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REVIEW

Engineering Antiviral Vaccines

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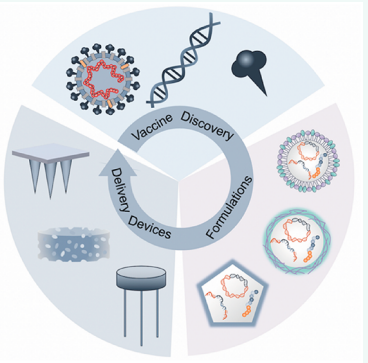
ABSTRACT: **Despite the vital role of vaccines in fighting viral pathogens, effective vaccines are still unavailable for many infectious diseases. The importance of vaccines cannot be overstated during the outbreak of a pandemic, such as the coronavirus disease 2019 (COVID-19) pandemic. The understanding of genomics, structural biology, and innate/adaptive immunity have expanded the toolkits available for current vaccine development. However, sudden outbreaks and the requirement of population-level immunization still pose great challenges in today's vaccine designs. Well-established vaccine development protocols from previous experiences are in place to guide the pipelines of vaccine development for emerging viral diseases. Nevertheless, vaccine development may follow different paradigms during a pandemic. For example, multiple vaccine candidates must be pushed into clinical trials simultaneously, and manufacturing capability must be scaled up in early stages. Factors from essential features of safety, efficacy, manufacturing, and distributions to administration approaches are taken into consideration based on advances in materials science and engineering technologies. In this review, we present recent advances in vaccine development by focusing on vaccine discovery, formulation, and delivery devices enabled by alternative administration approaches. We hope to shed light on developing better solutions for faster and better vaccine development strategies through the use of biomaterials, biomolecular engineering, nanotechnology, and microfabrication techniques.**



KEYWORDS: *COVID-19, pandemics, infectious disease, vaccine, immunotherapy, drug discovery, drug delivery, biomedical devices*

The appearance of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has caused unprecedented global disruption to health, economy, and social stability.[1] SARS-CoV-2 causes coronavirus disease 2019 (COVID-19) in which individuals can suffer from mild to severe symptoms once infected.[2] Global scientific communities are working together to fight the crisis caused by this pandemic. The initial efforts to combat this pandemic included identifying infected patients and ramping up clinical trials for repurposing existing drugs.[3] However, the development of effective vaccines to prevent SARS-CoV-2 infection is believed by many to be the most effective long-term solution.[4]

The pathway from identifying a virus to having a vaccine is lengthy and expensive. After billions of dollars spent and multiple years of trials, researchers generally face high rates of failure in the traditional vaccine development paradigms (Figure 1a).[4] Despite rapid responses from the scientific community and the pharmaceutical industry, vaccines have historically not been ready even after an epidemic becomes manageable, such as severe acute respiratory syndrome (SARS), Zika, and Ebola.[5] Considering economic factors, funding for vaccine development may be reallocated after a substantial drop in infection cases is observed, as in the 2015–2016 Zika and SARS epidemics. The 2014–2016 Ebola outbreak in Africa resulted in over 28,000 cases and 11,000 deaths.[6] After continuous research funding support, in December 2019, the FDA granted the first approval to Ervebo (a single-dose, live attenuated virus vaccine from Merck) for Ebola virus prevention.[7] Benefiting from egg- and cell-based platforms, vaccine approval for the most recent 2009 H1N1 pandemic was relatively fast following the peak of the outbreak, and the vaccine was ultimately incorporated in the seasonal influenza vaccine.[8] Since the initiation and ending of epidemics are highly unpredictable, the rapid deployment of vaccine development may still be too slow for a sudden outbreak.[4,5]

To tackle a pandemic, a different vaccine development process is needed to rapidly produce safe and effective vaccines against novel viruses. This process is characterized by overlapping phases, multiple potential vaccine candidates,

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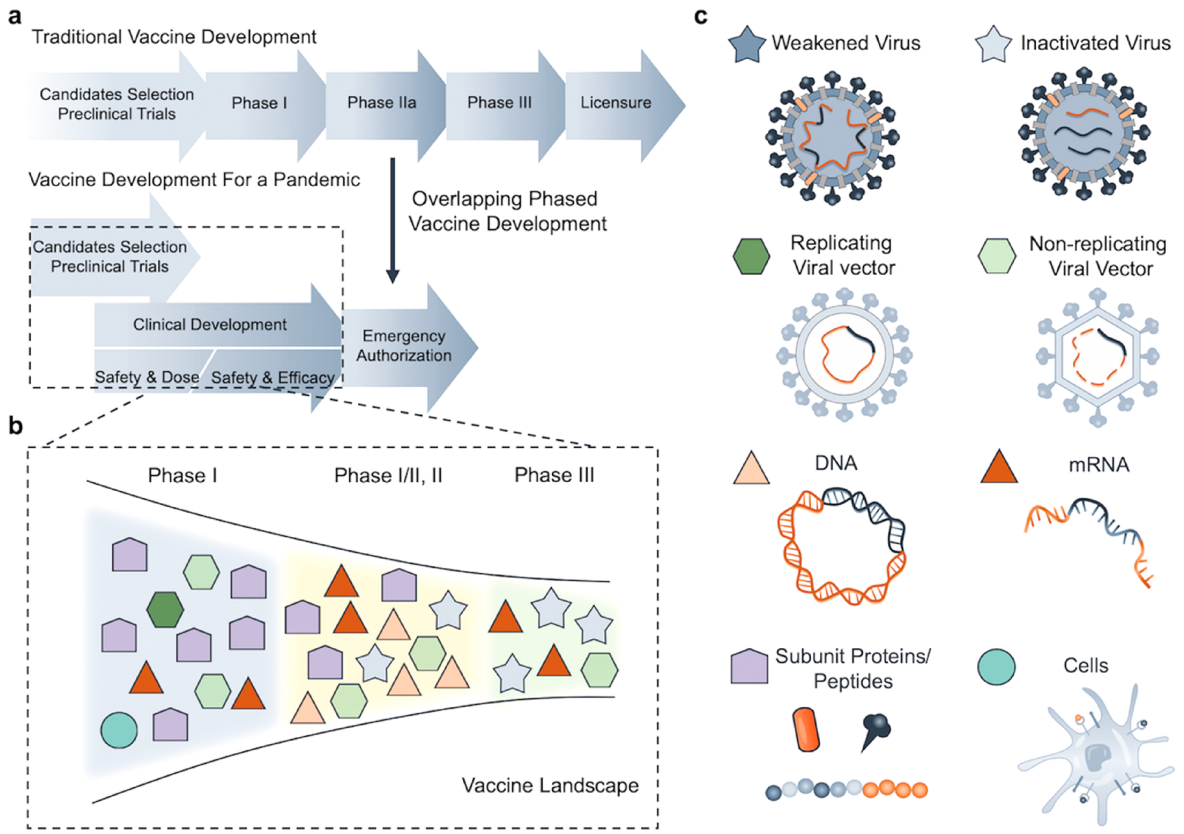

Figure 1. Summary of vaccine development paradigms and major types of vaccines. (a) Paradigms of vaccine development in a traditional condition *vs* during a pandemic.[5] (b) Snapshot of the vaccine landscape for COVID-19 with the different types of vaccine candidates in various clinical stages as of August 25, 2020.[10] (c) Schematic of major types of vaccines, including virus-based (weakened and inactivated), viral vector-based (replicating and nonreplicating), nucleic acid-based (DNA and mRNA), protein and peptide-based, and cell-based.[13]

and early ramp-up of manufacturing capacities (Figure 1a).[4] After the initial release of the genetic sequence of SARS-CoV-2, researchers worldwide started to develop vaccines for the prevention of COVID-19. The first dose was administered into humans by Moderna Inc. on March 16, 2020.[9] This method utilized lipid nanoparticles (LNPs) with a formulated mRNA vaccine.[9] This work was fast-tracked at an unprecedented pace, even though mRNA-based vaccines have never been licensed before.[9] A snapshot of the landscape of COVID-19 vaccines (Figure 1b) shows that around 31 vaccines have entered clinical trials as of August 25, 2020.[10] A high diversity of vaccine candidates is seen in these forerunners, which includes six mRNA-based, five inactivated virus-based, five nonreplicating viral vector-based, one replicating viral vector-based, four DNA-based, and one cell culture-based vaccines as well as nine protein-based vaccines (Figure 1b,c). The incomplete understanding of the SARS-CoV-2 interactions with the human immune system became one of the major obstacles in vaccine development. A recent report on a second COVID-19 infection in the same patient further raises the question of protective immunity and immune memory. For instance, it is still unknown how durable the immune memory response induced by SARS-CoV-2 could be or what is the threshold of antibodies titers that can protect the patients against reinfection.[11,12] In addition, protective immunity against SARS-CoV-2 should also be comprehensively studied, where the balance of cellular and humoral immunity, the ratio of effector to memory T cells, the maintenance of memory B cells, and functional features of activated T cells should be characterized.[11,12] These aspects could ultimately facilitate vaccine designs and evaluations.

In this review, we aim to provide engineering insights on vaccine development by covering vaccine discovery, vaccine formulations in terms of different materials (lipid, polymer, and inorganic particles), and vaccine delivery devices. We will present how some of the existing challenges could potentially be addressed by nano/micro/macroscale engineering approaches. We will also discuss some emerging technologies that may contribute to future vaccine development pipelines.

VACCINE DISCOVERY

After a virus is identified, different categories of vaccines can be developed, which includes virus-, viral vector-, nucleic acid-, protein/peptide-, and/or cell-based vaccines (Figure 1c).[13] Virus-based vaccines commonly require patient-derived viruses, while other types can utilize the viral genomic sequences to accelerate vaccine development. Previously approved vaccines mostly fall into the virus-based and the recombinant protein-based categories, but not into the nucleic acid-based one (Figure 2a).[14] The discovery process of each vaccine type is distinct with corresponding pros and cons (Figure 2b–d).

