A biomaterials viewpoint for the 2020 SARS-CoV-2 vaccine development

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Key Words:
biomaterials; COVID-19; nanomaterials; SARS-CoV-2; vaccine

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused a considerable loss of life, morbidity, and economic distress since its emergence in late 2019. In response to the novel virus, public and private institutions around the world have utilized novel technologies to develop a vaccine in the hopes of building herd immunity and ending the pandemic. This review provides an overview of mechanisms and available data on the nascent vaccine technologies undergoing clinical trials to combat SARS-CoV-2, namely, those using protein subunits, viral vectors, mRNA, and DNA. Furthermore, we discuss the potential uses of biomaterials in improving the immunogenicity and safety of these vaccine technologies with the goal of improving upon newly-available technologies to combat future SARS-CoV-2 strains and other emerging viral pathogens.

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Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and has spread widely across the world now.[1] As of December 21, 2020, SARS-CoV-2 had infected 75,704,857 people and resulted in 1,690,061 deaths worldwide.[2] SARS-CoV-2 is considered the causative agent of coronavirus disease 2019 (COVID-19), a respiratory disease characterized by a range of symptoms—or lack thereof—that vary with age and pre-existing health conditions, which can lead to hospitalization and strain the healthcare system.[1] Despite improvements in treatment and public policy aimed to curb the spread of the virus, cases remain high and have been rapidly increasing since November, 2020 in many regions across the world.

Like the previous human coronaviruses severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS), SARS-CoV-2 is a betacoronavirus that is likely of zoonotic origin, as suggested by its genetic similarity with betacoronaviruses found in bats and pangolins.[1] SARS-CoV-2 contains single-stranded RNA that is surrounded by a protein envelope, which contains crown-like spike proteins on the outer surface.[3] Structurally, SARS-CoV-2 is composed of four structural proteins, namely spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as the replicase open reading frame (ORF1a/ORF1b), which encodes a polypeptide that is cleaved to form assorted non-structural proteins involved in replication and transcription (**Figure 1**).[1, 4] Of interest is the S protein, which mediates the entry of SARS-CoV-2 into host cells. The viral S protein is composed of two subunits, designated S1 and S2, of which the former contains the N-terminal domain and the receptor-binding domain (RBD), while the latter contains the fusion peptide, transmembrane domain, cytoplasmic domain, and two heptapeptide repeat sequences (HR1 and HR2).[5] These two subunits are responsible for recognizing and binding to host angiotensin converting enzyme II receptors and subsequent cell fusion (**Figure 2**).[5, 6]

Angiotensin converting enzyme II receptors are commonly expressed by epithelial alveolar

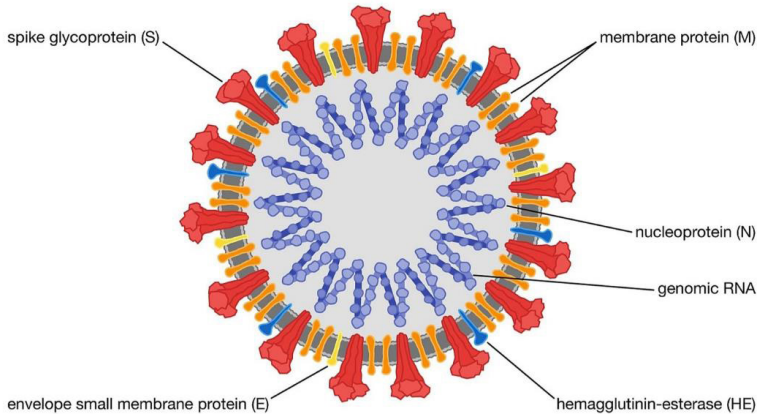

Figure 1. Structure of SARS-CoV-2. A graphic illustrating the structure of SARS-CoV-2, which shows the viral RNA along with the S, M, E, and N proteins. Figure reprinted from Shaikh et al.[4] Licensed under CC BY 4.0. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

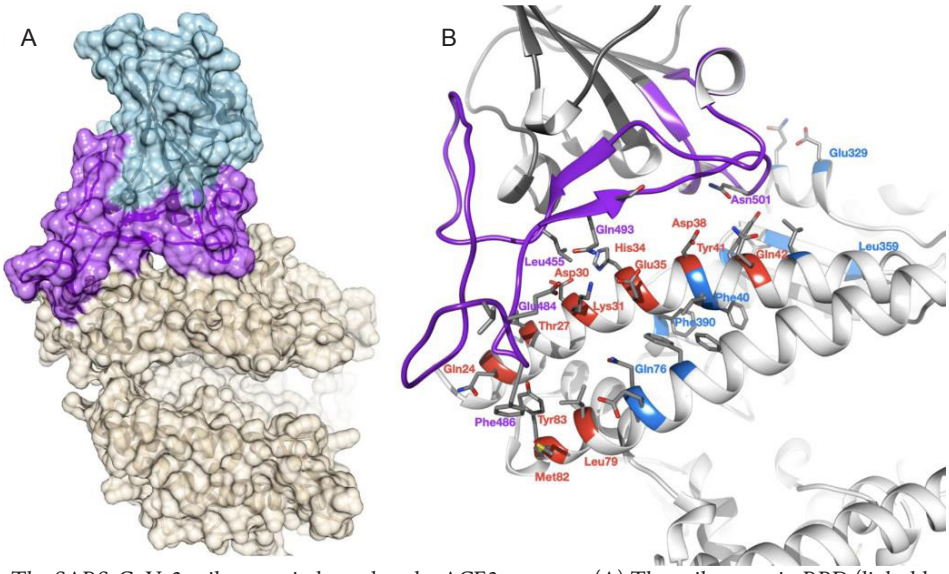

Figure 2. The SARS-CoV-2 spike protein bound to the ACE2 receptor. (A) The spike protein RBD (light blue, purple) is shown containing the receptor-binding motif (purple) while at the interface of the ACE2 receptor (tan). (B) Interface residues of the RBD (purple) are shown interacting with ACE2 residues in direct contact (red) or extended direct contact (blue) with the RBD. Figure reprinted from Lam et al.[6] Licensed under CC BY 4.0. ACE2: angiotensin converting enzyme II; RBD: receptor-binding domain; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

type II cells in the lungs, as well as in the heart, kidneys, and intestines.[7] Viral cell fusion is mediated by a number of host furin-like proteases, such as trypsin and transmembrane serine protease 2, which cleave the S protein into S1 and S2 subunits at furin cleavage sites. It is thought that the greater number of furin cleavage sites in the S protein of SARS-CoV-2 is responsible for its greater pathogenicity compared to SARS-CoV. After cleavage of the S protein, the fusion peptide binds to the host cell membrane and initiates fusion. The HR1 and HR2 domains then bring the two membranes together until they fuse, allowing the virus to release genetic material into the host cell.[5] The role of the S protein in SARS-CoV-2 pathogenicity makes it a key target for vaccine development, though E, M, N, and other accessory proteins may also hold potential to act as antigens.

