2017. Polyanhydride nanovaccine against swine influenza virus in pigs. Vaccine 35:1124–1131. https://doi.org/10.1016/j.vaccine.2017.01.019.

77. Kipper MJ, Wilson JH, Wannemuehler MJ, Narasimhan B. 2006. Single dose vaccine based on biodegradable polyanhydride microspheres can modulate immune response mechanism. J Biomed Mater Res A 76: 798–810. https://doi.org/10.1002/jbm.a.30545.

78. Thukral A, Ross K, Hansen C, Phanse Y, Narasimhan B, Steinberg H, Talaat AM. 2020. A single dose polyanhydride-based nanovaccine against paratuberculosis infection. NPJ Vaccines 5:15–10. https://doi.org/10.1038/s41541-020-0164-y.

79. Knight FC, Gilchuk P, Kumar A, Becker KW, Sevimli S, Jacobson ME, Suryadevara N, Wang-Bishop L, Boyd KL, Crowe JE, Jr, Joyce S, Wilson JT. 2019. Mucosal immunization with a pH-responsive nanoparticle vaccine induces protective CD8$^+$ lung-resident memory T cells. ACS Nano 13: 10939–10960. https://doi.org/10.1021/acsnano.9b00326.

80. Pandey AP, Sawant KK. 2016. Polyethylenimine: a versatile, multifunctional non-viral vector for nucleic acid delivery. Mater Sci Eng C Mater Biol Appl 68:904–918. https://doi.org/10.1016/j.msec.2016.07.066.

81. Shen C, Li J, Zhang Y, Li Y, Shen G, Zhu J, Tao J. 2017. Polyethylenimine-based micro/nanoparticles as vaccine adjuvants. Int J Nanomedicine (Lond) (Lond) 12:5443–5460. https://doi.org/10.2147/IJN.S137980.

82. Shim B-S, Park S-M, Quan J-S, Jere D, Chu H, Song MK, Kim DW, Jang Y-S, Yang M-S, Han SH, Park Y-H, Cho C-S, Yun C-H. 2010. Intranasal immunization with plasmid DNA encoding spike protein of SARS-coronavirus/polyethylenimine nanoparticles elicits antigen-specific humoral and cellular immune responses. BMC Immunol 11:65. https://doi.org/10.1186/1471-2172-11-65.

83. Parhamifar L, K Larsen A, Christy Hunter A, L Andresen T, Moein Moghimi S. 2010. Polycation cytotoxicity: a delicate matter for nucleic acid therapy—focus on polyethylenimine. Soft Matter 6:4001–4009. https://doi.org/10.1039/c000190b.

84. Kircheis R, Wightman L, Wagner E. 2001. Design and gene delivery activity of modified polyethylenimines. Adv Drug Deliv Rev 53:341–358. https://doi.org/10.1016/s0169-409x(01)00202-2.

85. Sahdev P, Ochyl LJ, Moon JJ. 2014. Biomaterials for nanoparticle vaccine delivery systems. Pharm Res 31:2563–2582. https://doi.org/10.1007/s11095-014-1419-y.

86. Bakshi PS, Selvakumar D, Kadirvelu K, Kumar NS. 2020. Chitosan as an environment friendly biomaterial—a review on recent modifications and applications. Int J Biol Macromol 150:1072–1083. https://doi.org/10.1016/j.ijbiomac.2019.10.113.

87. Lai WF, Lin MC. 2009. Nucleic acid delivery with chitosan and its derivatives. J Control Release 134:158–168. https://doi.org/10.1016/j.jconrel.2008.11.021.

88. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. 2001. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Deliv Rev 51:81–96. https://doi.org/10.1016/s0169-409x(01)00171-5.

89. Riteau N, Sher A. 2016. Chitosan: an adjuvant with an unanticipated STING. Immunity 44:522–524. https://doi.org/10.1016/j.immuni.2016.03.002.

90. Garg NK, Mangal S, Khambete H, Sharma PK, Tyagi RK. 2010. Mucosal delivery of vaccines: role of mucoadhesive/biodegradable polymers. Recent Pat Drug Deliv Formul 4:114–128. https://doi.org/10.2174/187221110791185015.

91. Jesus S, Marques AP, Duarte A, Soares E, Costa JP, Colaco M, Schmutz M, Som C, Borchard G, Wick P, Borges O. 2020. Chitosan nanoparticles: shedding light on immunotoxicity and hemocompatibility. Front Bioeng Biotechnol 8:100. https://doi.org/10.3389/fbioe.2020.00100.

92. Jung SM, Yoon GH, Lee HC, Shin HS. 2015. Chitosan nanoparticle/PCL nano-fiber composite for wound dressing and drug delivery. J Biomater Sci Polym Ed 26:252–263. https://doi.org/10.1080/09205063.2014.996699.

93. Elgadir MA, Uddin MS, Ferdosh S, Adam A, Chowdhury AJK, Sarker MZI. 2015. Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: a review. J Food Drug Anal 23:619–629. https://doi.org/10.1016/j.jfda.2014.10.008.

94. Lopes PD, Okino CH, Fernando FS, Pavani C, Casagrande VM, Lopez RV, Montassier MFS, Montassier HJ. 2018. Inactivated infectious bronchitis virus vaccine encapsulated in chitosan nanoparticles induces mucosal immune responses and effective protection against challenge. Vaccine 36:2630–2636. https://doi.org/10.1016/j.vaccine.2018.03.065.

95. Raghuwanshi D, Mishra V, Das D, Kaur K, Suresh MR. 2012. Dendritic cell targeted chitosan nanoparticles for nasal DNA immunization against SARS CoV nucleocapsid protein. Mol Pharm 9:946–956. https://doi.org/10.1021/mp200553x.

96. Wang WW, Das D, Tang XL, Budzynski W, Suresh MR. 2005. Antigen targeting to dendritic cells with bispecific antibodies. J Immunol Methods 306:80–92. https://doi.org/10.1016/j.jim.2005.07.023.

97. Raghuwanshi D, Mishra V, Suresh MR, Kaur K. 2012. A simple approach for enhanced immune response using engineered dendritic cell targeted nanoparticles. Vaccine 30:7292–7299. https://doi.org/10.1016/j.vaccine.2012.09.036.

98. Amidi M, Romeijn SG, Verhoef JC, Junginger HE, Bungener L, Huckriede A, Crommelin DJA, Jiskoot W. 2007. N-Trimethyl chitosan (TMC) nanoparticles loaded with influenza subunit antigen for intranasal vaccination: biological properties and immunogenicity in a mouse model. Vaccine 25:144–153. https://doi.org/10.1016/j.vaccine.2006.06.086.

99. Read RC, Naylor SC, Potter CW, Bond J, Jabbal-Gill I, Fisher A, Illum L, Jennings R. 2005. Effective nasal influenza vaccine delivery using chitosan. Vaccine 23:4367–4374. https://doi.org/10.1016/j.vaccine.2005.04.021.

100. Sui Z, Chen Q, Fang F, Zheng M, Chen Z. 2010. Cross-protection against influenza virus infection by intranasal administration of M1-based vaccine with chitosan as an adjuvant. Vaccine 28:7690–7698. https://doi.org/10.1016/j.vaccine.2010.09.019.

101. Gupta NK, Tomar P, Sharma V, Dixit VK. 2011. Development and characterization of chitosan coated poly-(varepsilon-caprolactone) nanoparticulate system for effective immunization against influenza. Vaccine 29:9026–9037. https://doi.org/10.1016/j.vaccine.2011.09.033.

102. Mohamed SH, Arafa AS, Mady WH, Fahmy HA, Omer LM, Morsi RE. 2018. Preparation and immunological evaluation of inactivated avian influenza virus vaccine encapsulated in chitosan nanoparticles. Biologicals 51:46–53. https://doi.org/10.1016/j.biologicals.2017.10.004.