Virus-Based Vaccines. Virus-based vaccines, including the weakened and inactivated types, are still the most widely used to date. The effective prevention of the polio epidemic by vaccination in the 20th century represented a landmark success in medical history.[18] This vaccination regimen included the use of the inactivated polio vaccine (IPV) and oral polio vaccine

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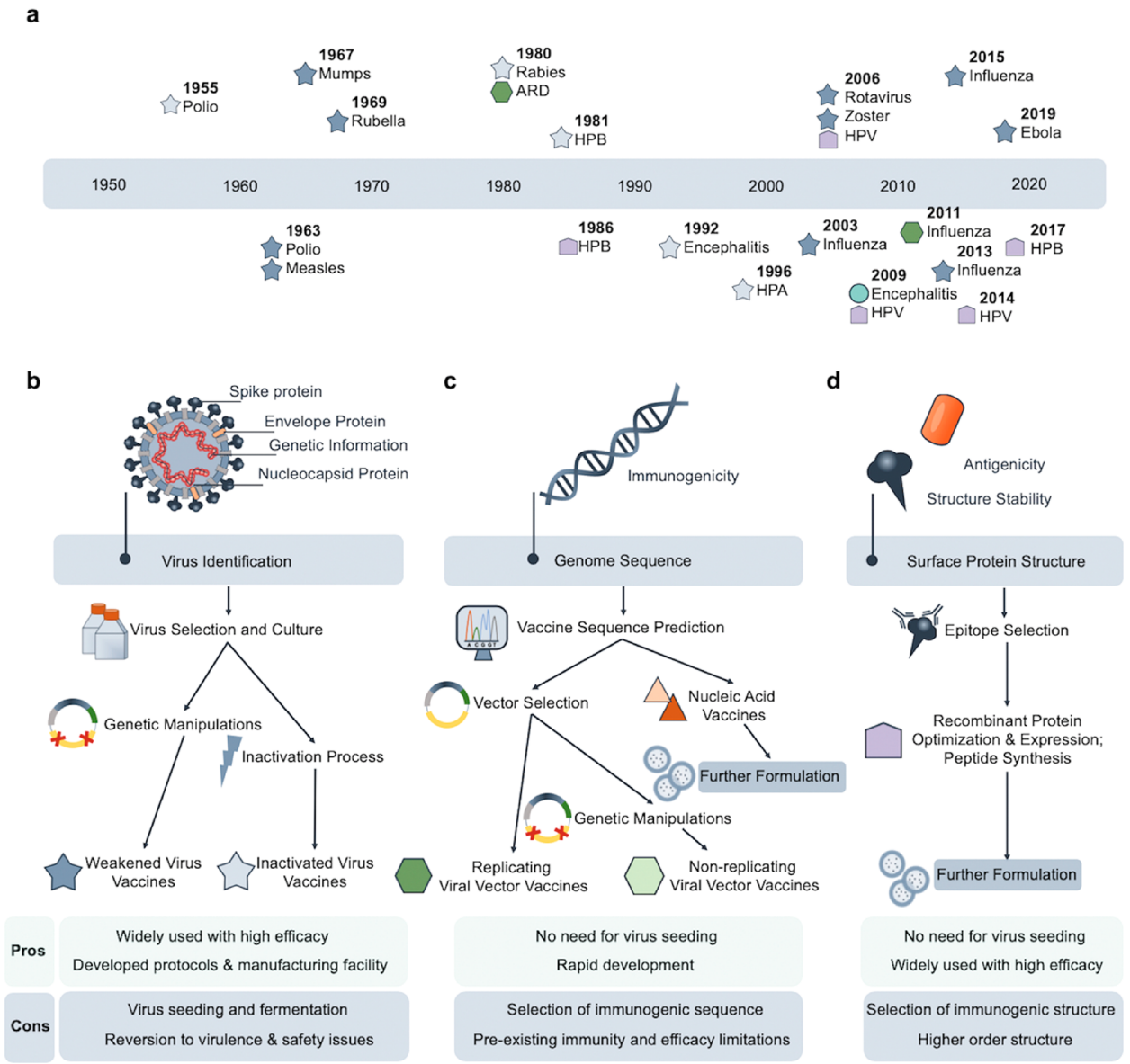

Figure 2. Vaccine discovery processes for different types of vaccines. (a) Timeline of previously approved antiviral vaccines.[14] (b) Virus-based vaccines require the seeding and culture of specific viruses derived from patients. By manipulating the genetic sequences of the virus or inactivating the virus directly with chemicals, weakened and inactivated virus vaccines can be produced.[15] (c) Viral vector-based and nucleic acid-based vaccines are independent of virus culture and rely on the genetic sequence of the virus and/or the selection of immunogenic sequences of the virus. By selecting commonly developed vectors, virus-specific sequences can be inserted.[16] Nucleic acid vaccines require further formulations for optimal efficacy. (d) Structure-based understanding of native viral proteins can validate the expressed recombinant proteins and predict the peptide sequences desirable as vaccines. Protein/peptide vaccines require further formulations for optimal efficacy.[17]

(OPV).[18] The IPV was made by inactivating lab-cultured poliovirus by formalin. The OPV was weakened live poliovirus.[18] Weakened viruses are highly immunogenic and are commonly developed by the deletion of viral genes. Nevertheless, mutations could cause virulence reversion as observed for vaccine-derived polioviruses that have been responsible for recent polio outbreaks.[19] This represents an obstacle for polio eradication and, more importantly, a concern for the weakened virus-based vaccines in general. Further genetic manipulation strategies are therefore required to prevent this issue. For instance, modifying the 5′ untranslated region (UTR), 2C coding region, and 3D polymerase region resulted in a genetically stabilized OPV strain that was less likely to regain virulence and exhibited limited viral adaptability.[20]

On the contrary, inactivated virus vaccines generally do not have virulence reversion issues. They also do not rely on extensive genetic manipulations, but the inactivation process may cause the loss of antigenicity of viral immunogens. This vaccine type has been quickly adapted to cope with COVID-19, where patient-derived viruses were inactivated with β-propiolactone, followed with purification by chromatography, and formulated with $Al(OH)_3$ as the adjuvant.[15] The vaccine protected nonhuman primates from SARS-CoV-2 challenges with no notable side effects.[15] Advantages of these virus-based methods include well-developed methodologies, well-established regulation policies, and existing manufacturing facilities from previous practices. Nevertheless, the requirement of virus seeding and culture along with unknown safety issues

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necessitates alternative practices for the development of other vaccines.

Viral Vector-Based Vaccines. Viral vector-based vaccines work by inserting viral antigen-encoding DNA sequences into host cells, which leads to the expression of viral antigens by the host cells.[16] The rationale is based on the capability of viruses to infect cells efficiently through viral genome integration mechanisms.[16] This method could also be problematic since the genome of the host could be altered and cause other diseases. The selection of viruses is therefore crucial in balancing the efficacy (efficient infectivity) and safety (limited pathogenicity). Another common obstacle is the host's pre-existing immunity to certain vectors that would mitigate the efficacy of a vaccine. The selected viral vectors could be further genetically modified to be nonreplicable by disrupting genes responsible for replication. Adenoviruses (Ad) are the most extensively used vectors for vaccines because of several advantages: infectious to various cell types, efficient transgene expression, high *in vitro* growth, lack of genome integration, and genetic stability.[21] For instance, Johnson & Johnson (J & J) deployed the Ad26 vector to deliver gene encoding surface proteins of the SARS-CoV-2, and CanSino Biological used the Ad5 vector.[22] The spike (S) glycoprotein of SARS-CoV-2 was cloned with the tissue plasminogen activator signal peptide gene in the early region 1 (E1) and early region 3 (E3) deleted Ad5 vector. The E1 and E3 deletions render the Ad5 vector nonreplicating. Even though rapid humoral and T cell responses were induced by the vaccine, pre-existing anti-Ad5 immunity partially diminished the immune responses among healthy adults.[22] To address the pre-existing immunity to Ad, chimpanzee Ad (ChAdOX1) represents an alternative solution that provided broad protective immunity against the Middle East Respiratory Syndrome Coronavirus (MERS-CoV).[23] An investigational vaccine for SARS-CoV-2 from the University of Oxford is based on this vector.[23] Their preclinical study showed the prevention of SARS-CoV-2 pneumonia in rhesus macaques with no evidence of subsequent immune-enhanced diseases.[23] However, viral loads were more significantly reduced in the lower respiratory tract but not in the nasal swabs. In general, because of previous vaccine programs, the manufacturing capacity and protocols for this method are already well-established; therefore, less effort may be needed to shift toward the large-scale production of vector-based vaccines. However, both the pre-existing immunity toward certain vectors and previously demonstrated safety concerns over the increased risk of human immunodeficient virus type 1 (HIV-1) acquisition should be carefully evaluated.[22]

Nucleic Acid-Based Vaccines. DNA- and mRNA-based vaccines have the greatest potential for rapid development because of their synthetic nature that circumvents the need for cell culture or fermentation of viruses. The synthetic nature of this method also results in a high safety profile and rapid manufacturing ability.[24] However, this method generally requires further delivery formulations or better delivery devices to boost vaccine efficacy.[24] In contrast to RNA, the increased stability of DNA decreases the need for frozen storage and low-temperature transportation.[24] One of the forerunners among COVID-19 vaccines is DNA-based (Inovio).[10] Similar DNA-based vaccines have had promising results for the prevention of MERS.[25] In the discovery phase of DNA-based vaccines, strategies such as codon/RNA optimization and the addition of highly efficient immunoglobulin leader sequences are needed to enhance the magnitude and breadth of the immune response.[26] For instance, Muthumani *et al.* designed a consensus DNA sequence for the S protein by analyzing S protein sequences, where sequences from clades A and B can be both involved.[26] To enhance the *in vivo* expression, the immunoglobulin E (IgE) leader sequence was added to the immunogen sequence. The insert was further incorporated into the pVax1 vector, which allowed a high-level transient expression of proteins of interest in mammalian cells.[26] The strategies of adding the IgE leader sequence and the use of the pVax1 vector could be quickly adapted to the different virus sequences as well.

Due to their high potency, rapid development, and low cost for manufacturing, mRNA vaccines have emerged as a promising alternative vaccination development strategy for various infectious diseases and cancers.[27] No mRNA-based vaccine is currently FDA-approved. Several strategies have been proposed to solve the common drawbacks of mRNA-based vaccines, which include mRNA instability and high innate immunogenicity.[28] The 5′ and 3′ UTR of mRNA has significant regulatory influences on both stability and translatability.[28] Half-life and expression can be enhanced when these sequences come from viral genes: A 5′ cap is essential for efficient protein expression, and a poly(A) tail affects mRNA translation and stability.[29] Furthermore, the replacement of rare codons with frequently used synonymous codons also enhances protein expression.[30] Additional optimization strategies include increasing G:C content and introducing modified nucleosides.[31,32] In spite of the innate immunostimulatory effect of exogenous or unpurified mRNA, further investigations are required to assess the advantages of their use for vaccines specifically.[33] Innate immune sensing mechanisms of mRNA have resulted in the inhibition of antigen expression and a decreased immune response.[34] Therefore, further formulations for vaccine delivery could potentially reduce innate mRNA-associated immunogenicity and favor immune activation.