Development of a safe and effective vaccine requires an understanding of the immune correlates of protection against SARS-CoV-2. It has been found that infection with SARS-CoV-2 induces both humoral and cellular immune responses.[1] The production of neutralizing antibodies seems to provide a good correlate of protection against SARS-CoV-2. A study using purified IgG antibodies from convalescent rhesus macaques was found to confer protection for rhesus macaques with no previous exposure to the virus, which seems to indicate their role in protecting against SARS-CoV-2 reinfection.[8] That study also found that $CD8^+$ T cells can mediate protection against SARS-CoV-2 reinfection in the wake of waning antibody titers.[8] A follow up with SARS-CoV patients six years post infection identified memory T cell responses, even with no detectable IgG antibodies or memory B cell responses.[9] These results may imply the potential for a long-lasting cellular response to SARS-CoV-2 even after waning antibody titres. Thus, vaccine candidates should induce both humoral and cellular responses against SARS-CoV-2.

In response to the COVID-19 pandemic, vaccines are being developed at an unprecedented speed using various novel materials and technologies representing the most advanced biomedical science. Countries, seeking to mitigate economic disruptions and loss of life, are committing to mass vaccination programs as soon as a vaccine candidate is deemed safe and effective. The goal of these programs is to quickly reach a state of herd immunity, which would likely require unnecessary loss of life and economic productivity if left to occur naturally, as in the 1918 Spanish influenza pandemic.[10] Thus, safe and effective vaccines seem to be the best method of ending the COVID-19 pandemic. In this paper we are going to summarize the development and mechanisms of several of the novel vaccine types that have been developed for SARS-CoV-2.

The articles used in this review of the COVID-19 vaccines were retrieved through an electronic search of the PubMed database. Literatures from 2019 to present with regards to COVID-19 and the COVID-19 vaccines were included. Initial searches were performed under the following conditions: ((COVID-19) OR (SARS-2-CoV)) AND (vaccines). Studies were screened by title, abstract, and date to include only human COVID-19 vaccines, as well as the most up-to-date studies. Subsequent searches were completed relevant to the different types of COVID-19 vaccine using the following terms: RNA-based vaccine, DNA-based vaccine, protein subunit vaccine, recombinant protein vaccine, viral vector vaccine, adenovirus vector vaccine, adjuvants, and cold-chain transport.

Different Types of COVID-19 Vaccine

According to the World Health Organization, there are 64 vaccine candidates already in clinical trials and 172 candidates in pre-clinical development as of January 6, 2021.[2] **Table 1** lists the vaccines currently undergoing clinical trials, as well as those authorized for limited or emergency use in certain regions. Of the vaccine mechanisms to be discussed, 30% utilize protein subunits, while 14% use an inactivated virus and 27% use a viral vector, either replicating or non-replicating. Additionally, of the 24% of vaccine candidates that use nucleic acids, 13% are DNA-based while 11% are RNA-based.[2]

Inactivated virus and live attenuated virus vaccines are well-established means of conferring protection against a novel pathogen. Chemical, temperature, and radiation treatments are used to "inactivate" viruses by altering proteins involved in pathogenesis or preventing genome reading, while antigen epitopes remain intact to stimulate an immune response.[11] Several inactivated virus vaccines are currently undergoing Phase 3 clinical trials or have emergency use authorization, primarily in China, as well as QazCovid-in in Kazakhstan.[2] Live attenuated viruses, on the other hand, are created by propagating viruses under novel conditions that render them less pathogenic and less virulent. The added mutations which arise when growing under these conditions leads to an attenuated strain; however, there is still potential for the attenuated strain to revert back to the virulent strain, which makes them less safe than other vaccine technologies.[12] Only one vaccine candidate currently in clinical trials uses the live attenuated virus, which is produced by Codagenix/Serum Institute of India.[2] As vaccines of this type have been extensively studied, and many licensed inactivated or live attenuated virus vaccines exist, we will instead focus on vaccines made with novel biotechnologies, particularly, protein subunit, viral vector, mRNA, and DNA vaccines. **Figure 3** summarizes the mechanisms of several of these vaccine technologies.[13]

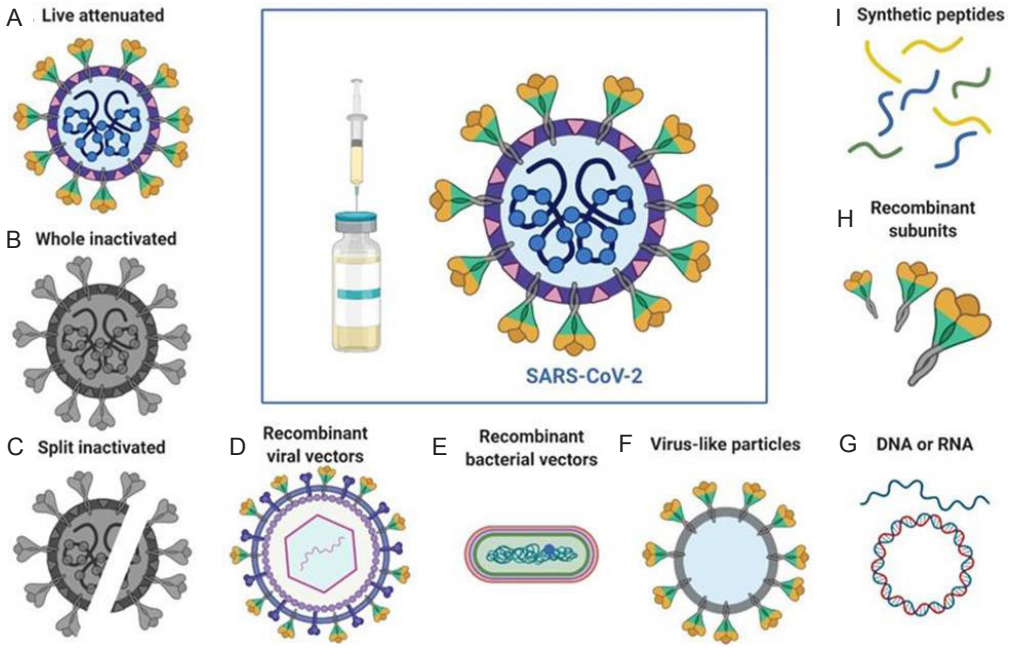

Figure 3. Summary of SARS-CoV-2 vaccine types. A summary of several of the major vaccine types being manufactured, including live attenuated (A), inactivated (B, C), viral vector (D), bacterial vector (E), virus-like particles (F), DNA- or RNA-based (G), recombinant protein subunit (H), and synthetic peptides vaccines (I). Figure reprinted from Liu et al.[13] Licensed under CC BY 4.0.