103. Zhao L, Seth A, Wibowo N, Zhao C-X, Mitter N, Yu C, Middelberg APJ. 2014. Nanoparticle vaccines. Vaccine 32:327–337. https://doi.org/10.1016/j.vaccine.2013.11.069.

104. Bose RJ, Kim M, Chang JH, Paulmurugan R, Moon JJ, Koh W-G, Lee S-H, Park H. 2019. Biodegradable polymers for modern vaccine development. J Ind Eng Chem 77:12–24. https://doi.org/10.1016/j.jiec.2019.04.044.

105. Chen N, Gallovic MD, Tiet P, Ting JPY, Ainslie KM, Bachelder EM. 2018. Investigation of tunable acetalated dextran microparticle platform to optimize M2e-based influenza vaccine efficacy. J Control Release 289:114–124. https://doi.org/10.1016/j.jconrel.2018.09.020.

106. Pifferi C, Fuentes R, Fernández-Tejada A. 2021. Natural and synthetic carbohydrate-based vaccine adjuvants and their mechanisms of action. Nat Rev Chem 5:197–216. https://doi.org/10.1038/s41570-020-00244-3.

107. Marques Neto LM, Kipnis A, Junqueira-Kipnis AP. 2017. Role of metallic nanoparticles in vaccinology: implications for infectious disease vaccine development. Front Immunol 8:239. https://doi.org/10.3389/fimmu.2017.00239.

108. Cao-Milan R, Liz-Marzan LM. 2014. Gold nanoparticle conjugates: recent advances toward clinical applications. Expert Opin Drug Deliv 11:741–752. https://doi.org/10.1517/17425247.2014.891582.

109. Ding Y, Jiang Z, Saha K, Kim CS, Kim ST, Landis RF, Rotello VM. 2014. Gold nanoparticles for nucleic acid delivery. Mol Ther 22:1075–1083. https://doi.org/10.1038/mt.2014.30.

110. Sanchez-Guzman D, Le Guen P, Villeret B, Sola N, Le Borgne R, Guyard A, Kemmel A, Crestani B, Sallenave JM, Garcia-Verdugo I. 2019. Silver nanoparticle-adjuvanted vaccine protects against lethal influenza infection through inducing BALT and IgA-mediated mucosal immunity. Biomaterials 217:119308. https://doi.org/10.1016/j.biomaterials.2019.119308.

111. Jazayeri SD, Ideris A, Zakaria Z, Shameli K, Moeini H, Omar AR. 2012. Cytotoxicity and immunological responses following oral vaccination of nanoencapsulated avian influenza virus H5 DNA vaccine with green synthesis silver nanoparticles. J Control Release 161:116–123. https://doi.org/10.1016/j.jconrel.2012.04.015.

112. Boisselier E, Astruc D. 2009. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev 38:1759–1782. https://doi.org/10.1039/b806051g.

113. Yang G, Phua SZF, Bindra AK, Zhao Y. 2019. Degradability and clearance of inorganic nanoparticles for biomedical applications. Adv Mater 31:e1805730. https://doi.org/10.1002/adma.201805730.

114. Marasini N, Ghaffar KA, Skwarczynski M, Toth I. 2017. Chapter Twelve—Liposomes as a vaccine delivery system, p 221–239. In Skwarczynski M, Toth I (ed), Micro and Nanotechnology in Vaccine Development. Elsevier, Amsterdam, the Netherlands.

115. Bozzuto G, Molinari A. 2015. Liposomes as nanomedical devices. Int J Nanomedicine (Lond) 10:975–999. https://doi.org/10.2147/IJN.S68861.

116. Schwendener RA. 2014. Liposomes as vaccine delivery systems: a review of the recent advances. Ther Adv Vaccines 2:159–182. https://doi.org/10.1177/2051013614541440.

117. Riaz MK, Riaz MA, Zhang X, Lin C, Wong KH, Chen X, Zhang G, Lu A, Yang Z. 2018. Surface functionalization and targeting strategies of liposomes in solid tumor therapy: a review. Int J Mol Sci 19:195. https://doi.org/10.3390/ijms19010195.

118. Bovier PA. 2008. Epaxal: a virosomal vaccine to prevent hepatitis A infection. Expert Rev Vaccines 7:1141–1150. https://doi.org/10.1586/14760584.7.8.1141.

119. Mischler R, Metcalfe IC. 2002. Inflexal®V a trivalent virosome subunit influenza vaccine: production. Vaccine 20:B17–B23. https://doi.org/10.1016/S0264-410X(02)00512-1.

120. Conne P, Gauthey L, Vernet P, Althaus B, Que JU, Finkel B, Glück R, Cryz SJ, Cryz SJ, Jr. 1997. Immunogenicity of trivalent subunit versus virosome-formulated influenza vaccines in geriatric patients. Vaccine 15:1675–1679. https://doi.org/10.1016/S0264-410X(97)00087-X.

121. Nagata T, Toyota T, Ishigaki H, Ichihashi T, Kajino K, Kashima Y, Itoh Y, Mori M, Oda H, Yamamura H, Taneichi M, Uchida T, Ogasawara K. 2007. Peptides coupled to the surface of a kind of liposome protect infection of influenza viruses. Vaccine 25:4914–4921. https://doi.org/10.1016/j.vaccine.2007.04.010.

122. Joseph A, Louria-Hayon I, Plis-Finarov A, Zeira E, Zakay-Rones Z, Raz E, Hayashi T, Takabayashi K, Barenholz Y, Kedar E. 2002. Liposomal immunostimulatory DNA sequence (ISS-ODN): an efficient parenteral and mucosal adjuvant for influenza and hepatitis B vaccines. Vaccine 20:3342–3354. https://doi.org/10.1016/s0264-410x(02)00295-5.

123. Ohno S, Kohyama S, Taneichi M, Moriya O, Hayashi H, Oda H, Mori M, Kobayashi A, Akatsuka T, Uchida T, Matsui M. 2009. Synthetic peptides coupled to the surface of liposomes effectively induce SARS coronavirus-specific cytotoxic T lymphocytes and viral clearance in HLA-A*0201 transgenic mice. Vaccine 27:3912–3920. https://doi.org/10.1016/j.vaccine.2009.04.001.

124. Kohyama S, Ohno S, Suda T, Taneichi M, Yokoyama S, Mori M, Kobayashi A, Hayashi H, Uchida T, Matsui M. 2009. Efficient induction of cytotoxic T lymphocytes specific for severe acute respiratory syndrome (SARS)-associated coronavirus by immunization with surface-linked liposomal peptides derived from a non-structural polyprotein 1a. Antiviral Res 84:168–177. https://doi.org/10.1016/j.antiviral.2009.09.004.

125. Ulmer JB, Mason PW, Geall A, Mandl CW. 2012. RNA-based vaccines. Vaccine 30:4414–4418. https://doi.org/10.1016/j.vaccine.2012.04.060.

126. Zhang C, Maruggi G, Shan H, Li J. 2019. Advances in mRNA Vaccines for infectious diseases. Front Immunol 10:594. https://doi.org/10.3389/fimmu.2019.00594.

127. Pardi N, Weissman D. 2017. Nucleoside modified mRNA vaccines for infectious diseases. Methods Mol Biol 1499:109–121. https://doi.org/10.1007/978-1-4939-6481-9_6.

128. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. 2016. mRNA vaccine delivery using lipid nanoparticles. Ther Deliv 7:319–334. https://doi.org/10.4155/tde-2016-0006.

129. Freyn AW, Ramos da Silva J, Rosado VC, Bliss CM, Pine M, Mui BL, Tam YK, Madden TD, de Souza Ferreira LC, Weissman D, Krammer F, Coughlan L, Palese P, Pardi N, Nachbagauer R. 2020. A multi-targeting, nucleoside-modified mRNA influenza virus vaccine provides broad protection in mice. Mol Ther 28:1569–1584. https://doi.org/10.1016/j.ymthe.2020.04.018.