Subunit Protein/Peptide Vaccines. Unlike nucleic acid-based vaccines, which produce viral protein after administration, the direct application of viral protein antigens may be a more straightforward method to trigger immunity. This approach currently represents the largest category of COVID-19 vaccines under preclinical investigation.[4,10] Full-length proteins are advantageous because they can induce the development of antibodies against multiple epitopes. Importantly, there is a higher probability that the developed antibodies could bind to the native conformation of the viral protein. However, this also increases the chance of inducing nonspecific cross-reactive antibodies.[35] In addition to the high cost of recombinant protein technologies, the recombinant protein products may not fully match all the molecular features of the corresponding viral proteins, which may lead to ineffective induction of broad neutralizing antibodies (bNAb).[35] Optimizing peptide sequences in the discovery phase can improve the stabilization of epitopes associated with bNAb while reducing the antigenicity of non-NAb epitopes.[17]

Given that only specific amino acid sequences of the full-length protein antigens are responsible for effective immune responses, minimally immunogenic peptides, mimicking B and T cell epitopes, have been proposed for vaccines.[17] Early approaches used short peptide sequences (8−10 amino acids) that could potentially be presented by major histocompatibility complex (MHC) I molecules. Importantly, the memory immune response of cytotoxic T cells could not be efficiently induced with such T cell epitope-mimicking peptides.[36] One

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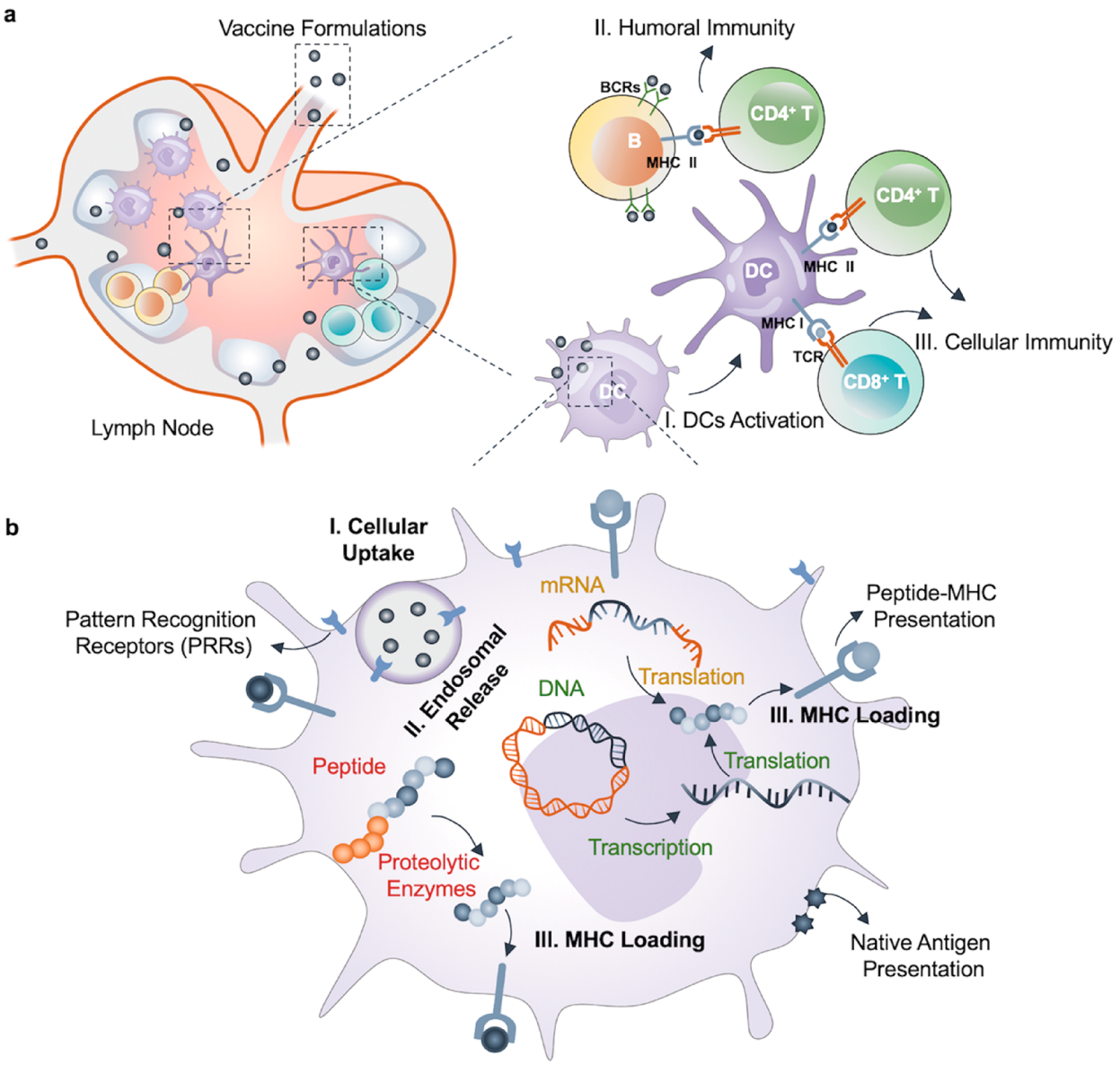

Figure 3. Action mechanisms of vaccine formulations. (a) Vaccines generate humoral and cellular immunity within lymph nodes (LNs): I. DCs can process the antigens and present the peptide fragments *via* both MHC class I and class II molecules. II. B cells can directly recognize the antigens *via* BCRs and present the antigenic peptide fragments by MHC class II to helper T cells ($CD4^+$). Stimulated B cells can subsequently initiate a humoral immune response. III. Cytotoxic T cells ($CD8^+$) can recognize the antigenic peptide fragments presented by MHC class I through TCRs and trigger the cellular immune response.[12,45] (b) Intracellular response of DCs to antigen presentation for different types of vaccines through PRRs. I. Vaccine formulations can be effectively internalized by cells, followed by II. Endosomal release. Before III. MHC loading of antigen peptide, peptide vaccine undergoes enzymatic processing, DNA vaccine undergoes transcription and translation, and mRNA vaccine undergoes translation.[24,27,37]

proposed rationale is that *in vivo* direct loading of short peptides occurs for all cells expressing MHC molecules, including T and B cells. Unlike DCs, they are incapable of generating an effective immune response.[37] These early findings benefitted the design of synthetic long peptide (SLP) vaccines where both helper T cells (T_H) peptides and toll-like receptor (TLR) ligand or peptides could be hybridized to enhance the immune response. For example, Jackson *et al.* demonstrated one single SLP vaccine that can target TLR 2 can serve as self-adjuvant and contain both MHC I- and II-specific peptides (T cell epitopes).[38] SLPs cannot directly bind with MHC I and have to be internalized by DCs for further presentation. Furthermore, *in silico* computer-based approaches can be used to benefit the discovery of peptide vaccines by enabling quicker screening and more accurate predictions of peptide sequences with high immunogenicity and binding affinities to MHC I and II molecules.[39] These approaches have been used to accelerate the development pipeline of vaccines, and particularly for emerging viral diseases such as COVID-19. These computational approaches can predict the binding affinity of specific peptides sequences to either MHC I and II molecules or B cell receptors/antibodies. In this regard, theoretical B and T cell epitopes specific to SARS-CoV-2 were synthesized to generate a multiepitope protein.[39] The antigenicity, allergenicity, physiochemical parameter, and secondary and tertiary structures of these multiepitope proteins were determined *in silico*. However, the processing in the endosomal/lysosomal compartments of the

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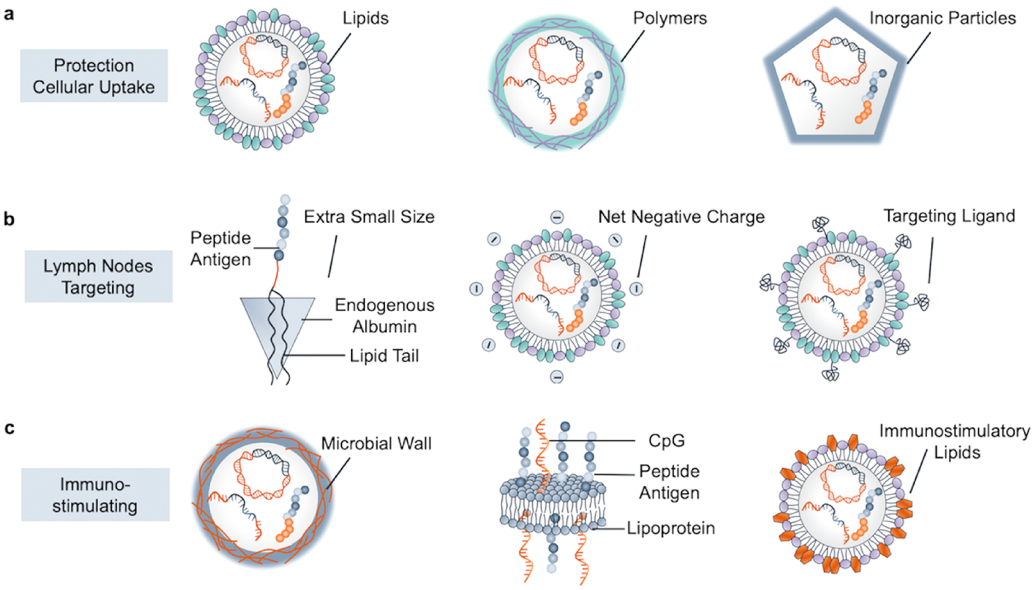

Figure 4. Materials-based vaccine formulations for important immunological functions. (a) Vaccine formulations that protect the active "cargo" and enhance cellular uptake may be based on lipids, polymeric materials, and/or inorganic particles. (b) Vaccine formulations that allow LN targeting by forming extra small-sized albumin-based vaccine complexes, tuning the net charge to be negative and incorporating targeting ligands. (c) Vaccine formulations that promote immunostimulatory effects by using microbial wall-derived polysaccharides as nanocarriers, co-delivering adjuvants with peptide antigens, and exploring innate immunostimulatory lipids.

designed multiepitope SARS-CoV-2 antigens remains to be addressed.