Table 1. Summary of COVID-19 vaccines currently in clinical trials

Vaccine candidate	Company	Mechanism	Phase
SARS-CoV-2 vaccine	Sinovac Research and Development Co., Ltd.	Inactivated	Phase 3
Inactivated SARS-CoV-2 vaccine	Sinopharm + China National Biotec Group Co. Ltd. + Wuhan Institute of Biological Products	Inactivated	Phase 3
Inactivated SARS-CoV-2 vaccine	Sinopharm + China National Biotec Group Co. Ltd. + Beijing Institute of Biological Products	Inactivated	Phase 3
ChAdOx1-S (AZD1222)	AstraZeneca + University of Oxford	Viral vector	Phase 3
Recombinant novel coronavirus vaccine (adenovirus type 5 vector)	CanSino Biologics Inc. + Beijing Institute of Biotechnology	Viral vector	Phase 3
Gam-COVID-Vac, Aden-based (rAd26-S+rAd5-S)	Gamaleya Research Institute, Health Ministry of the Russian Federation	Viral vector	Phase 3
AD26.COV2.S	Janssen Pharmaceuticals, Inc.	Viral vector	Phase 3
SARS-CoV-2 rS/Matrix M1-Adjuvant	Novavax	Protein subunit	Phase 3
mRNA-1273	Moderna + National Institute of Allergy and Infectious Diseases	RNA	Phase 3
BNT162 (3 LNP-mRNAs)	BioNTech + Fosun Pharma; Jiangsu Provincial Centre for Disease Prevention and Control + Pfizer	RNA	Phase 2/3
Recombinant SARS-CoV-2 vaccine	Anhui Zhifei Longcom Biopharmaceuticals + Institute of Microbiology, Chinese Academy of Sciences	Protein subunit	Phase 3
CVnCoV vaccine	CureVac AG	RNA	Phase 3
SARS-CoV-2 vaccine	Institute of Medical Biology, Chinese Academy of Medical Sciences	Inactivated	Phase 3
QazCovid-in – COVID-19 inactivated vaccine	Research Institute for Biological Safety Problems, Republic of Kazakhstan	Inactivated	Phase 3
INO-4800+electroporation	Inovio Pharmaceuticals + International Vaccine Institute, South Korea + Advaccine (Suzhou) Biopharmaceutical Co., Ltd.	DNA	Phase 2/3
AG0301-COVID19	AnGes + Takara Bio Inc. + Osaka University	DNA	Phase 2/3
nCov vaccine	Cadila Healthcare Ltd.	DNA	Phase 3
GX-19	Genexine Consortium	DNA	Phase 1/2
Whole-Virion Inactivated SARS-CoV-2 Vaccine (BBV152)	Bharat Biotech International Limited	Inactivated	Phase 3
KBP-COVID-19 (RBD-based)	Kentucky Bioprocessing Inc.	Protein subunit	Phase 1/2
SARS-CoV-2 vaccine formulation 1 with adjuvant	Sanofi Pasteur + GSK	Protein subunit	Phase 1/2
ARCT-021	Arcturus Therapeutics	RNA	Phase 2
RBD SARS-CoV-2 HBsAg VLP vaccine	Serum Institute of India + Accelagen Pty	Virus like particle	Phase 1/2
Inactivated SARS-CoV-2 vaccine	Shenzhen Kangtai Biological Products Co., Ltd.	Inactivated	Phase 2
GRAd-COV2	ReiThera + Leukocare + Univercells	Viral vector	Phase 1
VXA-CoV2-1 AD5 adjuvanted oral vaccine platform	Vaxart Inc.	Viral vector	Phase 1
MVA-SARS-2-S	University Medical Centre Hamburg-Eppendorf + Ludwig Maximilian University of Munich	Viral vector	Phase 2
SCB-2019 + AS03 or CpG 1018 adjuvant plus Alum adjuvant	Clover Biopharmaceuticals Inc./GSK/Dynavax	Protein subunit	Phase 2/3
COVID19 vaccine	Vaxine Pty Ltd. + Medytox	Protein subunit	Phase 1
MVC-COV1901 (S-2P protein + CpG 1018)	Medigen Vaccine Biologics + Dynavax + National Institute of Allergy and Infectious Diseases	Protein subunit	Phase 1
FINLAY-FR anti-SARS-CoV-2 Vaccine	Instituto Finlay de Vacunas	Protein subunit	Phase 2
EpiVacCorona	Federal Budgetary Research Institution, State Research Centre of Virology and Biotechnology "Vector"	Protein subunit	Phase 1/2
RBD Recombinant SARS-CoV-2 vaccine (Sf9 cell)	West China Hospital of Sichuan University	Protein subunit	Phase 2