130. Linares-Fernandez S, Lacroix C, Exposito JY, Verrier B. 2020. Tailoring mRNA vaccine to balance innate/adaptive immune response. Trends Mol Med 26:311–323. https://doi.org/10.1016/j.molmed.2019.10.002.

131. Pardi N, Parkhouse K, Kirkpatrick E, McMahon M, Zost SJ, Mui BL, Tam YK, Karikó K, Barbosa CJ, Madden TD, Hope MJ, Krammer F, Hensley SE, Weissman D, Kariko K, Barbosa CJ, Madden TD, Hope MJ, Krammer F, Hensley SE, Weissman D. 2018. Nucleoside-modified mRNA immunization elicits influenza virus hemagglutinin stalk-specific antibodies. Nat Commun 9:3361. https://doi.org/10.1038/s41467-018-05482-0.

132. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, Dowd KA, Sutherland LL, Scearce RM, Parks R, Wagner W, Granados A, Greenhouse J, Walker M, Willis E, Yu JS, McGee CE, Sempowski GD, Mui BL, Tam YK, Huang YJ, Vanlandingham D, Holmes VM, Balachandran H, Sahu S, Lifton M, Higgs S, Hensley SE, Madden TD, Hope MJ, Kariko K, Santra S, Graham BS, Lewis MG, Pierson TC, Haynes BF, Weissman D. 2017. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature 543:248–251. https://doi.org/10.1038/nature21428.

133. Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP, Flach B, O'Connell S, Bock KW, Minai M, Nagata BM, Andersen H, Martinez DR, Noe AT, Douek N, Donaldson MM, Nji NN, Alvarado GS, Edwards DK, Flebbe DR, Lamb E, Doria-Rose NA, Lin BC, Louder MK, O'Dell S, Schmidt SD, Phung E, Chang LA, Yap C, Todd J-PM, Pessaint L, Van Ry A, Browne S, Greenhouse J, Putman-Taylor T, Strasbaugh A, Campbell T-A, Cook A, Dodson A, Steingrebe K, Shi W, Zhang Y, Abiona OM, Wang L, Pegu A, Yang ES, Leung K, Zhou T, Teng I-T, Widge A, et al. 2020. Evaluation of the mRNA-1273 vaccine against SARS-CoV-2 in non-human primates. N Engl J Med 383:1544–1555. https://doi.org/10.1056/NEJMoa2024671.

134. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, McCullough MP, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott A, Flach B, Doria-Rose NA, Corbett KS, Morabito KM, O'Dell S, Schmidt SD, Swanson PA, II, Padilla M, Mascola JR, Neuzil KM, Bennett H, Sun W, Peters E, Makowski M, Albert J, Cross K, Buchanan W, Pikaart-Tautges R, Ledgerwood JE, Graham BS, Beigel JH; for the mRNA-1273 Study Group. 2020. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med 383:1920–1931. https://doi.org/10.1056/NEJMoa2022483.

135. Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott AB, Flach B, Lin BC, Doria-Rose NA, O'Dell S, Schmidt SD, Corbett KS, Swanson PA, II, Padilla M, Neuzil KM, Bennett H, Leav B, Makowski M, Albert J, Cross K, Edara VV, Floyd K, Suthar MS, Martinez DR, Baric R, Buchanan W, Luke CJ, Phadke VK, Rostad CA, Ledgerwood JE, Graham BS, Beigel JH, M RNASG. 2020. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med 383:2427–2438. https://doi.org/10.1056/NEJMoa2028436.

136. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Kehtan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T; Group CS, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M; for the COVE Study Group, et al. 2021. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med 384:403–416. https://doi.org/10.1056/NEJMoa2035389.

137. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, Laska ME, Smith M, Almarsson O, Thompson J, Ribeiro AM, Watson M, Zaks T, Ciaramella G. 2017. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. Mol Ther 25:1316–1327. https://doi.org/10.1016/j.ymthe.2017.03.035.

138. Feldman RA, Fuhr R, Smolenov I, Mick Ribeiro A, Panther L, Watson M, Senn JJ, Smith M, Almarsson Ö, Pujar HS, Laska ME, Thompson J, Zaks T, Ciaramella G. 2019. mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. Vaccine 37:3326–3334. https://doi.org/10.1016/j.vaccine.2019.04.074.

139. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Perez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW, Jr, Hammitt LL, Tureci O, Nell H, Schaefer A, Unal S, Tresnan DB, Mather S, Dormitzer PR, Sahin U, Jansen KU, Gruber WC, Group CCT, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW, Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U,

C4591001 Clinical Trial Group, et al. 2020. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. N Engl J Med 383:2603–2615. https://doi.org/10.1056/NEJMoa2034577.

140. Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, Baum A, Pascal K, Quandt J, Maurus D, Brachtendorf S, Lörks V, Sikorski J, Hilker R, Becker D, Eller AK, Grützner J, Boesler C, Rosenbaum C, Kühnle MC, Luxemburger U, Kemmer-Brück A, Langer D, Bexon M, Bolte S, Karikó K, Palanche T, Fischer B, Schultz A, Shi PY, Fontes-Garfias C, Perez JL, Swanson KA, Loschko J, Scully IL, Cutler M, Kalina W, Kyratsous CA, Cooper D, Dormitzer PR, Jansen KU, Türeci Ö. 2020. COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses. Nature 586:594–599. https://doi.org/10.1038/s41586-020-2814-7.

141. Walsh EE, Frenck RW, Jr, Falsey AR, Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K, Mulligan MJ, Bailey R, Swanson KA, Li P, Koury K, Kalina W, Cooper D, Fontes-Garfias C, Shi P-YY, Tureci O, Tompkins KR, Lyke KE, Raabe V, Dormitzer PR, Jansen KU, Sahin U, Gruber WC, Türeci Ö, Tompkins KR, Lyke KE, Raabe V, Dormitzer PR, Jansen KU, Şahin U, Gruber WC. 2020. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Engl J Med 383:2439–2450. https://doi.org/10.1056/NEJMoa2027906.

142. Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M, Kranz LM, Walzer KC, Hein S, Güler A, Loschko J, Maddur MS, Ota-Setlik A, Tompkins K, Cole J, Lui BG, Ziegenhals T, Plaschke A, Eisel D, Dany SC, Fesser S, Erbar S, Bates F, Schneider D, Jesionek B, Sänger B, Wallisch AK, Feuchter Y, Junginger H, Krumm SA, Heinen AP, Adams-Quack P, Schlereth J, Schille S, Kröner C, de la Caridad Güimil Garcia R, Hiller T, Fischer L, Sellers RS, Choudhary S, Gonzalez O, Vascotto F, Gutman MR, Fontenot JA, Hall-Ursone S, Brasky K, Griffor MC, Han S, Su AAH, Lees JA, Nedoma NL, Mashalidis EH, Sahasrabudhe PV, et al. 2021. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. Nature 592:283–289. https://doi.org/10.1038/s41586-021-03275-y.

143. Chang X, Augusto GS, Liu X, Kündig TM, Vogel M, Mohsen MO, Bachmann MF. 2021. BNT162b2 mRNA COVID-19 vaccine induces antibodies of broader cross-reactivity than natural infection, but recognition of mutant viruses is up to 10-fold reduced. Allergy 76:2895–2998. https://doi.org/10.1111/all.14893.

144. Rauch S, Roth N, Schwendt K, Fotin-Mleczek M, Mueller SO, Petsch B. 2021. mRNA-based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus-neutralising antibodies and mediates protection in rodents. NPJ Vaccines 6:57. https://doi.org/10.1038/s41541-021-00311-w.