Cell-Based Vaccines. DCs are the most potent antigen-presenting cells (APCs) at the interface of innate and adaptive immunity to sense the "danger signals", process the antigens, and present the nonself-antigens.[40] Unlike other types of vaccines, DC vaccines are manipulated *ex vivo* to be antigen-specific, and thus they are ready to initiate immune responses after being reinfused *in vivo*. The DC vaccine is quality controllable and natively able to traffic efficiently *in vivo*. Different methodologies have been developed to produce modified DC vaccines *ex vivo*, including loading DCs with immunogenic peptides or proteins (subunit DCs), viral transduction to express immunogenic peptides,[41] or using mRNA-based DCs.[42] Different strategies may produce DC vaccines with different potency, for instance, genetically modified DCs may be more efficacious in immune activation than subunit DC vaccines.[41] In addition, supplementary immunomodulatory genes could be incorporated to enhance the potency. However, the process of producing cell-based vaccines is labor-intensive and expensive.[43] Thus, this method could be the most challenging to be scaled up as a universal vaccine for a global pandemic.[43]

VACCINE FORMULATIONS

Action Mechanisms. Vaccines are commonly administered intramuscularly, making it less efficient in interacting with the abundant immune cells present within the skin. Alternative strategies, including oral or intranasal deliveries, are also widely studied because of their ease of use.[44] The effectiveness of alternative strategies is based on mucosal immunity.[44] Nonetheless, different strategies rely on the activation of the adaptive immune system, which involves the interactions between T cells and APCs (DCs and B cells).[12] As shown in Figure 3a, APCs can internalize exogenous antigens and process them into antigenic peptide fragments. The peptide fragments can subsequently be loaded onto the MHC II molecules and displayed on the surface of the APCs.[12,45] $CD4^+$ helper T cells could recognize peptides presented by MHC class II through T cell receptors (TCRs), which could induce the production of cytokines, such as IL-2, that are essential to the normal function of immune cells.[12,45] B cells can directly capture antigens through B-cell receptors (BCRs). The antigens can be internalized and subsequently processed into antigenic peptides for presentation by MHC class II to $CD4^+$ helper T cells. This process is required for the clonal expansion and differentiation of B cells into IgG antibody-secreting plasma cells and memory B cells, which is classified as humoral immunity.[12,45] Generally, only endogenous antigens could be degraded into peptides and loaded onto MHC class I molecules for presentation to $CD8^+$ T cells.[12] Cross-presentation is the ability of certain APCs to uptake, process, and present extracellular antigens in an MHC class I-dependent manner to $CD8^+$ T cells. Cross-presentation can be achieved by several cell types, including DCs (the primary cell type), neutrophils, macrophages, and endothelial cells.[12] The MHC class I presented peptide fragments can activate $CD8^+$ cytotoxic T cells through TCR recognition. Antigen-specific $CD8^+$ cytotoxic T cells can then recognize these peptide fragments presented by target cells and kill them, which refers to cellular immunity.[12,45] Lastly, DCs are also capable of detecting "danger signals" *via* an array of receptors called pattern recognition receptors (PRRs). PRRs are capable of recognizing pathogenic molecules as part of the innate immunity for a response to virus infection by identifying pathogen-associated molecular patterns (PAMPs) from viruses or microbes. The understanding of innate immunity could benefit the development of adjuvants to enhance the immune response further. Together, the understanding of the action mechanisms facilitates the design of vaccine formulations to boost their efficacy.[46]

Requirement of Formulations. Based on the current understanding of eliciting optimal immune responses, materi-

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Table 1. List of the Representative Formulations Used for Vaccines

materials	formulations	cargos (vaccine types)	functions	refs
lipids	ionizable lipid	mRNA (glycoprotein 100 (gp100) and tyrosinase-related protein 2 (TRP 2)) and adjuvants lipopolysaccharide (LPS)	stabilization, enhanced cellular uptake	54
	covalently cross-linked lipid bilayers	protein/adjuvants (Monophosphoryl lipid A (MPLA))	stabilization, sustained delivery, co-delivery	55
	net negative charged formulations by tuning cationic lipids and mRNA ratio	mRNA (influenza virus hemagglutinin (HA) and OVA)	LN targeting, stabilization	56
	nanodiscs *via* sHDL	peptide neoantigens/adjuvants (CpG); dual adjuvants (CpG and MPLA)	co-delivery, enhanced uptake	57, 58
	lipids with cyclic amino headgroup	mRNA (OVA and E7)	immunostimulatory function	59
	modified dendrimer	mRNA (H1N1 influenza, Ebola viruses, and *Toxoplasma gondii* parasite)	enhanced loading, cellular uptake, and endosomal release	60
polymers	stearic acid conjugated low MW PEI	peptide (TRP 2) and mRNA (HIV-1)	lowered charge density, higher biocompatibility	61, 62
	cyclodextrin conjugated PEI	mRNA (HIV)	lowered charge density, higher biocompatibility	63
	PLGA NPs	protein (OVA)	controlled antigen release	64
	PEI/chitosan/poly lysine coated PLGA/PLA	protein (HBV surface antigen)	enhanced loading and cellular uptake, intrinsic immunostimulatory function	65−67
	PBAE and PLGA	plasmid DNA (Luciferase)	enhanced loading and lower cytotoxicity	68
	ionizable amino polyester	mRNA (luciferase)	spleen targeting	69
	oligopeptide-modified PBAE	mRNA (enhanced green fluorescent protein)	APC targeting	70
	albumin and albumin-binding lipids	peptide (OVA) /adjuvant (CpG)	LN targeting	71
	polysaccharide from microbial cell wall and PEI	mRNA (OVA)	immunostimulatory, enhanced loading and endosomal release	72
	aluminum hydroxide	phosphoserine conjugated protein antigen (HIV trimer)	immunostimulatory, sustained release	73
inorganic particles	chemically functionalized Au NPs with hydrophobicity	−	intrinsic immunostimulatory function	74
	aspect ratio optimized and surface modified Au NRs	plasmid DNA (HIV-1)	enhanced cellular uptake, intrinsic immunostimulatory function	75, 76
	sized optimized Au NPs	peptide (OVA) and adjuvant (CpG)	enhanced DC activation	77
	mesoporous Si NPs	adjuvant; protein (E2 of diarrhea virus)	intrinsic immunostimulatory function	78, 79
	antigen density tunable/Size controllable QDs	peptide (self-antigen from multiple sclerosis)	LN homing and higher density of antigens leads to more effective tolerance, intrinsic antiviral effects	80, 81
	oxidized multiwalled CNTs	protein (NY-ESO-1) and adjuvant (CpG)	co-delivery, enhanced uptake	82

als-based nanoengineering approaches could improve the efficacy of vaccines. In this regard, different methodologies could be applied based on different types of vaccines, with either distinct or shared requirements. Virus and viral vector-based vaccines generally do not require further formulations, and their efficacy and safety profiles are largely dependent on the purification and inactivation strategies, the optimization of culturing of viral particles, and the choice of vectors.[47] They have efficient innate advantages in delivery due to their small sizes, and they are easily recognized by the immune system. Although inactivated or weakened virus-based vaccines still make up most of the effective vaccines today, they are not effective for all pathogens.[47] Similarly, the potency of DC vaccines is mostly dependent on how DCs are manipulated *ex vivo*. Strategies in further potentiating cell-based immunotherapies, such as the decoration with nanoparticles (NPs),[48] are mainly developed for cancer immunotherapies, but they may be less suitable for large-scale manufacturing that is needed for the production of antiviral vaccines.

In contrast, synthetic vaccines (nucleic acid-based and protein/peptide-based ones) generally require additional formulation designs for optimal efficacy.[49] These synthetic molecules all have essential secondary or higher-order structures that could be disrupted in physiological conditions.[12] Various endogenous enzymes (such as nuclease or protease) can digest these synthetic molecules before reaching immunocompetent cells, such as APCs, or immunocompetent sites, such as secondary lymphoid organs.[12] Furthermore, physiochemical features of these synthetic molecules may pose challenges for cells to efficiently uptake and process them. For instance, DNA and mRNA are too large to directly diffuse across the cellular membrane and their dense negative charge repels them from cell membranes, which further prevents efficient cellular uptake.[27] Even after penetrating the membrane, the vaccine formulations need to reach the endosomal compartments either to be processed into peptides (protein/peptide-based vaccines), released for further transcription (DNA-based vaccines), or translation (mRNA-based vaccines). The resulting peptide fragments can be further loaded onto MHC molecules and exported to the membrane surface for antigen presentation to T cells (Figure 3b).[50]

We refer vaccine formulations to engineering approaches that could facilitate the *in vivo* delivery of vaccines and enhance immune responses.[51] We categorize the vaccine formulations in terms of commonly used materials: lipids, polymeric materials, and inorganic particles (Figure 4). Commonly pursued feature goals include protection of cargo, improving loading capacity, and enhancing cellular uptake (Figure 4a).[52] In addition, the ideal microenvironment for initiating and amplifying immune responses is secondary lymphoid organs

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where APCs, T cells, and B cells are in close proximity for cell-to-cell interactions. Therefore, targeting DC or LN can be one type of approach to pursue (Figure 4b).[53] To further boost immune responses, immunostimulatory components or adjuvants are usually required, and coformulation approaches have been widely explored for developing immunostimulatory vaccine formulations (Figure 4c). Relevant studies have been summarized in Table 1.