Table 1. Continued

Vaccine candidate	Company	Mechanism	Phase
IMP CoVac-1 (SARS-CoV-2 HLA-DR peptides)	University Hospital Tübingen	Protein subunit	Phase 1
UB-612	COVAXX + United Biomedical Inc.	Protein subunit	Phase 2/3
V591-001 – Measles-vector based (TMV-o38)	Merck & Co. Inc. + Themis + Merck Sharp & Dohme Ltd. + Institut Pasteur + University of Pittsburgh	Viral vector (replicating)	Phase 1/2
DelNS1-2019-nCoV-RBD-OPT1	Jiangsu Provincial Centre for Disease Prevention and Control	Viral vector (replicating)	Phase 2
LNP-nCoVsaRNA	Imperial College London	RNA	Phase 1
SARS-CoV-2 mRNA vaccine	Shulan Hospital + Guangxi Centre for Disease Prevention and Control	RNA	Phase 1
Coronavirus-like particle COVID-19	Medicago Inc.	Viral like particle	Phase 2/3
Covid-19/aAPC vaccine	Shenzhen Geno-Immune Medical Institute	Viral vector (replicating) + APC	Phase 1
LV-SMENP-DC vaccine	Shenzhen Geno-Immune Medical Institute	Viral vector (non-replicating) + APC	Phase 1/2
AdimrSC-2f	Adimmune Corporation	Protein subunit	Phase 1
Covigenix VAX-001	Entos Pharmaceuticals Inc.	DNA	Phase 1
CORVax	Providence Health & Services	DNA	Phase 1
ChulaCov19 mRNA vaccine	Chulalongkorn University	RNA	Phase 1
bacTRL-Spike	Symvivo Corporation	DNA	Phase 1
hAd5-S-Fusion+N-ETSD vaccine	ImmunityBio, Inc.	Viral vector	Phase 1
COH04S1 (MVA-SARS-2-S)	City of Hope Medical Center + National Cancer Institute	Viral vector	Phase 1
rVSV-SARS-CoV-2-S vaccine	Israel Institute for Biological Research	Viral vector (replicating)	Phase 1/2
Dendritic cell vaccine AV-COVID-19	Avita Biomedical, Inc. + National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia	Viral vector (replicating) + APC	Phase 1/2
COVI-VAC	Codagenix/Serum Institute of India	Live attenuated virus	Phase 1
CIGB-669 (RBD+AgnHB)	Center for Genetic Engineering and Biotechnology	Protein subunit	Phase 1/2
CIGB-66 (RBD + aluminium hydroxide)	Center for Genetic Engineering and Biotechnology	Protein subunit	Phase 1/2
VLA2001	Valneva + National Institute for Health Research, United Kingdom	Inactivated	Phase 1/2
BECOV2	Biological E., Ltd.	Protein subunit	Phase 1/2
AdCLD-CoV19	Cellid Co. Ltd.	Viral vector (replicating)	Phase 1/2
GLS-5310	GeneOne Life Science, Inc.	DNA	Phase 1/2
Recombinant SARS-CoV-2 spike protein, aluminium adjuvanted	Nanogen Pharmaceutical Biotechnology	Protein subunit	Phase 1/2
S-268019	Shionogi Co., Ltd.	Protein subunit	Phase 1/2
AdCOVID	Altimmune, Inc.	Viral vector	Phase 1
SARS-CoV-2-RBD-Fc fusion protein	University Medical Center Groningen + Akston Biosciences Inc.	Protein subunit	Phase 1/2
ERUCOV-VAC	Erciyes University	Inactivated	Phase 1

Note: This table is adapted from the list of vaccines currently undergoing clinical trials published by the World Health Organization,[2] organized by candidate, company, mechanism, and phase of the clinical trial. This table is up to date as of January 6, 2021. Ad5: adenovirus type 5 vector; COVID-19: coronavirus disease 2019; LNP: lipid nanoparticle; RBD: receptor-binding domain; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Recombinant protein-based vaccines

As mentioned earlier, protein subunit vaccines are the most frequently-chosen vaccine type among the candidates currently undergoing clinical trials.[2] Protein subunit vaccines, instead of using the whole virus, often utilize a specific antigenic protein. In the case of SARS-CoV-2, this is often a recombinant form of the full-length S protein, or specific domains on the S protein, such as the RBD.[14]

For instance, Novavax, which is testing a protein subunit-based SARS-CoV-2 vaccine in a Phase 3 study, utilizes a recombinant form of the full-length spike protein in conjunction with a Matrix-M1 adjuvant.[15] The recombinant spike protein includes a mutation in the furin-cleavage site as well as two proline substitutions at residues K986P and V987P in order to prevent cleavage into the post-fusion form.[15] This keeps epitopes present in the pre-fusion conformation accessible, allowing them to elicit neutralizing antibody responses.[16] These mutations are made to the S-gene through cloning via the baculovirus expression system for expression in SF9 cells prior to extraction and purification.[17] Anhui Zhifei Longcom Biopharmaceuticals, working with the Institute of Microbiology, Chinese Academy of Sciences, also has a protein subunit vaccine candidate currently under Phase 2 study.[2] Their vaccine, however, uses a recombinant dimeric RBD.[14]

One hindrance in the development of protein subunit vaccines is that they display low immunogenicity without the addition of adjuvants.[18] Thus, adjuvants need to be added to protein subunit vaccines in order to promote strong humoral and cellular immune responses. As mentioned earlier, the Novavax vaccine uses the Matrix-M1 adjuvant along with its recombinant spike protein subunit. Matrix-M1 is a saponin-based adjuvant that has been found to upregulate major histocompatibility complex class II as well as induce the recruitment and activation of dendritic cells, which go on to activate humoral and cellular immune responses.[19, 20] A more extensive discussion of other adjuvants used in protein subunit vaccines can be found later in this paper.

Another biomaterial that has seen use in conjunction with protein subunits in SARS-CoV-2 vaccine candidates is virus-like particles (VLPs). VLPs are highly-structured arrangements of proteins from the viral capsid that mimic the virus structure but do not contain actual genetic material.[14] VLPs have been shown to elicit both B cell and cytotoxic T cell immune responses, and as a result of mimicking viral structure, they often require lower doses of antigen than vaccines consisting of the protein subunit alone.[21] In order to be safely mass produced, VLPs must be formed through an expression system, such as with hepatitis B virus in yeast cells or the baculovirus expression system that utilizes certain lepidopteran species.[21] The nanoparticles that display the modified S protein subunit in the Novavax vaccine are one example of such VLPs.[22] A vaccine produced by Medicago Inc. in Phase 2/3 clinical trials also uses a VLP produced in tobacco to display a recombinant SARS-CoV-2 spike protein.[18]

Viral vector vaccines

Viral vector-based vaccines are an emerging technology that clone specific SARS-CoV-2 antigens into the genetic material of either replicating or non-replicating virus vectors.[23] Several non-replicating vector vaccines have entered Phase 3 clinical trials, and some have gained emergency use authorization in certain regions. These vaccines utilize adenovirus vectors with E1 gene deletions, preventing replication.[22] Such vaccines currently under Phase 3 clinical trials are being produced by AstraZeneca/University of Oxford in the United Kingdom, Gamaleya Research Institute in Russia, CanSino Biologics, Inc. in China, and Janssen Pharmaceuticals, Inc. in the USA.[2] Vaccines based on viral vectors work by transducing the antigenic gene via the vector into the host cell nucleus, where the gene is transcribed and later exported back into the cytoplasm to be translated and to elicit an immune response (**Figure 4A**).[22] These vaccines hold potential to induce a highly-specific and efficient immune response against SARS-CoV-2.