145. Kremsner P, Mann P, Bosch J, Fendel R, Gabor JJ, Kreidenweiss A, Kroidl A, Leroux-Roels I, Leroux-Roels G, Schindler C, Schunk M, Velavan TP, Fotin-Mleczek M, Müller S, Quintini G, Schönborn-Kellenberger O, Vahrenhorst D, Verstraeten T, Walz L, Wolz O-O, Oostvogels L, CV-NCOV-001 Study Group. 2021. Safety and immunogenicity of an mRNA-lipid nanoparticle vaccine candidate against SARS-CoV-2: a phase 1 randomized clinical trial. Wien Klin Wochenschr 133:931–941. https://doi.org/10.1007/s00508-021-01922-y.

146. Hong HC, Kim KS, Park SA, Chun MJ, Hong EY, Chung SW, Kim HJ, Shin BG, Braka A, Thanappan J, Jang S, Wu S, Cho YJ, Kim S-H. 2021. An mRNA vaccine against SARS-CoV-2: lyophilized, liposome-based vaccine candidate EG-COVID induces high levels of virus neutralizing antibodies. bioRxiv https://doi.org/10.1101/2021.03.22.436375.

147. Ballesteros-Briones MC, Silva-Pilipich N, Herrador-Cañete G, Vanrell L, Smerdou C. 2020. A new generation of vaccines based on alphavirus self-amplifying RNA. Curr Opin Virol 44:145–153. https://doi.org/10.1016/j.coviro.2020.08.003.

148. Brito LA, Kommareddy S, Maione D, Uematsu Y, Giovani C, Berlanda Scorza F, Otten GR, Yu D, Mandl CW, Mason PW, Dormitzer PR, Ulmer JB, Geall AJ. 2015. Chapter Seven—Self-amplifying mRNA vaccines, p 179–233. In Huang L, Liu D, Wagner E (ed), Advanced Genetics. Academic Press, New York, NY.

149. Blakney AK, Ip S, Geall AJ. 2021. An update on self-amplifying mRNA vaccine development. Vaccines (Basel) 9:97. https://doi.org/10.3390/vaccines9020097.

150. Lundstrom K. 2020. Nanoparticle-based delivery of self-amplifying RNA. Gene Ther 27:183–185. https://doi.org/10.1038/s41434-020-0132-1.

151. Maruggi G, Chiarot E, Giovani C, Buccato S, Bonacci S, Frigimelica E, Margarit I, Geall A, Bensi G, Maione D. 2017. Immunogenicity and protective efficacy induced by self-amplifying mRNA vaccines encoding bacterial antigens. Vaccine 35:361–368. https://doi.org/10.1016/j.vaccine.2016.11.040.

152. McKay PF, Hu K, Blakney AK, Samnuan K, Brown JC, Penn R, Zhou J, Bouton CR, Rogers P, Polra K, Lin PJC, Barbosa C, Tam YK, Barclay WS, Shattock RJ. 2020. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle

vaccine candidate induces high neutralizing antibody titers in mice. Nat Commun 11:3523. https://doi.org/10.1038/s41467-020-17409-9.

153. de Alwis R, Gan ES, Chen S, Leong YS, Tan HC, Zhang SL, Yau C, Low JGH, Kalimuddin S, Matsuda D, Allen EC, Hartman P, Park K-JJ, Alayyoubi M, Bhaskaran H, Dukanovic A, Bao Y, Clemente B, Vega J, Roberts S, Gonzalez JA, Sablad M, Yelin R, Taylor W, Tachikawa K, Parker S, Karmali P, Davis J, Sullivan BM, Sullivan SM, Hughes SG, Chivukula P, Ooi EE. 2021. A single dose of self-transcribing and replicating RNA-based SARS-CoV-2 vaccine produces protective adaptive immunity in mice. Mol Ther https://doi.org/10.1016/j.ymthe.2021.04.001.

154. Ramaswamy S, Tonnu N, Tachikawa K, Limphong P, Vega JB, Karmali PP, Chivukula P, Verma IM. 2017. Systemic delivery of factor IX messenger RNA for protein replacement therapy. Proc Natl Acad Sci U S A 114: E1941–E1950. https://doi.org/10.1073/pnas.1619653114.

155. Erasmus JH, Khandhar AP, O'Connor MA, Walls AC, Hemann EA, Murapa P, Archer J, Leventhal S, Fuller JT, Lewis TB, Draves KE, Randall S, Guerriero KA, Duthie MS, Carter D, Reed SG, Hawman DW, Feldmann H, Gale M, Jr, Veesler D, Berglund P, Heydenburg Fuller D. 2020. An alphavirus-derived replicon RNA vaccine induces SARS-CoV-2 neutralizing antibody and T cell responses in mice and nonhuman primates. Sci Transl Med 12:eabc9396. https://doi.org/10.1126/scitranslmed.abc9396.

156. Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M, Giusti F, Ferlenghi I, Johnson RN, Roberts CW, O'Hagan DT, Baudner BC, Perrie Y. 2020. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: the impact of cationic lipid selection. J Control Release 325:370–379. https://doi.org/10.1016/j.jconrel.2020.06.027.

157. Bogers WM, Oostermeijer H, Mooij P, Koopman G, Verschoor EJ, Davis D, Ulmer JB, Brito LA, Cu Y, Banerjee K, Otten GR, Burke B, Dey A, Heeney JL, Shen X, Tomaras GD, Labranche C, Montefiori DC, Liao HX, Haynes B, Geall AJ, Barnett SW. 2015. Potent immune responses in rhesus macaques induced by nonviral delivery of a self-amplifying RNA vaccine expressing HIV type 1 envelope with a cationic nanoemulsion. J Infect Dis 211:947–955. https://doi.org/10.1093/infdis/jiu522.

158. Geall AJ, Verma A, Otten GR, Shaw CA, Hekele A, Banerjee K, Cu Y, Beard CW, Brito LA, Krucker T, O'Hagan DT, Singh M, Mason PW, Valiante NM, Dormitzer PR, Barnett SW, Rappuoli R, Ulmer JB, Mandl CW. 2012. Nonviral delivery of self-amplifying RNA vaccines. Proc Natl Acad Sci U S A 109:14604–14609. https://doi.org/10.1073/pnas.1209367109.

159. Wang D, Christopher ME, Nagata LP, Zabielski MA, Li H, Wong JP, Samuel J. 2004. Intranasal immunization with liposome-encapsulated plasmid DNA encoding influenza virus hemagglutinin elicits mucosal, cellular and humoral immune responses. J Clin Virol 31:S99–S106.

160. Liu J, Wu J, Wang B, Zeng S, Qi F, Lu C, Kimura Y, Liu BJ. 2014. Oral vaccination with a liposome-encapsulated influenza DNA vaccine protects mice against respiratory challenge infection. J Med Virol 86:886–894. https://doi.org/10.1002/jmv.23768.

161. Yeh PY, Ellens H, Smith PL. 1998. Physiological considerations in the design of particulate dosage forms for oral vaccine delivery. Adv Drug Deliv Rev 34:123–133. https://doi.org/10.1016/S0169-409X(98)00036-2.

162. Schalk JAC, Mooi FR, Berbers GAM, van Aerts L, Ovelgönne H, Kimman TG. 2006. Preclinical and clinical safety studies on DNA vaccines. Hum Vaccin 2:45–53. https://doi.org/10.4161/hv.2.2.2620.

163. Roldão A, Mellado MCM, Castilho LR, Carrondo MJT, Alves PM. 2010. Virus-like particles in vaccine development. Expert Rev Vaccines 9: 1149–1176. https://doi.org/10.1586/erv.10.115.

164. Fuenmayor J, Gòdia F, Cervera L. 2017. Production of virus-like particles for vaccines. N Biotechnol 39:174–180. https://doi.org/10.1016/j.nbt.2017.07.010.