Lipids. Lipid molecules are suitable for vaccine formulations because of their high safety profile and tunable physiochemical features.[55,59] These advantages also make them the leading platform in developing vaccines quickly. Currently, there are two mRNA vaccine forerunners in development for COVID-19 that are based on lipid formulations (Moderna and BioNTech).[10] Lipids are a class of amphiphiles that contain hydrophilic head groups and hydrophobic tails. Interactions between those molecules can drive the formation of spherical vesicles to encapsulate vaccine formulations either with one (unilamellar) or more (multilamellar) lipids bilayers.[55] Lipid molecules have also been explored to achieve distinct biological functions.[59] For instance, cationic lipids are commonly used for efficient cellular uptake and increase the loading of negatively charged mRNA and DNA.[56] DOTMA, DOTAP, and DOPE are common examples.[56] However, cationic lipids are commonly associated with high toxicity and neutralization by anionic serum proteins, which reduce the delivery efficacy.[83] Therefore, ionizable lipids that can change their charge state at different pH conditions can be utilized to maintain efficacy, to reduce toxicity, and to facilitate the endosomal release of cargos.[84] Some common strategies to improve the efficacy of LNPs include enhancing cellular uptake and promoting endosomal escape by incorporating cholesterol, polyethylene glycol (PEG) lipids, or helper lipids DOPE.[56] Hydrophobic cholesterol can fill the gaps between lipid tails to stabilize the vesicle. PEG lipids can shield the vesicles and protect them from clearance when systemically delivered.[56] Helper lipids have unsaturated bonds to form an unstable hexagonal lamellar phase that enhances the endosomal escape.[85] In addition, good biocompatibility of lipids makes it possible for expedited FDA approvals.[86] Therefore, LNPs can be optimized in vaccine formulations to stabilize and enhance uptake of cargo, to improve immunostimulatory functions for antigens, and for targeted delivery. Oberli *et al.* utilized an ionizable lipid, a phospholipid, cholesterol, and a PEG anchored lipid as LNP formulations for mRNA vaccine delivery, which resulted in stable nanoformulations, efficient antigen presentation, and elicitation of a strong T cell response.[54] However, the *in vivo* potency of LNP-based vaccines could be limited by their suboptimal physiological stability, which can cause the fast release of antigens before initiating any immune response. Moon *et al.* developed a hyperstabilized lipid-based vaccine formulation by covalently cross-linking two lipid molecule layers between their head groups using a mild chemical condition that is compatible with loaded antigens.[55] The resulting vesicles are stabilized multilamellar structures that could trap high levels of protein antigens. Combined with PEGylation as a well-established stabilization strategy, the hyperstabilized NPs can sustain the release of antigens up to 30 days in the presence of serum, which significantly enhanced the humoral immune response.[87] By adding the lipid-like monophosphoryl lipid A (MPLA) as adjuvants, the vaccine formulation can further enhance T cell-mediated humoral responses.

In addition, LNPs formulations can be optimized for LN targeting, thereby enhancing the immune response *in situ*. One notable study by Kranz *et al.* demonstrated that a designed mRNA-lipoplexes (RNA-LPX) could target DCs *in vivo* simply by using lipid molecules.[56] They established a library of RNA lipoplexes by tuning the lipid to RNA ratios. Cationic lipids DOTMA or DOTAP and the helper lipid DOPE or cholesterol were used. The charge ratio, size, ζ potential, colloidal properties, and RNA stability also varied with the shifting of lipid to RNA ratios. Interestingly and unexpectedly, all negatively charged particles displayed selective targeting to the spleen. The negatively charged formulation (1.3:2 lipid:RNA) displayed effective targeting of DCs and formed monodisperse NPs (~300 nm) that protected encapsulated mRNA. The authors demonstrated the *in vivo* efficacy using RNA-LPX encoding for the influenza virus hemagglutinin (HA), where DCs, NK, B, and T cells were activated by a single intravenous injection of HA-LPX. They also observed an enhanced IFN-α production by vaccination, which is a typical hallmark of APCs sensing for antiviral environment development induced by RNA virus infections through TLR3 and TLR7.[88]

To enhance immunostimulatory effects, a common strategy is to co-deliver adjuvants to stimulate immune responses. Kuai *et al.* designed an antigen and adjuvant co-delivery nanodisc platform based on synthetic high-density lipoprotein (sHDL).[89] One of the striking features of the nanodisc is its extra-small size: ~10 nm in diameter, which contributed to the enhanced trafficking to LNs.[57] Exploiting the endogenous role of HDL as a cholesterol carrier, adjuvant CpG was modified with cholesterol to efficiently insert into preformed nanodiscs. This strategy also allowed the co-loading of more than one adjuvant, which promoted a synergistic immune activation.[58] Together, these features contributed to the sustained antigen presentation by DCs. Recently, there is a growing interest in exploring innate immunostimulatory functions of lipid molecules. To expand the diversity of lipid molecules, Miao *et al.* developed a library of lipids through a one-step three-component reaction that included amines, isocyanides, and alkyl ketones.[59] This strategy significantly increased the diversity of synthesized lipids compared to traditional two-component reactions.[59] They showed that the immunostimulatory effect of lipids was dependent on the head groups. The identified lipids with cyclic amino head groups displayed immune cell activation efficacy through the intracellular stimulator of interferon genes (STING) pathway. The lipids could be further formulated with mRNA to form LNPs as mRNA vaccine formulations to efficiently deliver the oligonucleotide products. Another advantage is that the formulation could activate the mRNA-independent intracellular STING pathway rather than TLRs, which could reduce systemic toxicity. In addition, inhibition of the antigen expression related to the TLRs binding by exogenous mRNA was prevented to favor an optimal immune response.[27]

Polymers. Aside from the lipids system, polymers have been widely explored for the delivery of different vaccine candidates. Specifically, cationic and ionizable polymers are promising in nucleic acid-based vaccine formulations. Positively charged polymers can condense nucleic acids as nanocomplexes for efficient cellular uptake and endosomal release. In addition, early studies showed that cationic

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materials tend to induce an inflammatory response.[90] One commonly applied polymer category is polyamines, such as polyethylene imine (PEI) and its derivatives.[91] Chahal *et al.* utilized modified dendrimer for antigen-encoding mRNA replicon complexation to form monodisperse NPs.[60] This delivery system, with self-amplifying mRNA, enabled immunization in mice, which protected them from various virus challenges, including H1N1 influenza, Ebola virus, and the *Toxoplasma gondii* parasite.[60] However, their high molecular weight (MW) and branched polymer structure (needed to allow a massive loading of vaccine therapeutics) also lead to severe cytotoxicity, which limits wider applications.[92] Zhao *et al.* developed a low MW PEI with modifications (stearic acid and 2 kDa PEI conjugates) to form self-assembled micelles for delivering mRNA encoding HIV-1 group-specific antigen (gag).[62] Li *et al.* used 2 kDa PEI conjugated with cyclodextrin for intranasal delivery of HIV antigen-encoding mRNA that was able to stimulate HIV-specific immune response.[63] Cyclodextrin modification provided mucosal permeation and mitigated cytotoxicity by lowering the charge density. Other commonly used polymers include polyester-based, such as poly(lactic-*co*-glycolic acid) (PLGA) and poly(lactic acid) (PLA), because of their biocompatibility and biodegradability.[93] Several studies have demonstrated the delivery of protein antigens in PLGA NPs with enhanced immune responses as compared to soluble formulations.[94,95] In addition, such solid polymer particles can control the kinetics of antigens released from the polymer core based on different biodegradation profiles.[64,96] However, nonmodified PLGA is generally negatively charged, and as a result, it has a limited cellular uptake efficiency and loading of anionic antigens.[66] Therefore, various coating or modification strategies (such as PEI,[65,97] chitosan,[66] and poly-L-lysine (PLL) coatings) have been demonstrated to enable the delivery of vaccines and promote interactions with negatively charged cellular membranes.[67] Notably, the cationic polymeric delivery carrier itself could induce T helper cell responses through TLR-4 dependent IL-12 secretion.[67,98] Poly(β-amino esters) (PBAE) is also extensively used in delivering plasmids DNA or mRNA vaccines because of its nontoxic and biodegradable nature.[99] Fields *et al.* developed NPs formulations for plasmid DNA delivery by combining PLGA and PBAE to reconcile the drawbacks from each component: the low DNA loading efficiency of PLGA and the cytotoxicity of PBAE.[68] Other polymeric materials have also been explored, such as polysaccharides[100] and block copolymers.[101]

In terms of targeted delivery to immune cells, versatility in polymer chemistry shows great potential in developing tissue-selective formulations. For instance, a large library of PBAE-based polymers could be easily synthesized and screened for desirable target polymers.[69] Kowalski *et al.* designed an ionizable amino polyester (APE) for mRNA delivery through the ring opening of lactones with amino alcohols.[69] Tissue-selective delivery was achieved by different APE candidates, including APCs within the spleen.[69] Oligopeptides have been used to modify PBAE to allow the tuning of the charge status of NPs.[102] Initially, this was done to optimize cargo loading and mitigate cytotoxicity. Later, Fornaguera *et al.* demonstrated that specific oligopeptide modifications lead to APCs targeting within the spleen and exhibited an efficient transfection of mRNA.[70] Inspirations also come from using native LN trafficking molecules. Clinically, visualization of sentinel LNs is achieved by injecting dyes that bind to albumin because of the LN trafficking capability of albumin particles.[103] Inspired by the endogenous role of albumin, Liu *et al.* designed a vaccine by conjugating the adjuvants or antigens to the albumin-binding lipid tail.[71] The vaccines displayed a notable increased accumulation in the LNs, which decreased the systemic distribution of the vaccines and prolonged the vaccine bioavailability. These features limited the systemic toxicity while increasing the potency of vaccination for OVA antigen, HIV antigen, and human papillomavirus (HPV) antigen.[71]

Some polymeric delivery carriers have been demonstrated to have immunostimulatory effects, such as polymers with cationic charge and hydrophobic domains.[104,105] In addition, inspired by microbes' immunostimulatory effect, Son *et al.* designed sugar capsules that were derived from microbial cell walls for vaccine NPs coating.[72] The coating process was achieved by using silicate NPs (Si NPs) as a template, followed by its removal after the mRNA was loaded and the sugar capsules were entirely coated. Notably, the removal of Si NPs renders relatively flexible nanostructures that are beneficial for lymphatic drainage and accumulation.[72] The mRNA cargos can be efficiently loaded by PEI coating due to electrostatic interactions. The PEI coating was also expected to facilitate the endosomal release for mRNA translation and antigen presentation. The sugar capsule itself functioned as a strong DC activator by eliciting the production of a variety of pro-inflammatory factors, including IL-6, TNF-α, and IL-12 p40, without the use of exogenous adjuvants.[72]