Of the four viral vector-based vaccines currently in Phase 3 clinical trials, all use some form of a non-replicating adenovirus vector. Adenoviruses have double-stranded DNA and cause common cold symptoms in humans.[22] Adenovirus vectors have several features that make them an attractive choice for vaccine developers. First, adenovirus vectors stimulate potent innate and adaptive immune responses while maintaining a high safety profile.[24] Furthermore, transgenes can be inserted into adenovirus genomes, allowing for the expression of the target peptide as well as other immune response enhancers, such as cytokines and danger signals.[24] The development of this biotechnology allows for high adaptability and can be exploited to increase the speed at which vaccines are produced. Also of interest is the separation of cellular attachment and entry processes in adenovirus vectors.[24] The proteins responsible for recognition and attachment to certain receptors on host cells can be altered to increase specificity for receptors elsewhere without disrupting viral entry or gene transduction. Current SARS-CoV-2 vaccine candidates utilize both human and non-human adenovirus vectors.

For instance, CanSino Biologics, Inc. utilizes a recombinant human adenovirus type 5 vector (Ad5) in their vaccine, and AstraZeneca/University of Oxford's AZD1222 vaccine uses the recombinant chimpanzee ChAdOx1 adenovirus vector. The Gamaleya-produced vaccine uses a combination of recombinant Ad5 and Ad26, while Janssen solely uses the Ad26 vector.[14] All of these vaccines use the adenovirus vector to carry the full-length spike glycoprotein, where it is produced using the host cell's machinery to be recognized and presented by antigen-presenting cells (APCs) to induce an immune response. The Janssen vaccine differs from the other three that use wild-type spike protein in that the S protein contains proline substitutions at K986P and V987P and two furin cleavage site mutations.[23] One limitation to the adenovirus vector is the potential for immunity to certain vectors as a result of previous exposure. Indeed, a Phase 2 trial by CanSino Biologics, Inc. of their Ad5-vectored vaccine found that 52% of study participants had high pre-existing immunity to the Ad5 vector, which resulted in a two-fold decrease in neutralizing antibodies compared to those with

minimal pre-existing immunity.[25] A possible solution to this is using adenovirus vectors that have a lower seroprevalence in humans, such as Ad26 as used by Gamaleya Research Institute and Janssen Pharmaceuticals, Inc., or using a non-human adenovirus vector with very low human seroprevalence, as used by AstraZeneca/University of Oxford.[26, 27]

Although the majority of SARS-CoV-2 vaccine candidates using a viral vector mechanism in clinical trials are non-replicating and use adenovirus vectors, it is worth briefly discussing the candidates that use a replicating vector and/or non-adenovirus vector. A notable vaccine candidate of this type includes TMV-083, which is being produced by the Institut Pasteur in conjunction with Themis, Merck & Co. Inc., the University of Pittsburgh, and Merck Sharp & Dohme Ltd., and uses the measles virus as a vector.[2] Recent studies have shown the measles vector platform, based on the established measles vaccine, to be safe and effective in Phase 1 and 2 clinical trials in formulating a vaccine against Chikungunya virus.[28] Furthermore, current evidence shows that pre-existing immunity does not affect the vaccine functionality, indicating the potential of this viral vector to rapidly formulate a vaccine against novel pathogens.[28]

mRNA-based vaccines

Despite their novelty, several mRNA-based vaccine candidates have been developed and are currently undergoing clinical trials for SARS-CoV-2.[2] mRNA-based vaccines offer high flexibility and adaptability, which allow them to be rapidly developed in the face of emerging pandemics.[29] Indeed, the first two vaccines to receive emergency use authorizations from the United States Food and Drug Administration were mRNA-based vaccines produced by BioNTech/Pfizer and Moderna/NIAID. mRNA-based vaccines also offer the advantage of being self-adjuvanting. It has been shown that stabilized mRNA carries the ability to activate Toll-like receptors 7/8 and 3, which are essential for a primed immune response against viral targets.[22] The innate immunostimulatory properties of mRNA can be utilized to elicit immune responses without the addition of an adjuvant, which can save resources by avoiding the need for additional safety testing or studying synergistic effects. Finally, mRNA vaccines only require the nucleic acid-encoded antigen to reach the cytosol of the target cells for translation