165. Wu CY, Yeh YC, Chan JT, Yang YC, Yang JR, Liu MT, Wu HS, Hsiao PW. 2012. A VLP vaccine induces broad-spectrum cross-protective antibody immunity against H5N1 and H1N1 subtypes of influenza A virus. PLoS One 7:e42363. https://doi.org/10.1371/journal.pone.0042363.

166. Kang SM, Song JM, Quan FS, Compans RW. 2009. Influenza vaccines based on virus-like particles. Virus Res 143:140–146. https://doi.org/10.1016/j.virusres.2009.04.005.

167. Kato T, Takami Y, Kumar Deo V, Park EY. 2019. Preparation of virus-like particle mimetic nanovesicles displaying the S protein of Middle East respiratory syndrome coronavirus using insect cells. J Biotechnol 306: 177–184. https://doi.org/10.1016/j.jbiotec.2019.10.007.

168. Wang C, Zheng X, Gai W, Wong G, Wang H, Jin H, Feng N, Zhao Y, Zhang W, Li N, Zhao G, Li J, Yan J, Gao Y, Hu G, Yang S, Xia X. 2017. Novel chimeric virus-like particles vaccine displaying MERS-CoV receptor-binding domain induce specific humoral and cellular immune response in mice. Antiviral Res 140:55–61. https://doi.org/10.1016/j.antiviral.2016.12.019.

169. Lokugamage KG, Yoshikawa-Iwata N, Ito N, Watts DM, Wyde PR, Wang N, Newman P, Kent Tseng CT, Peters CJ, Makino S. 2008. Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SCoV) S protein protect mice against challenge with SCoV. Vaccine 26:797–808. https://doi.org/10.1016/j.vaccine.2007.11.092.

170. Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, Peters CJ, Couch RB. 2012. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. PLoS One 7:e35421. https://doi.org/10.1371/journal.pone.0035421.

171. Cecil JD, Sirisaengtaksin N, O'Brien-Simpson NM, Krachler AM. 2019. Outer membrane vesicle-host cell interactions. Microbiol Spectr 7. https://doi.org/10.1128/microbiolspec.PSIB-0001-2018.

172. Mancini F, Rossi O, Necchi F, Micoli F. 2020. OMV vaccines and the role of TLR agonists in immune response. Int J Mol Sci 21:4416. https://doi.org/10.3390/ijms21124416.

173. Giuntini S, Lujan E, Gibani MM, Dold C, Rollier CS, Pollard AJ, Granoff DM. 2017. Serum bactericidal antibody responses of adults immunized with the MenB-4C vaccine against genetically diverse serogroup B meningococci. Clin Vaccine Immunol 24:e00430-16. https://doi.org/10.1128/CVI.00430-16.

174. Marsay L, Dold C, Green CA, Rollier CS, Norheim G, Sadarangani M, Shanyinde M, Brehony C, Thompson AJ, Sanders H, Chan H, Haworth K, Derrick JP, Feavers IM, Maiden MC, Pollard AJ. 2015. A novel meningococcal outer membrane vesicle vaccine with constitutive expression of FetA: a phase I clinical trial. J Infect 71:326–337. https://doi.org/10.1016/j.jinf.2015.05.006.

175. McQuaid F, Snape MD, John TM, Kelly S, Robinson H, Yu L-M, Toneatto D, D'Agostino D, Dull PM, Pollard AJ. 2015. Persistence of specific bactericidal antibodies at 5 years of age after vaccination against serogroup B meningococcus in infancy and at 40 months. CMAJ 187:E215–E223. https://doi.org/10.1503/cmaj.141200.

176. Snape MD, Saroey P, John TM, Robinson H, Kelly S, Gossger N, Yu LM, Wang H, Toneatto D, Dull PM, Pollard AJ. 2013. Persistence of bactericidal antibodies following early infant vaccination with a serogroup B meningococcal vaccine and immunogenicity of a preschool booster dose. CMAJ 185:E715–E724. https://doi.org/10.1503/cmaj.130257.

177. Saez-Llorens X, Beltran-Rodriguez J, Novoa Pizarro JM, Mensi I, Keshavan P, Toneatto D. 2018. Four-year antibody persistence and response to a booster dose of a pentavalent MenABCWY vaccine administered to healthy adolescents and young adults. Hum Vaccin Immunother 14: 1161–1174. https://doi.org/10.1080/21645515.2018.1457595.

178. Stoddard MB, Pinto V, Keiser PB, Zollinger W. 2010. Evaluation of a whole-blood cytokine release assay for use in measuring endotoxin activity of group B *Neisseria meningitidis* vaccines made from lipid A acylation mutants. Clin Vaccine Immunol 17:98–107. https://doi.org/10.1128/CVI.00342-09.

179. Shehata MM, Mostafa A, Teubner L, Mahmoud SH, Kandeil A, Elshesheny R, Frantz R, La Pietra L, Pleschka S, Osman A, Kayali G, Chakraborty T, Ali MA, Mraheil MA. 2019. Bacterial outer membrane vesicles (OMVs)-based dual vaccine for influenza A H1N1 virus and MERS-CoV. Vaccines (Basel) 7:46. https://doi.org/10.3390/vaccines7020046.

180. Bae EH, Seo SH, Kim CU, Jang MS, Song MS, Lee TY, Jeong YJ, Lee MS, Park JH, Lee P, Kim YS, Kim SH, Kim DJ. 2019. Bacterial outer membrane vesicles provide broad-spectrum protection against influenza virus infection via recruitment and activation of macrophages. J Innate Immun 11:316–329. https://doi.org/10.1159/000494098.

181. Vanaja SK, Russo AJ, Behl B, Banerjee I, Yankova M, Deshmukh SD, Rathinam VAK. 2016. Bacterial outer membrane vesicles mediate cytosolic localization of LPS and caspase-11 activation. Cell 165:1106–1119. https://doi.org/10.1016/j.cell.2016.04.015.

182. Wang Z, Gao H, Zhang Y, Liu G, Niu G, Chen X. 2017. Functional ferritin nanoparticles for biomedical applications. Front Chem Sci Eng 11: 633–646. https://doi.org/10.1007/s11705-017-1620-8.

183. Wang L, Xing D, Le Van A, Jerse AE, Wang S. 2017. Structure-based design of ferritin nanoparticle immunogens displaying antigenic loops of Neisseria gonorrhoeae. FEBS Open Bio 7:1196–1207. https://doi.org/10.1002/2211-5463.12267.

184. Kanekiyo M, Wei CJ, Yassine HM, McTamney PM, Boyington JC, Whittle JR, Rao SS, Kong WP, Wang L, Nabel GJ. 2013. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. Nature 499:102–106. https://doi.org/10.1038/nature12202.

185. Yao D, Lao F, Liu Y, Ouyang F, Cheng J, Ding H, Ke T. 2020. Human H-ferritin presenting RBM of spike glycoprotein as potential vaccine of SARS-CoV-2. bioRxiv https://doi.org/10.1101/2020.05.25.115618.

186. Powell AE, Zhang K, Sanyal M, Tang S, Weidenbacher PA, Li S, Pham TD, Pak JE, Chiu W, Kim PS. 2021. A single immunization with spike-functionalized ferritin vaccines elicits neutralizing antibody responses against SARS-CoV-2 in mice. ACS Cent Sci 7:183–199. https://doi.org/10.1021/acscentsci.0c01405.

187. Ueda G, Antanasijevic A, Fallas JA, Sheffler W, Copps J, Ellis D, Hutchinson GB, Moyer A, Yasmeen A, Tsybovsky Y. 2020. Tailored design of protein nanoparticle scaffolds for multivalent presentation of viral glycoprotein antigens. Elife 9:e57659. https://doi.org/10.7554/eLife.57659.