Inorganic Particles. Inorganic materials have been used in vaccine formulations for a long time. Back in 1926, an aluminum compound (alum) was discovered to be able to precipitate the toxoid to enhance the immunogenicity.[106] Alum is still the most widely used adjuvant today. In spite of over 80 years of development, the underlying mechanisms for alum as an adjuvant are still controversial.[107] Popular explanations include the "depot theory" for extended antigen release[106] and enhanced uptake by APCs through antigen absorption on alum.[108] Various immunity signaling and activation pathways have also been identified to contribute to the function of the adjuvants.[109] Recently, Moyer *et al.* utilized the interaction between alum and phosphoserine (pSer) to engineer a short peptide-immunogen conjugate to significantly prolong the immunogen release from pSer-immunogen:alum complexes.[73] The complexes could form NPs for efficient trafficking to LNs and initiated antigen processing and presentation by APCs.[73] Site-specific pSer modification on the base of the HIV trimer could enable the immunogen (HIV trimer) to be displayed with a specific orientation, which could prevent the induction of undesired NAbs.[73] Apart from alum, other inorganic materials have been proposed that may stimulate vaccine-specific immune responses, such as silica crystal[110] and calcium phosphate.[109]

In addition to the traditional use as immunostimulatory adjuvants, a wide range of biocompatible inorganic particles have also been developed as vaccine formulations,[111] including quantum dots (QDs),[80] gold nanoparticles (Au NPs),[77] carbon nanotubes (CNTs),[82] and Si NPs.[79] The physical properties of formulations, such as surface charge, hydrophobicity, and aspect ratios, are closely associated with the efficacy of vaccines.[112] Inorganic materials have a high degree of tunability to allow them to fulfill the requirements of vaccine formulations. Easy preparation and good storage stability are additional advantages of inorganic particles.[113] For example, Au NPs have been chemically functionalized to investigate the

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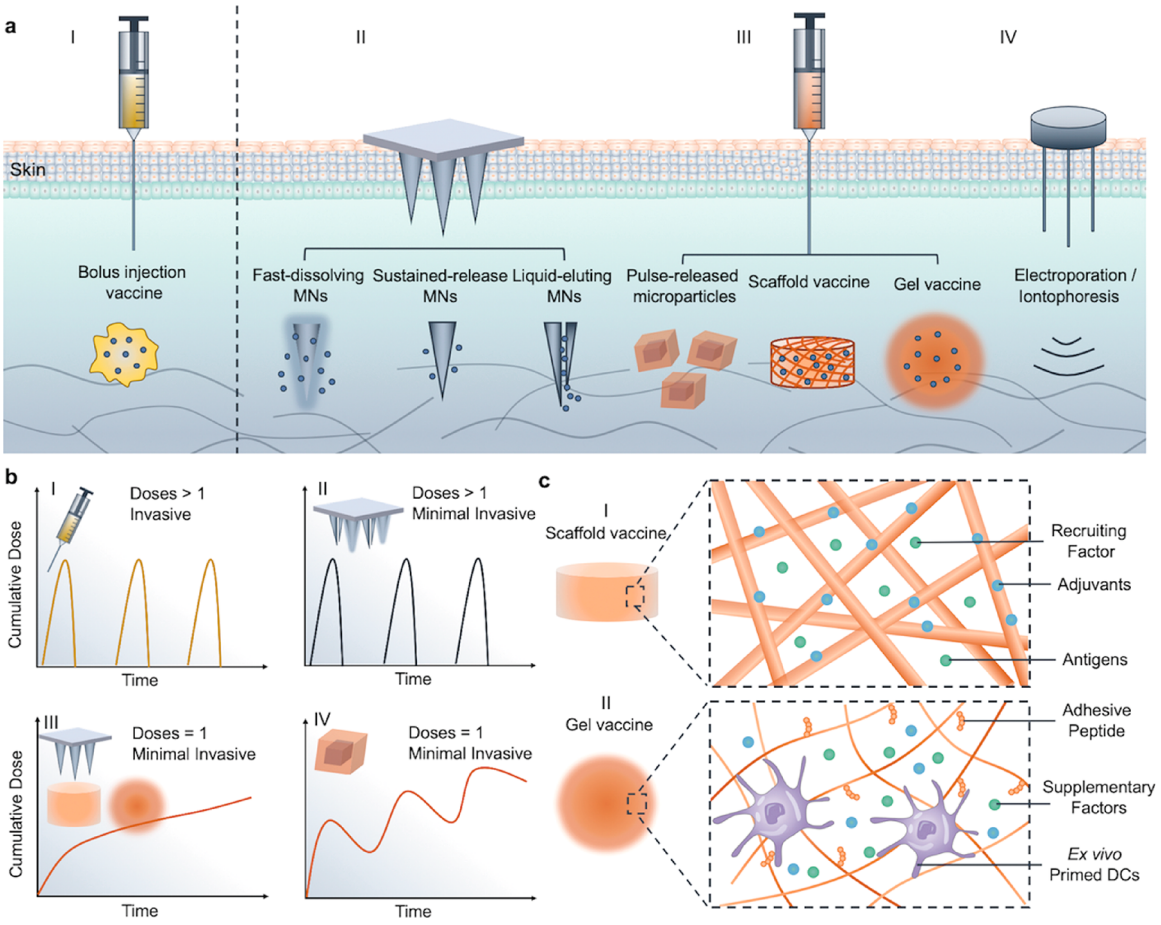

Figure 5. Summary and characteristics of various vaccine delivery devices. (a) Drug delivery devices developed for vaccine administration: I. Traditional vaccine-containing solutions for bolus injection. II. Minimally invasive microneedle devices (including fast-dissolving, sustained-released, and liquid-eluting hollow microneedles). III. Injectable drug delivery devices (such as microparticles, solid scaffolds, and hydrogel-based). IV. Other devices for enhancing vaccine efficacy (such as electroporation and iontophoresis). (b) Schematic for representative PKs of vaccine and administration features for different vaccine delivery devices: I. Traditional bolus injection is invasive and may require multiple doses. II. Fast dissolving or liquid eluting MNs can be self-administrable and minimally invasive but still require multiple doses.[125] III. Sustained released MNs can be both minimally invasive, with only one dose necessary,[122] while scaffold and gel vaccines could be injectable to avoid implantation and also only require one dose. IV. Pulse-released microparticles can mimic a multiple-dose regimen with only one injection.[127,128] (c) Schematic of representative mechanisms for vaccine delivery devices. I. Scaffold vaccines: The scaffold could sustainably release recruiting factors to home DCs, and encapsulated antigens could further be processed by DCs *in situ*.[129] II. Gel vaccines: Hydrogel microenvironment can be engineered to support encapsulated cell vaccines (such as DC vaccines) by incorporating cell adhesive peptides and supplementary factors.[136]

relationship between surface hydrophobicity and the associated immune activity.[74] It is also expected that other functional groups may bring a pathway-dependent immune activation that is specifically desirable for antiviral defense. Xu *et al.* described surface-engineered Au nanorods (NRs) for plasmid DNA vaccine delivery.[75] With certain surface modifications and aspect ratios, cellular toxicity of Au NRs can be lowered, internalization can be enhanced, and intrinsic adjuvant features can be utilized.[76] *In vivo* vaccination with HIV-1 envelope glycoprotein (Env) plasmids demonstrated enhanced cellular and humoral immunity.[75] Zhou *et al.* synthesized a series of Au NPs (15 to 80 nm) for co-delivery of antigens (OVA peptides) and adjuvants (CpG) by surface conjugation.[77] Particles of 60 and 80 nm outperformed other sizes (15, 30, and 40 nm) in activating DCs; this was most likely due to a higher payload of antigens. Notably, they promoted DC homing to the LNs and induced potent cellular immune response.[77] High tunability is also achievable in other organic particles, such as adjuvant-based mesoporous Si NPs,[78] mesoporous Si NP-based antigen/adjuvant co-delivery systems,[79] antigen density tunable QDs,[80] size-controlled antiviral QDs,[81] and surface oxidized antigen/adjuvant-based multiwall CNTs.[82]

VACCINE DELIVERY DEVICES

If the vaccine formulations are considered as utilizing nanoscale engineering, then vaccine delivery devices utilize micro/macroscale engineering to improve vaccine efficiency and efficacy. Beyond the optimization of the vaccine itself and designing appropriate delivery carriers, the efficacy of a vaccine also depends on pharmacokinetics (PK) of the antigens after administration.[114] When the body is exposed to an exogenous antigen, both humoral and cellular immune responses are initiated to neutralize it, which also leads to immunological memory.[12] During a virus infection, the replication process typically takes one to several weeks to sustainably stimulate the immune system with a continuous supplement of antigens.[115] In contrast, typical vaccines show a rapid clearance and can only be detected in LNs within days.[114] Although still poorly

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understood, the PK of a vaccine, which refers to the dynamics of the antigens presence during immunization, has a profound impact on immune response.[87] One study indicated that increasing the dosage of vaccines over 2 weeks at both priming and boost stages induced a durable immune response and increased antibody production compared to traditional bolus immunization.[87] Also, computational models suggested that antigens can be better captured in germinal centers (GCs) if antigen availability is extended.[116] In addition to the advantages of extended antigen availability, traditional vaccination strategies often fail to yield the desired optimized immune responses in a single dose. Furthermore, vaccine regimes are often designed to be multiple shots, thus achieving an even longer immune protection by rechallenging the immune system with the same antigens in secondary "boosting" shots administered after the primary shots.[117] Therefore, vaccine delivery devices have been widely investigated, to develop single-dose vaccines, minimal invasively administered vaccines, and self-administrable vaccines (Figure 5a,b). However, its potential can only be realized if various design principles are met (Table 2).