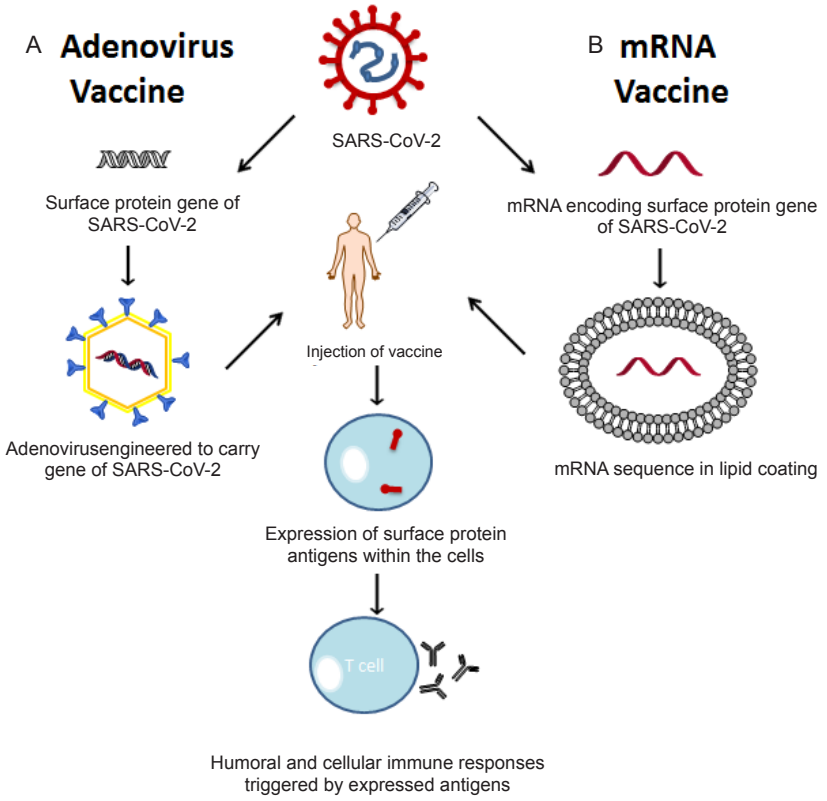

Figure 4. Schematic mechanism of manufacturing of viral vector vaccines (A, adenovirus as example) and mRNA vaccines (B). The RNA of SARS-CoV-2 was sequenced, which identified the coding of surface proteins. Using endonuclease methods, an engineered mutated adenovirus vector that carries the SARS-CoV-2 surface protein gene was made. Different from the preparation of adenovirus, the mRNA sequences that encode the spike protein were directly generated. To enhance the stabilities of mRNA and to escape from human immunities, lipid nanoparticles were used to envelope the mRNA. After injection of both viral vector and mRNA vaccines, cells will read the mRNA sequence express the epitope of the surface protein (red within cell) in the cytoplasm or in the nucleus. This will trigger the host's humoral and cellular immune responses that could potentially contribute to specific immunity to SARS-CoV-2.

to occur. This provides an additional safety element, especially compared to other nucleic acid-based vaccines, as there is no potential for integration into the genome (**Figure 4B**).[18]

Two limitations to RNA-based vaccines are the inherent instability of mRNA *in vivo* and the low translatability of "naked" mRNA. Several strategies have been developed in order to circumvent this issue and deliver the antigenic RNA without rapid degradation by RNases. Stabilization of mRNA can be achieved through modifications to the 5′- and 3′-untranslated region elements, which surround the ORF containing the antigenic gene.[30] These modifications include synthetically adding a 5′ cap, regulating the poly(A) tail length, and optimizing codon sequences.[29] In addition to stabilizing mRNA, these modifications can increase protein translation.[30] Another modification of interest is the use of protamine, a polycationic peptide that protects mRNA from degradation.[31] Protamine, however, shows limited efficacy when complexing mRNA in and of itself, but efficacy is improved when it is included as part of an mRNA vaccine platform.[30] mRNA vaccine platforms often include encapsulation by lipid nanoparticles (LNPs). LNPs contain ionizable cationic lipids that aid *in vivo* delivery of mRNA to target cells.[32]

Due to the high adaptability of mRNA-based vaccines, various approaches have been taken to develop such a vaccine against SARS-CoV-2. For instance, the mRNA-1273 vaccine produced by Moderna/NIAID encodes the full-length, pre-cleavage stabilized spike protein within an LNP capsule.[18] Four lipids are used in a fixed ratio with the mRNA, although the exact composition of the lipids is unknown.[33] BNT162, the mRNA vaccine candidate produced by BioNTech/Pfizer, uses nucleoside-modified RNA that encodes the RBD of the SARS-CoV-2 spike protein.[34] The addition of 1-methyl-pseudouiridine has been found to reduce the immunogenicity of mRNA, while increasing stability and protein translation.[35] Additionally, BNT162 utilizes a T4 fibritin-derived "foldon" trimerization domain, which allows for a multivalent display of the RBD antigen, thus increasing the number of binding sites and immunogenicity.[34] Like the Moderna/NIAID vaccine, BNT162 is encapsulated within LNPs and does not mention any use of adjuvant. Both vaccine formulations have been found to cause minimal negative side effects and high efficacy thus far.[36, 37]

DNA vaccines

Like RNA-based vaccines, DNA vaccines utilize genetic material that codes for specific antigenic proteins on SARS-CoV-2 and can be rapidly developed against novel pathogens for mass production. Likewise, DNA-based vaccines work in a similar manner to mRNA-based vaccines. The antigen is encoded by a sequence incorporated into a DNA plasmid, which is then transfected into host cells. There, host machinery is used to transcribe and translate the antigen into a functional peptide.[23] The use of DNA rather than mRNA comes with both advantages and disadvantages. For instance, while mRNA is intrinsically unstable and can be degraded by RNases, DNA offers greater stability meaning DNA expression is longer-lived, thus potentially conferring a more potent immune response, and cold chain transport is not required.[18] However, a major disadvantage to the use of DNA-based vaccines is the potential for host genome integration, as the antigenic DNA must enter the host cell nucleus to be transcribed.[18]

Although several DNA-based vaccines are currently undergoing clinical trials, to the best of the authors' knowledge only Inovio Pharmaceuticals has begun Phase 3 clinical trials and published data on their INO-4800 vaccine.[38] Inovio Pharmaceuticals, which is also currently testing a DNA-based vaccine against MERS-CoV, developed their SARS-CoV-2 vaccine INO-4800 to encode the full-length spike glycoprotein along with an N-terminal IgE leader sequence. This optimized DNA sequence is encoded on a plasmid labelled pGX9501 and has been shown to elicit both cellular and humoral responses against the spike protein following immunization of mice and guinea pigs.[38] One interesting aspect of INO-4800 is the use of electroporation to administer the vaccine intradermally. Electroporation is an interesting biotechnology that uses short electrical pulses to increase cell membrane permeability and pDNA uptake at the vaccine administration site, which has been associated with a greater recruitment of APCs and inflammatory cells.[39]

Another interesting DNA-based vaccine, bacTRL-Spike, has been developed by Symvivo Corporation and is currently undergoing a Phase 1 clinical trial set to be completed in February 2022 (NCT04334980). The bacTRL-spike vaccine, which is taken orally, marks the first in-human use of the *Bifidobacterium longum* vector to deliver a modified DNA plasmid containing the SARS-CoV-2 spike protein. *B. longum* is an anaerobic bacterium present in the human microbiome; therefore, it does not present a risk for virulence.[39] Additionally, strains of *B. longum* have previously been tested as carriers of hepatitis C virus and enterovirus, but not in human hosts.[39]

Importance of Formulation

Adjuvants

As mentioned earlier, adjuvants are immunostimulatory agents that are often added to vaccines to improve the ability of antigens to induce an immune response. While nucleic acid-based vaccines are considered self-adjuvanting given their high immunogenicity, and viral vectors prime the immune response through the vector, protein subunit vaccines require the use of adjuvants.[22] Some adjuvants that are seeing use in the development of protein subunit-based SARS-CoV-2 vaccines include alum, Matrix-M1, and CpG.[2] **Figure 5** summarizes the various mechanisms of adjuvants for improving high immunogenicity.