188. Boyoglu-Barnum S, Ellis D, Gillespie RA, Hutchinson GB, Park Y-J, Moin SM, Acton OJ, Ravichandran R, Murphy M, Pettie D, Matheson N, Carter L, Creanga A, Watson MJ, Kephart S, Ataca S, Vaile JR, Ueda G, Crank MC, Stewart L, Lee KK, Guttman M, Baker D, Mascola JR, Veesler D, Graham BS, King NP, Kanekiyo M. 2021. Quadrivalent influenza nanoparticle vaccines induce broad protection. Nature 592:623–628. https://doi.org/10.1038/s41586-021-03365-x.

189. Tian JH, Patel N, Haupt R, Zhou H, Weston S, Hammond H, Logue J, Portnoff AD, Norton J, Guebre-Xabier M, Zhou B, Jacobson K, Maciejewski S, Khatoon R, Wisniewska M, Moffitt W, Kluepfel-Stahl S, Ekechukwu B, Papin J, Boddapati S, Jason Wong C, Piedra PA, Frieman MB, Massare MJ, Fries L, Bengtsson KL, Stertman L, Ellingsworth L, Glenn G, Smith G. 2021. SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 immunogenicity in baboons and protection in mice. Nat Commun 12:1–14. https://doi.org/10.1038/s41467-020-20653-8.

190. Bangaru S, Ozorowski G, Turner HL, Antanasijevic A, Huang D, Wang X, Torres JL, Diedrich JK, Tian JH, Portnoff AD, Patel N, Massare MJ, Yates JR, Nemazee D, Paulson JC, Glenn G, Smith G, Ward AB. 2020. Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate. Science 370:1089–1094. https://doi.org/10.1126/science.abe1502.

191. Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Smith GE, Frieman MB. 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 32:3169–3174. https://doi.org/10.1016/j.vaccine.2014.04.016.

192. Guebre-Xabier M, Patel N, Tian JH, Zhou B, Maciejewski S, Lam K, Portnoff AD, Massare MJ, Frieman MB, Piedra PA, Ellingsworth L, Glenn G, Smith G. 2020. NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge. Vaccine 38:7892–7896. https://doi.org/10.1016/j.vaccine.2020.10.064.

193. Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, Plested JS, Zhu M, Cloney-Clark S, Zhou H, Smith G, Patel N, Frieman MB, Haupt RE, Logue J, McGrath M, Weston S, Piedra PA, Desai C, Callahan K, Lewis M, Price-Abbott P, Formica N, Shinde V, Fries L, Lickliter JD, Griffin P, Wilkinson B, Glenn GM. 2020. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. N Engl J Med 383:2320–2332. https://doi.org/10.1056/NEJMoa2026920.

194. Heath PT, Galiza EP, Baxter DN, Boffito M, Browne D, Burns F, Chadwick DR, Clark R, Cosgrove C, Galloway J, Goodman AL, Heer A, Higham A, Iyengar S, Jamal A, Jeanes C, Kalra PA, Kyriakidou C, McAuley DF, Meyrick A, Minassian AM, Minton J, Moore P, Munsoor I, Nicholls H, Osanlou O, Packham J, Pretswell CH, San Francisco Ramos A, Saralaya D, Sheridan RP, Smith R, Soiza RL, Swift PA, Thomson EC, Turner J, Viljoen ME, Albert G, Cho I, Dubovsky F, Glenn G, Rivers J, Robertson A, Smith K, Toback S. 2021. Safety and efficacy of NVX-CoV2373 COVID-19 vaccine. N Engl J Med. 385:1172–1183. https://doi.org/10.1056/NEJMoa2107659.

195. Formica N, Mallory R, Albert G, Robinson M, Plested JS, Cho I, Robertson A, Dubovsky F, Glenn GM. 2021. Evaluation of a SARS-CoV-2 vaccine NVX-CoV2373 in younger and older adults. medRxiv https://doi.org/10.1101/2021.02.26.21252482.

196. Shinde V, Bhikha S, Hoosain Z, Archary M, Bhorat Q, Fairlie L, Lalloo U, Masilela MSL, Moodley D, Hanley S, Fouche L, Louw C, Tameris M, Singh N, Goga A, Dheda K, Grobbelaar C, Kruger G, Carrim-Ganey N, Baillie V, de Oliveira T, Lombard Koen A, Lombaard JJ, Mngqibisa R, Bhorat AE, Benadé G, Lalloo N, Pitsi A, Vollgraaff P-L, Luabeya A, Esmail A, Petrick FG, Oommen-Jose A, Foulkes S, Ahmed K, Thombrayil A, Fries L, Cloney-Clark S, Zhu M, Bennett C, Albert G, Faust E, Plested JS, Robertson A, Neal S, Cho I, Glenn GM, Dubovsky F, Madhi SA, 2019nCoV-501 Study Group. 2021. Efficacy of NVX-CoV2373 COVID-19 vaccine against the B.1.351 variant. N Engl J Med 384:1899–1909. https://doi.org/10.1056/NEJMoa2103055.

197. Toback S, Galiza E, Cosgrove C, Galloway J, Goodman AL, Swift PA, Rajaram S, Graves-Jones A, Edelman J, Burns F, Minassian AM, Cho I, Kumar L, Plested JS, Rivers EJ, Robertson A, Dubovsky F, Glenn G, Heath PT. 2021. Safety, immunogenicity, and efficacy of a COVID-19 vaccine (NVX-CoV2373) co-administered with seasonal influenza vaccines. medRxiv https://doi.org/10.1101/2021.06.09.21258556.

198. Prausnitz MR, Langer R. 2008. Transdermal drug delivery. Nat Biotechnol 26:1261–1268. https://doi.org/10.1038/nbt.1504.

199. Schoellhammer CM, Blankschtein D, Langer R. 2014. Skin permeabilization for transdermal drug delivery: recent advances and future prospects. Expert Opin Drug Deliv 11:393–407. https://doi.org/10.1517/17425247.2014.875528.

200. Mistilis MJ, Joyce JC, Esser ES, Skountzou I, Compans RW, Bommarius AS, Prausnitz MR. 2017. Long-term stability of influenza vaccine in a dissolving microneedle patch. Drug Deliv Transl Res 7:195–205. https://doi.org/10.1007/s13346-016-0282-2.

201. Chu LY, Ye L, Dong K, Compans RW, Yang C, Prausnitz MR. 2016. enhanced stability of inactivated influenza vaccine encapsulated in dissolving microneedle patches. Pharm Res 33:868–878. https://doi.org/10.1007/s11095-015-1833-9.

202. Mistilis MJ, Bommarius AS, Prausnitz MR. 2015. Development of a thermostable microneedle patch for influenza vaccination. J Pharm Sci 104:740–749. https://doi.org/10.1002/jps.24283.

203. Fernando GJP, Hickling J, Jayashi Flores CM, Griffin P, Anderson CD, Skinner SR, Davies C, Witham K, Pryor M, Bodle J, Rockman S, Frazer IH, Forster AH. 2018. Safety, tolerability, acceptability and immunogenicity of an influenza vaccine delivered to human skin by a novel high-density microprojection array patch (Nanopatch). Vaccine 36:3779–3788. https://doi.org/10.1016/j.vaccine.2018.05.053.

204. Forster AH, Witham K, Depelsenaire ACI, Veitch M, Wells JW, Wheatley A, Pryor M, Lickliter JD, Francis B, Rockman S, Bodle J, Treasure P, Hickling J, Fernando GJP. 2020. Safety, tolerability, and immunogenicity of influenza vaccination with a high-density microarray patch: results from a randomized, controlled phase I clinical trial. PLoS Med 17:e1003024. https://doi.org/10.1371/journal.pmed.1003024.