Microneedles Vaccines. Transdermal microneedles (MNs) patches have been proposed as a promising drug delivery device for immunotherapies[144−147] based on the minimally invasive nature of MNs and the highly active immune environment of the skin. Additionally, since MNs patch-based vaccines can be easily administered without the need for significant medical training, these vaccines can be easily deployed both in areas with limited medical resources, such as developing countries and during times of significant medical crisis, as seen during an outbreak or pandemic. Early studies include the use of vaccine-coated metal MNs[118] and fast-dissolving polymer-based MNs.[119] Recently, the dissolving MNs (carboxymethyl cellulose-based) were used for adjuvants and Ad5-based vaccine co-delivery in an attempt to address COVID-19.[147] A 1 month storage of MNs vaccine at 4 °C maintained the immunogenicity of Ad, where no significant loss in antibody responses was shown in mice studies.[147] Similar dissolving MNs are also being investigated to deliver the SARS-CoV-2 subunit spike protein (S1).[120] Potent virus-specific antibody responses were elicited in mice as early as 2 weeks after the immunization with MNs. The dissolvable MNs for influenza vaccine have been previously evaluated in a phase I clinical trial compared to the traditional intramuscular hypodermic injection of a single-dose vaccine.[121] The MN patch was well-tolerated and immunogenic after a single-dose vaccination. In addition, self-administration exhibited efficacies comparable to the administration by medical workers. The dissolving feature leaves no sharp wastes, and self-administration could significantly relieve the pressure on medical resources, which could be especially critical in coping with a pandemic. Beyond these milestones, advances in microfabrication, materials engineering, and vaccine formulation optimization have provided additional opportunities to enhance vaccine efficacies.[148]

Recent advances in microfabrication, such as 3D printing and stereolithography, can allow for more sophisticated MNs designs.[125] Liquid-based vaccine formulations are still the most widely used.[125] However, liquid-based vaccine formulations are incompatible with most solid-based MNs forms. Inspired by the capability of snake-fangs to efficiently inject venom, Bae *et al.* used an exposure lithography-based strategy to produce MNs patches with snake-fang architectures for efficient liquid

Table 2. Summary of Typical Designs, Advantages, and Limitations of Different Vaccine Delivery Devices

delivery devices		designs	advantages	limitations	refs
MNs vaccine (minimally invasive; minimal requirement on medical professionals and medical equipment)	fast-dissolving MNs	fast-dissolving polymers as MNs matrix	fast and enhanced transdermal delivery; could be cold-chain free	multiple administrations; vaccine formulations may get denatured from MNs fabrication	118−121
	sustained-release MNs	controlled biodegradable materials as MNs matrix	potential to be single shot; protect the vaccine formulations in the physiological environment; could be cold-chain free	vaccine formulations may get denatured from MNs fabrication	122−124
	liquid-eluting MNs	hollow MNs with different architectures	compatible with liquid vaccine formulation; fast and enhanced transdermal delivery	multiple administrations; requirement of cold-chain transport	125
	pulse-released microparticle	materials with different biodegradation profiles	optimal release for a potent immune responses; release kinetic can be optimized by materials engineering	vaccine formulations may get denatured during the fabrication	126−128
injectable materials assisted vaccine (potential for single-shot vaccines to reduce the requirement of medical resources)	scaffold vaccine	solid scaffolds that could tune the release of different immunomodulatory factors	continuously modulate the immune response *in situ*	injectability need to be achieved to avoid surgical implantation	129−134
	hydrogel vaccine	biomimicking gellable materials with tunable biophysical properties and high cytocompatibility	compatible with various types of vaccines, including cell-based vaccines; simple fabrication process and the water environment help protect the cargos	*in situ* gelation may be weak to achieve sustained release	135−143

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formulations delivery.[125] The design was compatible with most existing vaccine formulations, and the patch can be applied with the pressure of the thumb and achieve an efficient transdermal delivery.[125] Nevertheless, solid-based vaccines, including MNs-based ones, also attracted significant attention because of the decreased cost of vaccine manufacturing, transport, and storage when compared to the liquid-based formulations.[149] One of the key challenges faced by these MNs patches is the loss of vaccine activity during the drying or heating process of MNs patch fabrication,[150] where molecular structures of vaccines may be disrupted.[151] Different strategies can be adopted, such as the use of lyoprotective polymers as MNs matrices, which include silk fibroin,[122] gelatin, dextran, and PVP,[152] or adding sugar molecules as stabilizing excipients, including trehalose and sucrose.[147,152] In the vaccine discovery phase, the introduction of certain mutations can improve the thermal stability of vaccines as well,[148] which can be further applied to specific MNs formulations. Additionally, a hyper-stabilized vaccine formulation, such as the interbilayer-cross-linked multilamellar vesicles, helped protect vaccines during the drying process of MNs fabrication and have outperformed other traditional delivery strategies in inducing a potent antigen-specific immune response.[123]

MNs also provide advantages in tuning optimal release profiles of vaccines. As mentioned earlier, the sustained availability of vaccines is a desirable feature for immunization. Vaccines targeting different infectious diseases may vary in terms of optimal release profiles based on the unique features of each virus. Initially, MNs for vaccination were mainly an upgrade to traditional bolus delivery by using a fast-dissolving MNs matrix,[119] which requires multiple doses for sufficient immunity. With the exploration of the desired PK of vaccines, MNs can play a much more important role. For example, a silk matrix with crystallized structures could be used as an MNs matrix to sustainably release loaded vaccines over 2 weeks and to elicit potent immune responses with one single administration.[122] In addition, biodegradable polymers with known degradation kinetics can be used to encapsulate the vaccines and tune antigen release profiles. These polymers include biodegradable cationic PBAE[123] and PLGA.[124]

Injectable Material-Assisted Vaccines. Macroscale biomaterials strategies have recently boosted the field of immunotherapy.[153] There are many advantages in controlled release and encapsulation of various cargos, ranging from small molecules to proteins and cells.[153] These varied cargo elements can help to meet the need for diverse types of vaccine candidates. In terms of controlling PKs of vaccines, both sustained and pulsatile releases could be possibly achieved.[153] In addition, materials engineering can enable devices that do not require surgically invasive administrations.[153] Together, they could minimize the requirement of medical resources without mitigating the vaccine efficacy.

PLGA has also been developed for a single-shot vaccine based on the tunability of its biodegradation profile.[126] In the case of polio vaccines, two to three administrations over several months are required. Tzeng *et al.* demonstrated that IPV could be encapsulated within PLGA microspheres stably and released through two bursts that were one-month apart.[126] They examined the effect of adding cationic excipients (Eudragit E, PLL, and branched PEI) to stabilize IPV antigens, protecting them from denaturation by acidic byproducts, and modulate degradation profiles of PLGA microspheres.[126] Notably, a single shot of PLGA microsphere-based vaccines could elicit the production of NAb as potent as two bolus injections.[126] Combined with the advances in microfabrication and additive manufacturing, McHugh *et al.* fabricated microscale (~400 μm) hollow PLGA-based microparticles that were injectable.[127] The microparticles can be filled with vaccine formulations, and the degradation rate of the microparticle shell (a lactic/glycolic copolymer) can be tuned to achieve controlled release spanning from a few days to a few months.[127] Such a design can accomplish the goals of general transdermal vaccine injection and achieve temporally controlled pulsed delivery of antigens in a single injection.[127] Recently, a similar PLGA-based microparticles platform was applied to release a STING agonist (cyclic guanosine monophosphate-adenosine monophosphate (cGAMP)) in a pulsatile manner over a long period (~16 days).[128] One single injection of the pulsatile drug-releasing microparticles induced potent immunity as effective as three separate injections. These engineered platforms are versatile in terms of encapsulating different cargos required by vaccine formulations and tuning release kinetics correspondingly. However, even though the use of FDA-approved PLGA could facilitate the clinical translation to a certain degree, several design limitations need to be overcome for translating PLGA-based drug delivery devices into clinical applications. For instance, the pulsatile release was achieved with hollow microparticles that have thick PLGA shells to encapsulate the drugs in the hollow core for controlled release. This design significantly sacrificed their loading efficiency due to the limited volume of the core. Clinically, patients need a high drug dosage, and this formulation needs further optimization to meet the dosage requirement. In addition, PLGA can create an acidic microenvironment during biodegradation, damaging the encapsulated drugs.[126] Furthermore, the encapsulated vaccines need to survive the fabrication process; brief heating requires thermally stable vaccines.[127,128] All these aspects could compromise their applications for a broader collection of drugs or vaccines.

Traditionally, *ex vivo* modification of antigen-specific DC vaccines is a multistep process associated with high costs and high levels of complexity.[43] It was proposed that *in situ* DC recruitment, activation, and further trafficking to LNs would be more desirable compared to the *ex vivo* modification strategy (Figure 5c).[129] The early study by Ali *et al.* demonstrated the use of macroporous scaffold vaccines based on PLGA, which presented cytokines (GM-CSF), danger signals (CpG), and antigens in a spatiotemporally defined manner. GM-CSF can be sustainably released from the scaffolds over 20 days for effective DC recruitment.[129] By co-loading the CpG through PEI-mediated electrostatic interactions, CpG was immobilized on the scaffolds for local internalization by the recruited DCs. This process mimicked an infection and was able to continuously recruit and activate DCs.[129] This specific scaffold vaccine was surgically implanted; nevertheless, it encouraged further development of injectable vaccines that work similarly. Kim *et al.* demonstrated the use of mesoporous silica rods (MSRs) with a high-aspect-ratio that could be injected with a syringe needle.[130] They could self-assemble *in vivo* as macroporous scaffolds to initiate potent immune responses.[130] The DCs could be recruited into the scaffolds where the inflammatory signals (GM-CSF), adjuvants (CpG), and antigens (OVA) were sustainably released from the scaffolds for *in situ* DC activation and maturation. It was also demonstrated that locally injected scaffold vaccines based on

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MSRs could elicit strong systemic immune responses with the increase in levels of antigen-specific B cells, helper T cells, and cytotoxic T cells.[130] A PEI coating was later added to the design to enable antigen loading through adsorption for enhanced immunogenicity.[131] This specific system was recently utilized for synthetic peptide vaccines and small antigens.[132] This strategy was able to bypass multiple immunization requirements but sustained a more robust antibody response, compared to traditional bolus or alum vaccine formulations.[132]

Scaffold-based vaccines have displayed efficacy in inducing systemic immune response through *in situ* immune modulations. *In vivo* self-assembled scaffolds provide a good alternative to bypass surgically implantation; however, the self-assembling process is commonly undefined in terms of how long it could take for self-assembling.[133] Materials engineering facilitates the fabrication of scaffolds maintaining injectability. Bencherif *et al.* described a strategy for fabricating injectable scaffolds with shape memory based on cryotropic gelation.[134] The cryogels were based on alginate methacrylate, and they were capable of being reversibly deformed, over 90% strain, after being chemically cross-linked in a frozen condition (−20 °C).[134] The ice crystals hindered the cross-linking in frozen spots and therefore formed the macroporous pores that not only enabled the shape memory for the gel but also allowed the DCs infiltration.[134]