Alum is an aluminium-based adjuvant that has a long history of use as a clinical adjuvant. The addition of alum adjuvants promotes the adaptive immune response through uric acid, which induces the differentiation of dendritic cells.[40] Several current vaccines against COVID-19 utilize alum adjuvants, such as SCB-2019 and CIGB-66.[2] Matrix-M1, which was discussed in the context of the Novavax protein subunit vaccine, is a saponin-based adjuvant in an immune-stimulating complex-matrix conformation. This conformation includes a specific fraction of saponin, cholesterol, phospholipids, and the antigen

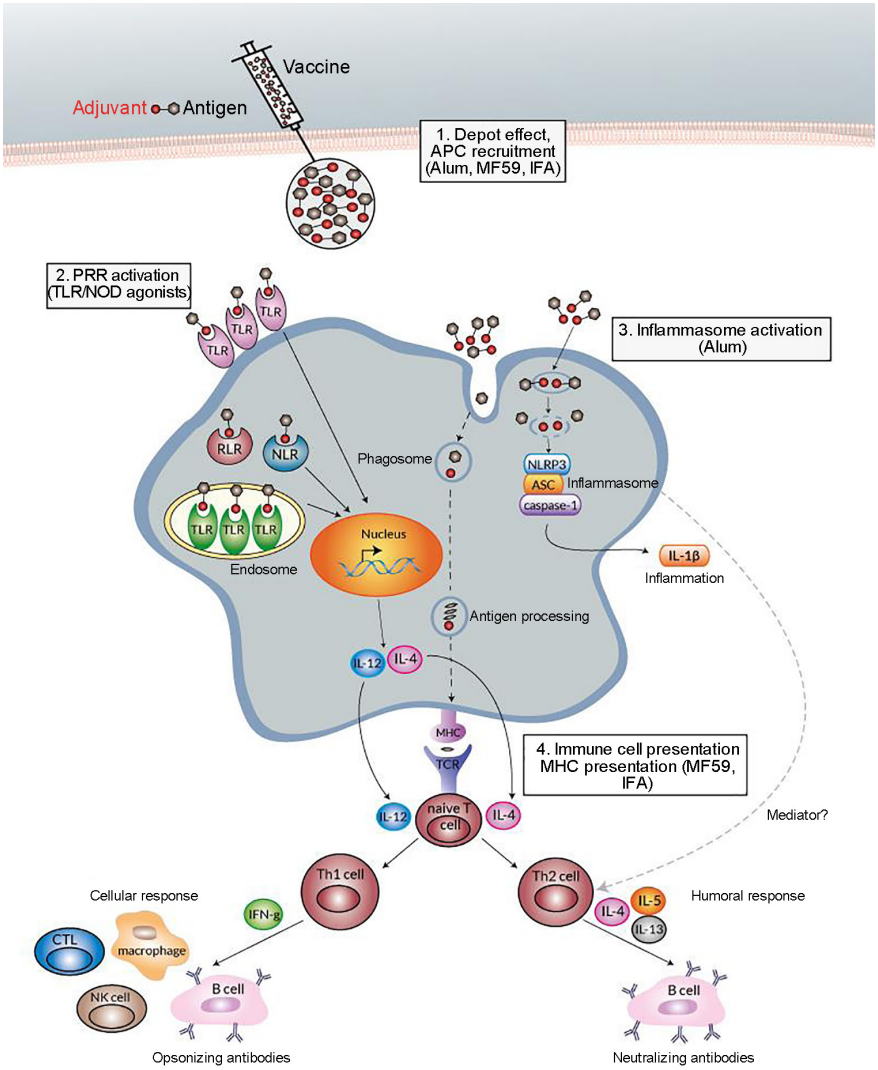

Figure 5. Adjuvants improve immunogenicity via different mechanisms. 1. Alum and emulsion such as MF59 generate depots to trap and recruit antigen presenting cells (APCs). 2. By utilizing TLR/NOD agonists, pattern recognition receptors (PRR) were covalently bound to their ligands, followed by the activation of downstream pathways. 3. Aside from APC recruitment, Alum could also induce NLRP3 inflammasome. 4. Depot generation and induction of MHC responses could be obtained by application of MF59 and Freund's Incomplete Adjuvant (IFA). The image is licensed and authorized by InvivoGen.

of choice, with the Matrix-M1 adjuvant including a mix of two different matrices (Matrix-A and Matrix-C) that have different saponin fractions.[19] Among the vaccines currently undergoing clinical trials, only Novavax uses the Matrix-M1 adjuvant to the best of our knowledge.[2] An interesting adjuvant is the use of CpG, which consists of unmethylated CG dinucleotides derived from bacterial DNA.[41] As CpG is expressed more highly in bacteria than eukaryotes, it is naturally recognized by Toll-like receptor-9 to trigger an innate immune response.[41] As DNA-based vaccines use recombinant bacterial DNA, they naturally contain CpG sequences which promote the innate immune response. Protein subunit-based vaccines currently in clinical trials, like SCB-2019 and MVC-COV1901, also exploit the use of these sequences to boost their immunogenicity.[2]

Cold chain transport

Since the widespread use of vaccination as a public health measure in the 1960s and 1970s, the necessity for a "vaccine cold chain" to transport temperature-sensitive vaccines has been underscored. Difficulties in storing and shipping these vaccines is particularly the case in tropical climates, where electricity is unstable, appropriate equipment is unavailable, and there is a lack of sufficiently trained staff.[42] However, these issues are primarily true for inactivated and live attenuated vaccines, which require storage at approximately 2°C to 8°C, with the exception of varicella vaccines which require storage at –50°C to –15°C.[43] In contrast, many of the new mRNA-based vaccines developed during the COVID-19 pandemic require storage at temperatures below these ranges. For instance, the BioNTech/Pfizer mRNA-based vaccine requires storage and transport at temperatures as low as –80°C to –60°C to remain stable for up to 6 months, while the Moderna/NIAID vaccine requires storage at –20°C for up to 6 months.[44] While stability at the more attractive 2°C to 8°C is possible for brief periods of

time (5 days for Pfizer/BioNTech and 30 days for Moderna/NIAID), this requires that all the vaccine doses are used quickly and presents a problem in developing countries where such freezers are not available.[44]