205. Kim E, Erdos G, Huang S, Kenniston TW, Balmert SC, Carey CD, Raj VS, Epperly MW, Klimstra WB, Haagmans BL, Korkmaz E, Falo LD, Jr, Gambotto A. 2020. Microneedle array delivered recombinant coronavirus vaccines: immunogenicity and rapid translational development. EBioMedicine 55:102743. https://doi.org/10.1016/j.ebiom.2020.102743.

206. Prausnitz MR. 2017. Engineering microneedle patches for vaccination and drug delivery to skin. Annu Rev Chem Biomol Eng 8:177–200. https://doi.org/10.1146/annurev-chembioeng-060816-101514.

207. Kwon KM, Lim SM, Choi S, Kim DH, Jin HE, Jee G, Hong KJ, Kim JY. 2017. Microneedles: quick and easy delivery methods of vaccines. Clin Exp Vaccine Res 6:156–159. https://doi.org/10.7774/cevr.2017.6.2.156.

208. Amante DH, Smith TR, Mendoza JM, Schultheis K, McCoy JR, Khan AS, Sardesai NY, Broderick KE. 2015. Skin transfection patterns and expression kinetics of electroporation-enhanced plasmid delivery using the CELLECTRA-3P, a portable next-generation dermal electroporation device. Hum Gene Ther Methods 26:134–146. https://doi.org/10.1089/hgtb.2015.020.

209. Todorova B, Adam L, Culina S, Boisgard R, Martinon F, Cosma A, Ustav M, Kortulewski T, Le Grand R, Chapon C. 2017. Electroporation as a vaccine delivery system and a natural adjuvant to intradermal administration of plasmid DNA in macaques. Sci Rep 7:4122. https://doi.org/10.1038/s41598-017-04547-2.

210. Diehl MC, Lee JC, Daniels SE, Tebas P, Khan AS, Giffear M, Sardesai NY, Bagarazzi ML. 2013. Tolerability of intramuscular and intradermal delivery by CELLECTRA® adaptive constant current electroporation device in healthy volunteers. Hum Vaccin Immunother 9:2246–2252. https://doi.org/10.4161/hv.24702.

211. Bagarazzi ML, Yan J, Morrow MP, Shen X, Parker RL, Lee JC, Giffear M, Pankhong P, Khan AS, Broderick KE, Knott C, Lin F, Boyer JD, Draghia-Akli R, White CJ, Kim JJ, Weiner DB, Sardesai NY. 2012. Immunotherapy against HPV16/18 generates potent T_H1 and cytotoxic cellular immune responses. Sci Transl Med 4:155ra138. https://doi.org/10.1126/scitranslmed.3004414.

212. Modjarrad K, Roberts CC, Mills KT, Castellano AR, Paolino K, Muthumani K, Reuschel EL, Robb ML, Racine T, Oh M-O, Lamarre C, Zaidi FI, Boyer J, Kudchodkar SB, Jeong M, Darden JM, Park YK, Scott PT, Remigio C, Parikh AP, Wise MC, Patel A, Duperret EK, Kim KY, Choi H, White S, Bagarazzi M, May JM, Kane D, Lee H, Kobinger G, Michael NL, Weiner DB, Thomas SJ, Maslow JN. 2019. Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial. Lancet Infect Dis 19:1013–1022. https://doi.org/10.1016/S1473-3099(19)30266-X.

213. Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J, Gary EN, Walker SN, Schultheis K, Purwar M, Xu Z, Walters J, Bhojnagarwala P, Yang M, Chokkalingam N, Pezzoli P, Parzych E, Reuschel EL, Doan A, Tursi N, Vasquez M, Choi J, Tello-Ruiz E, Maricic I, Bah MA, Wu Y, Amante D, Park

DH, Dia Y, Ali AR, Zaidi FI, Generotti A, Kim KY, Herring TA, Reeder S, Andrade VM, Buttigieg K, Zhao G, Wu JM, Li D, Bao L, Liu J, Deng W, Qin C, Brown AS, Khoshnejad M, Wang N, Chu J, Wrapp D, McLellan JS, Muthumani K, Wang B, et al. 2020. Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun 11:2601. https://doi.org/10.1038/s41467-020-16505-0.

214. Tebas P, Yang S, Boyer JD, Reuschel EL, Patel A, Christensen-Quick A, Andrade VM, Morrow MP, Kraynyak K, Agnes J, Purwar M, Sylvester A, Gillespie E, Maricic I, Zaidi FI, Kim KY, Dia Y, Frase D, Pezzoli P, Schultheis K, Smith TRF, Ramos SJ, McMullan T, Buttigieg K, Carroll MW, Ervin J, Diehl MC, Blackwood E, Mammen MP, Lee J, Dallas MJ, Brown AS, Shea JE, Kim JJ, Weiner DB, Broderick KE, Humeau LM. 2020. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: a preliminary report of an open-label, phase 1 clinical trial. E Clinical Medicine 31: 100689. https://doi.org/10.1016/j.eclinm.2020.100689.

215. Andrade VM, Christensen-Quick A, Agnes J, Tur J, Reed C, Kalia R, Marrero I, Elwood D, Schultheis K, Purwar M, Reuschel E, McMullan T, Pezzoli P, Kraynyak K, Sylvester A, Mammen MP, Tebas P, Kim JJ, Weiner DB, Smith TRF, Ramos SJ, Humeau LM, Boyer JD, Broderick KE. 2021. INO-4800 DNA vaccine induces neutralizing antibodies and T cell activity against global SARS-CoV-2 variants. bioRxiv https://doi.org/10.1101/2021.04.14.439719.

216. Riddell S, Goldie S, McAuley AJ, Kuiper MJ, Durr PA, Blasdell KR, Tachedjian M, Druce JD, Smith TRF, Broderick KE, Vasan SS. 2021. Live virus neutralisation of the 501Y.V1 and 501Y.V2 SARS-CoV-2 variants following INO-4800 vaccination of ferrets. Front Immunol 12:2475. https://doi.org/10.3389/fimmu.2021.694857.

217. Sokolowska E, Blachnio-Zabielska AU. 2019. A critical review of electroporation as a plasmid delivery system in mouse skeletal muscle. Int J Mol Sci 20:2776. https://doi.org/10.3390/ijms20112776.

218. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. 2020. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nat Nanotechnol 15:313–320. https://doi.org/10.1038/s41565-020-0669-6.

219. Holmgren J, Czerkinsky C. 2005. Mucosal immunity and vaccines. Nat Med 11:S45–S53. https://doi.org/10.1038/nm1213.

220. Thakur A, Foged C. 2020. Nanoparticles for mucosal vaccine delivery, p 603–646. In Mozafari M (ed), Nanoengineered biomaterials for advanced drug delivery. Elsevier, Amsterdam, the Netherlands.

221. Kim J, Li WA, Choi Y, Lewin SA, Verbeke CS, Dranoff G, Mooney DJ. 2015. Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy. Nat Biotechnol 33: 64–72. https://doi.org/10.1038/nbt.3071.

222. Shi L, Khondee S, Linz TH, Berkland C. 2008. Poly(N-vinylformamide) nanogels capable of pH-sensitive protein release. Macromolecules 41: 6546–6554. https://doi.org/10.1021/ma800812z.

223. Kwon YJ, James E, Shastri N, Frechet JM. 2005. In vivo targeting of dendritic cells for activation of cellular immunity using vaccine carriers based on pH-responsive microparticles. Proc Natl Acad Sci U S A 102: 18264–18268. https://doi.org/10.1073/pnas.0509541102.

224. Keller S, Wilson JT, Patilea GI, Kern HB, Convertine AJ, Stayton PS. 2014. Neutral polymer micelle carriers with pH-responsive, endosome-releasing activity modulate antigen trafficking to enhance CD8$^+$ T cell responses. J Control Release 191:24–33. https://doi.org/10.1016/j.jconrel.2014.03.041.

225. Duong HTT, Kim NW, Thambi T, Giang Phan VH, Lee MS, Yin Y, Jeong JH, Lee DS. 2018. Microneedle arrays coated with charge reversal pH-sensitive copolymers improve antigen presenting cells-homing DNA vaccine delivery and immune responses. J Control Release 269:225–234. https://doi.org/10.1016/j.jconrel.2017.11.025.

226. Hajj KA, Melamed JR, Chaudhary N, Lamson NG, Ball RL, Yerneni SS, Whitehead KA. 2020. A potent branched-tail lipid nanoparticle enables multiplexed mRNA delivery and gene editing in vivo. Nano Lett 20: 5167–5175. https://doi.org/10.1021/acs.nanolett.0c00596.

227. Hajj KA, Ball RL, Deluty SB, Singh SR, Strelkova D, Knapp CM, Whitehead KA. 2019. Branched-tail lipid nanoparticles potently deliver mRNA in vivo due to enhanced ionization at endosomal pH. Small 15:e1805097. https://doi.org/10.1002/smll.201805097.

228. Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, Sohail M, Luo S, Um SH, Khant H, Goodwin JT, Ramos J, Chiu W, Irvine DJ. 2011. Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. Nat Mater 10:243–251. https://doi.org/10.1038/nmat2960.

229. Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. 2017. The complexity and cost of vaccine manufacturing—an overview. Vaccine 35: 4064–4071. https://doi.org/10.1016/j.vaccine.2017.06.003.

230. Bernal-Chávez SA, Del Prado-Audelo ML, Caballero-Florán IH, Giraldo-Gomez DM, Figueroa-Gonzalez G, Reyes-Hernandez OD, Carmen MG-D, González-Torres M, Cortés H, Leyva-Gómez G. 2021. Insights into terminal sterilization processes of nanoparticles for biomedical applications. Molecules 26:2068. https://doi.org/10.3390/molecules26072068.

231. Holy CE, Cheng C, Davies JE, Shoichet MS. 2000. Optimizing the sterilization of PLGA scaffolds for use in tissue engineering. Biomaterials 22: 25–31. https://doi.org/10.1016/S0142-9612(00)00136-8.

232. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, Madden TD, Hope MJ, Weissman D. 2015. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Release 217:345–351. https://doi.org/10.1016/j.jconrel.2015.08.007.

233. Shen X, Tang H, Pajon R, Smith G, Glenn GM, Shi W, Korber B, Montefiori DC. 2021. Neutralization of SARS-CoV-2 variants B.1.429 and B.1.351. N Engl J Med 384:2352–2354. https://doi.org/10.1056/NEJMc2103740.

234. Keech C, Albert G, Reed P, Neal S, Plested JS, Zhu M, Cloney-Clark S, Zhou H, Patel N, Frieman MB, Haupt RE, Logue J, McGrath M, Weston S, Piedra PA, Cho I, Robertson A, Desai C, Callahan K, Lewis M, Price-Abbott P, Formica N, Shinde V, Fries L, Linkliter JD, Griffin P, Wilkinson B, Smith G, Glenn GM. 2020. First-in-human trial of a SARS CoV 2 recombinant spike protein nanoparticle vaccine. medRxiv https://doi.org/10.1101/2020.08.05.20168435.

Shalini Iyer is a Ph.D. student at the Université de Namur, Namur, Belgium. She did her undergraduate degree in biotechnology at the University of Madras, Chennai, India, followed by an M.Sc. in Human Genetics at Sri Ramachandra University, Chennai, India. Her master's thesis was on the toxicity assessment of titanium dioxide nanoparticles. She then did another specialized master's degree in immunology and cancer biology at Université Grenoble-Alpes, Grenoble, France. Her master's thesis at the Institute of Advanced Biosciences, Grenoble, France, was on cell adhesion and migration mechanisms. Shalini worked as a project assistant in the field of biomaterial-based vaccine development at the Drug Delivery Lab at the Indian Institute of Science, Bengaluru, India. Her scientific interests are in the manipulation of the immune system and use of biomaterials in cancer therapy. Her doctoral research in cancer immunology focuses on the reprogramming of tumor-associated macrophages through gold nanoparticles and proton therapy.

Rajesh Yadav is a Ph.D. Scholar from the Center for Biosystems Science and Engineering Department of the Indian Institute of Science, Bengaluru. As part of his Ph.D. project, he is addressing the decades-old unmet clinical need to develop a universal influenza vaccine. For this, he is using computation-guided immunogen targets to boost both humoral and cellular immune responses against conserved antigenic proteins of influenza virus. The optimized targets will be formulated in a biomaterial-based vaccine. He completed his master's degree in biotechnology at the Indian Institute of Technology Bombay, where he worked in the Molecular Virology Laboratory of Bioscience and Bioengineering Department. His major work was on the metagenomic analysis of giant viruses. He discovered a new virus called Bandra megavirus, the largest giant virus reported from India. He has also undergone a biodesign healthcare fellowship program at the Centre for Healthcare Entrepreneurship, Indian Institute of Technology Hyderabad.

Smriti Agarwal did her undergraduate studies at Vellore Institute of Technology, Vellore, in Biotechnology. During her undergraduate studies, she did a summer internship in the field of genetic diagnostics at the Center for Genetic Disorders, Banaras Hindu University. She did her thesis project in the field of functional genomics at the CSIR Institute of Genomics and Integrative Biology, Delhi, where she worked on the development of the diagnostic test for facioscapulohumeral muscular dystrophy using Southern blotting and long-range PCR techniques. After undergraduate study, she worked as a project assistant at the Indian Institute of Sciences, Bengaluru, in the drug delivery lab, where she worked on poly(lactic-*co*-glycolic acid)- and liposome-based sustained-delivery carriers for the treatment of osteoarthritis. She then briefly worked at the Indian Institute of Technology, Delhi, where she performed a literature study on using impedance spectroscopy to explore the real-time response of bacteria to antibiotics to design biosensors. She will soon be an intern in the design team of a leading unicorn startup, Razorpay.

Shashank Tripathi, Ph.D., is an Assistant Professor in the Microbiology and Cell Biology Department of the Indian Institute of Science. His lab is situated in the Centre for Infectious Disease research, where he also heads the viral biosafety level 3 (BSL3) facility. He is also a Wellcome Trust India Alliance Intermediate Fellow. He is a virologist by training and did his Ph.D. on influenza A virus-host interactions, working in the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, in collaboration with the Influenza Division, Centers for Disease Control (Atlanta, GA). Later, he did his postdoctoral training in Professor Adolfo Garcia-Sastre's lab in the Microbiology Department of the Icahn School of Medicine at Mount Sinai (New York, NY). There, he studied influenza A virus-host interactions at a systems level and the immune evasion and pathogenesis of Zika viruses. His current research program is focused on developing novel vaccines and antivirals against human respiratory viruses like influenza A virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Rachit Agarwal, Ph.D., is an Assistant Professor at the Indian Institute of Science, Bengaluru, India, since 2017. He did his undergraduate studies at the Indian Institute of Technology, Kharagpur, India, in biotechnology. He then worked in the field of biomaterial-based drug delivery for his Ph.D. at the University of Texas at Austin (Austin, Texas). His postdoctoral fellowship was on regenerative medicine at the Georgia Institute of Technology (Atlanta, GA). His scientific interests are in developing biomaterial-based delivery vehicles for the treatment of inflammatory and infectious diseases. He is a recipient of a prestigious Ramanujan fellowship, a Har-Gobind Khorana Young Biotechnologist award, and an DBT/Wellcome Trust India Alliance Intermediate Fellowship. Two of his major areas of interest are in the treatment of osteoarthritis and in using inhalable biomaterials for prevention and treatment of lung infections with the goal of translating science into technologies that can be used in the clinic. More information is available at https://be.iisc.ac.in/~rachit/.