As mentioned by Crommelin et al.[44] in their review of mRNA vaccine thermostability, liquid and lyophilized formulations of mRNA vaccines could provide refrigerated stability. One mRNA vaccine candidate, referred to as ARCov, uses a liquid formulation to deliver the LNP-encapsulated antigenic mRNA. A study using mice demonstrated that this vaccine induces both neutralizing antibodies and T-cell immune responses, as well as displaying thermostability at 2°C and 25°C for up to a week, though the authors acknowledge that the persistence of neutralizing antibodies is known as well as long-term thermostability at 2°C and 25°C.[45] Participants are currently being recruited for a Phase 1 study of this vaccine at Shulan Hospital.[2] Other vaccine formulations, such as those with viral vectors or DNA, also provide the high adaptability and scalability of mRNA-based vaccines combined with greater thermostability, which may aid in their distribution in developing countries to fight the global COVID-19 pandemic.[15, 46]

Summary and Perspective

The global COVID-19 pandemic has seen the adoption of several novel technologies in vaccine development as companies race to produce and deliver a safe and effective vaccine against SARS-CoV-2. Although conventional inactivated and live attenuated vaccines are being produced and approved by many countries, the COVID-19 pandemic has notably provided the opportunity to utilize protein subunit, viral vector, mRNA-, and DNA-based vaccine technologies due to their high adaptability and potential to be scaled up rapidly. Additionally, the development of nanoscale biomaterials has greatly enhanced the delivery, immunogenicity, and safety of these novel vaccines. As discussed earlier, the design of VLPs to mimic live or inactivated viruses has helped to increase the potency of immune responses for protein subunit vaccines, as well as nanoparticle-based adjuvants like Matrix-M1.[21] LNPs, used to encapsulate the mRNA antigen, aid in the delivery to target cells as well as the stability of mRNA, thereby increasing the potency of these vaccines through reduced mRNA degradation and increased protein translation. Modifications to the mRNA nucleotide sequence, particularly in the untranslated regions, can also improve stability and decrease innate immunogenicity that could trigger inflammation and other severe immune responses.[47]

Despite the many successes in the development of biomaterials, several avenues of research remain to be utilized in the rapid formulation, testing, production, and distribution of vaccines against novel pathogenic agents. First, despite their scalability and potential to be rapidly developed, the distribution of mRNA-LNP vaccines in developing nations is hindered by their instability and the requirement for the "cold chain" for vaccine distribution. Further research and development would be warranted in improving mRNA vaccine thermostability while retaining safety and efficacy, such as through lyophilized or liquid formulations.[44] Additionally, just as the development of refrigerators and thermal sensors to monitor the status of vaccines was necessary for the eradication of smallpox and the ongoing effort to eradicate polio, the formulation of ultra-cold freezers that can be adapted to developing nations is necessary for future widespread adoption of mRNA-based vaccines.[42]

Secondly, much potential remains in the application of nanobiotechnology to increase the structure and polyvalency of vaccine platforms. The seminal study by Bachmann et al.[48] demonstrated that high-density, organized antigen displays resulted in higher IgM titres and created better B cell activation in transgenic mice compared to less-ordered displays. These results highlight the potential for highly-ordered scaffolds, such as virus nanoparticles and VLPs, for application in presenting organized antigens that mimic the pathogen. An interesting recent study showed that rod-like viral particles outperformed icosahedral viral scaffolds in eliciting a long-lasting immune response when small and weakly-immunogenic haptens were displayed on the external surface of the viral capsids.[49] Additionally, it is possible to apply self-assembling polymeric particles to present high-density antigens to enhance the immune responses.[50] Equally relevant is the importance of polyvalent interactions between the selected antigen and APCs during antigen recruitment. Such interactions have been found to be stronger than their monovalent counterparts, increase the biological lifetime of the polyvalent molecules, and aid in the binding specificity of receptors to particular ligands.[51] Therefore, using bioconjugation technologies, a highly structured, polyvalent antigen presentation can be designed on the surfaces of VLPs or similar polyvalent scaffolds to boost immunogenicity and improve immune response of proteins or small molecular antigens.

Thirdly, as the COVID-19 pandemic marks the first time mRNA, DNA, and viral vector vaccines are seeing widespread use in humans, there is some hesitation on the safety of these vaccines. According to a study by the Pew Research Center in September 2020, only 51% of U.S. adults who responded stated that they would get the vaccine if it were available, and only 21% responded that they would definitely get vaccinated.[52] Although a variety of factors affect the reception of vaccines, including political and religious beliefs, demonstrating long-term safety and efficacy is essential for widespread adoption. With the rigorous and extensive clinical testing these novel vaccines are receiving around the world, their long-term safety will likely be demonstrated in the years to come. Nonetheless, it will be useful to develop methodologies that can more rapidly determine long-term efficacy and safety.

Furthermore, the recent announcement of the emergence of SARS-CoV-2 strains with increased infectivity in South Africa and the United Kingdom have heralded some worries over the efficacy of the newly-developed vaccines.[53, 54] While both have mutations in the spike protein, which is the target of many vaccines in development and clinical trials, it is likely the vaccines will still work, as they bind to multiple epitopes to induce protection. In the case that key epitopes contain the mutations, the adaptability of these vaccines should allow them to be quickly modified to provide protection against these

strains as well.

Finally, a plethora of bionanotechnologies has been utilized to produce safe and efficacious vaccines against SARS-CoV-2. Advances in these technologies allowed for their development and deployment against a novel pathogen at record speed. Vaccine platforms, such as LNP-encapsulated nucleic acid sequences, non-pathogenic viral vectors, and protein subunits, have a high degree of scalability and adaptability that will allow them to be readily put to use against future strains of SARS-CoV-2 or other novel pathogens. Biomaterials research should seek to utilize innovative technologies to enhance the immunogenicity and stability of vaccines while reducing deleterious reactions. Future biomaterials research should focus on developing novel adjuvants that improve safety profiles while heightening immune response, improving efficient interaction of nanoparticles with APCs, and generating expression systems that improve scalability and distribution in developing nations.[55]

In summary, the future seems bright for the development and application of novel vaccination strategies. Nonetheless, the continued refinement and development of nanotechnologies and biomaterials to modify these vaccines is warranted in order to improve their safety, efficacy, immunogenicity, and delivery to combat emerging strains of SARS-CoV-2 and prevent future pandemics.

Author contributions

IJ and JF provided the concept and design of the review. IJ was responsible for searching the literature and manuscript preparation. Both IJ and JF participated in manuscript editing and manuscript review. Both authors approved the final version of this manuscript.

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