Hydrogels have been extensively explored in the field of immunotherapy.[153] They have advantages in terms of controllable delivery of multiple cargos, *in situ* immunomodulation, and mild fabrication process for various vaccine formulations (Figure 5c). Early pioneering work includes the loading of *ex vivo* antigen-primed DC vaccines within Ca^{2+} cross-linked alginate gel by Hori *et al.*[135] Activated DCs have a short lifespan, and continuous immune responses may be suppressed if no sufficient host DCs and T cells could be recruited and primed *in vivo*.[135] Therefore, cell-based vaccines delivered by gels are expected to improve cell survival and prolong the presence of DCs.[136] Both host DCs and cytotoxic T cells can be recruited to the injection site by cytokines and chemokines secreted from the injected DCs. In addition, the enhanced efficacy of transplanted cells can be enhanced by supplementing the gels with other immunomodulatory molecules, such as cytokines (IL-2) or adjuvants (CpG).[137] The increased persistence of transplanted cells could be achieved by designing gels mimicking ECM by the incorporation of cell-adhesive peptides, such as RGD.[154]

Apart from cell-based vaccines, other types of vaccines could also be incorporated into gel systems, where the release profiles can be tuned. Roy *et al.* utilized the *in situ* cross-linking mechanism of tetrafunctional poly(ethylene oxide) amine and poly(ethylene oxide) succinimidyl glutarate to encapsulate plasmid DNA.[138] The gelation did not interfere with the supercoiled structure of plasmid DNA, and the gelling ability can be tuned by adjusting the concentrations of these two components. Notably, gene expression levels were comparable to bare DNA, but the duration of expression was significantly longer. Other *in situ* gel systems explored for vaccination include temperature-responsive gels[139] and Michael-addition-type hydrogels.[140] Singh *et al.* engineered dextran and PEG-based hydrogels that could be cross-linked *in situ* for antigen/adjuvant delivery.[141,142] Umeki *et al.* designed an immunostimulatory CpG DNA-based hydrogel for antigen delivery.[143] To achieve a sustained release of antigens, they proposed to cationize the antigens by ethylenediamine conjugation where antigens could form complexes with a negatively charged DNA hydrogel matrix. The innate immunostimulatory function of gel and prolonged antigen presence leads to an enhanced antigen-specific immune response with less toxicity.[143] *In situ* gelling strategies or physically cross-linked gels from these studies generally take minutes to hours, which renders limited control on cargos release and material properties during the gelling period.[143] Therefore, the development of gel systems that are both injectable and display defined cargo release/biodegradation profiles could be desirable for vaccine administration.

Other Delivery Devices. Many more investigations are currently ongoing to engineer alternative drug delivery devices. For instance, to promote the uptake of DNA vaccine by cells, electroporation is one of the traditional and effective strategies to enhance the uptake of plasmids.[155] Different parameters, such as electrical features (voltage, current, and frequency) and length of the electrode (intradermal or intramuscular targets), need to be optimized to ensure efficacy and safety and to minimize pain. This process has recently demonstrated promising clinical phase I results for MERS.[25] The same electroporation device (CELLECTRA) is also being investigated for a DNA vaccine of SARS-CoV-2 from Inovio.[156] The S protein of SARS-CoV-2 encoding DNA vaccine demonstrated potent antigen-specific T cell responses and the production of neutralization antibodies in preclinical mice and guinea pigs studies.[156] In order to incorporate the advantages of MNs, electroactive MNs devices have also been developed for the electroporation of DNA vaccine in a minimally invasive manner.[157] Similar devices using iontophoresis can also enhance the transdermal delivery of vaccines.[158] Recently, Tadros *et al.* designed millimeter-scale star-shaped particles, termed STAR particles, based on aluminum oxide for topical vaccine delivery,[159] and the minimally invasive STAR particles elicited an immune response comparable to intramuscular injection.[159] Even though STAR particles are simple to manufacture and well-tolerated by the immune system, a much higher dosage was administered compared to intramuscular injection. In addition, potential human-to-human variability in administration and drug leftovers on the skin may result in differential efficacy and undesirable toxicity.

In terms of reducing the demands for medical resources, vaccines that could be intranasally or orally administered are desired if their efficacy is comparable to transdermal delivery.[44] The main strategy for intranasal or oral vaccine development is currently limited to weakened viral vaccines mimicking natural infections. Nonliving vaccines are safer but less immunogenic and thus may benefit greatly from drug delivery formulations and devices.[160] A variety of drug delivery devices have been proposed for oral vaccination delivery. For instance, the micromotor-driven microparticles for oral delivery of antigens.[161,162] The micromotor vaccine can efficiently enter the intestine to enhance the antigen uptake. Aran *et al.* designed a needle-free oral microjet where the high-pressure liquid jet of the vaccine was produced by a self-contained gas-producing reaction.[163] It was shown that a buccal immunity response was enhanced, and this method was able to elicit a potent humoral immune response both locally and systemically.[163] Abramson *et al.* engineered a self-orienting system for oral delivery that could attach to the gastric wall by autonomously positioning to maximize the delivery efficiency.[164] Innovations in drug

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delivery devices will significantly transform traditional vaccination regimes.

SUMMARY AND FUTURE DIRECTIONS

The devastating disruptions caused by the sudden outbreak of COVID-19 has urged vaccine development at an unprecedented speed.[4] Within 70 days of the first release of the genetic information on SARS-CoV-2, the first dose of a vaccine was administered to human participants.[9] As of August 25, 2020, 31 vaccine candidates are in various stages of clinical trials, and around 142 candidates are in preclinical trials.[10] Apart from the high diversity of the vaccine candidates, many candidates are based on different technological platforms, such as nanotechnology delivered mRNA vaccine, electroporation device-enabled DNA vaccine, and MN-delivered vaccine. The COVID-19 pandemic has accelerated the deployment of different vaccine formulations and delivery devices. It is reasonable to expect that more efforts will be dedicated to further optimize the vaccine designs to help fight future outbreaks.

Here, we reviewed recent research efforts in the field of vaccine discovery, vaccine formulation development, and vaccine delivery device designs. Because of the deployment of various engineering technologies, it is expected that future vaccines will not only need to demonstrate safety and efficacy but also to be relatively convenient and simple to use for better coping with population-level vaccinations or sudden outbreaks worldwide. Nanoengineered formulations could enhance the immune response by delivering the required components together and maximizing their uptake by immunocompetent cells. To expedite the future preparedness of vaccines for the occurrence of a pandemic, synthetic vaccines have demonstrated great potential for rapid clinical applications because they are molecularly defined and independent of the time-consuming culture of viruses, which can accelerate the initial vaccine development. However, synthetic vaccines are also the ones that need to be formulated the most, which constitutes the major part of their time costs. To accelerate the development of synthetic vaccines, the nanoengineered formulations need to be simple and robust to manufacture in terms of chemistry or the selection of raw materials.[165,166] In terms of vaccine delivery devices, self-administrable vaccines, single-shot vaccines, and solid MN-based vaccines could further enhance the availability of vaccines to a broader population. For instance, self-administrable vaccines can be mailed to patients at home without the need of medical experts.[165] Single-shot vaccines can be much more convenient compared to the traditional immunization regimens that require multiple shots. Furthermore, solid MN-based vaccines that are cold chain free could simplify the vaccine transportation and distribution process. All of these aspects could be enabled by engineering approaches to accelerate the development of vaccines at a pandemic speed.[165,166] Although combining the advantages of vaccine formulations and vaccine delivery devices is promising, micro/macro-engineering still needs to develop solutions that do not require "harsh" conditions to be compatible with nanoengineered formulations.[167] Expectedly, platform technologies that can be quickly adapted to address emerging viral diseases could speed up the pace of vaccine development.

However, several challenges remain before these goals can be fully achieved. First, a differently identified virus is unknown in terms of its interactions with our immune system and which type of immune responses are most desirable to trigger prolonged immunity. An improved understanding of our immune system and its interactions with viruses are the basis for designing an effective vaccine, which also guides the steps for designing corresponding vaccine administration regimens. Second, innovative formulations or delivery devices for vaccines are mostly in the preclinical stage, which have not been approved by the FDA yet. For example, MN-based vaccines showed encouraging results from a phase I trial of dissolvable MN-based influenza vaccine and indicated a promise of using it as an alternative strategy for vaccination.[121] However, patient compliance with this approach and its efficacy needs to be tested in a larger population, considering only 100 human subjects are currently involved. Also, the efficacy of vaccines by self-administration should be further validated in comparison to those performed by medical experts. In addition, the proposed formulations and delivery devices should be easy to manufacture with minimal batch-to-batch variability to simplify the quality control process. The raw materials used could prioritize the FDA-approved ones to simplify the regulatory process, and the required chemistry is preferred to be simple and robust. Even though advanced manufacturing technologies excel in reducing batch-to-batch variability, a high cost may be associated. The improved efficacy of a vaccine from these formulations and delivery devices should not be complicated by the fabrication process so that it can facilitate the clinical translation and regulatory approvals.[165]

Additional challenges for vaccine development also exist during a pandemic. Multiple vaccine candidates flush into different stages of clinical trials that could burden the regulatory process by the FDA for selecting promising candidates and deciding final ones.[4] Furthermore, human trials are starting even before the most suitable animal models for safety and efficacy are defined.[168] Based on previous successful preclinical and early stage clinical data for a similar platform, animal studies are even skipped directly for human administrations.[10] Other engineering approaches such as organs-on-a-chip[169−171] or screening using organoids[172] may serve as a promising tool in future clinical trials for efficacy and toxicity determination. Ethically controversial human challenge trials by deliberately infecting humans with a virus have been proposed and pursued to accelerate the process of vaccine development.[173] Therefore, potentially we should have vaccine clinical trials specifically designed for different scenarios by incorporating engineering advances and regulatory guidelines readily outlined for unusual trials. In conclusion, human health and societal stability are constantly challenged by sudden virus outbreaks, and the rapid development of effective vaccines is believed to be an ultimate solution. We expect to observe faster and more efficient vaccine development paradigms in the future by combining the advances in different engineering fields.

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VOCABULARY

Coronavirus disease 2019 (COVID-19), a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been declared as a pandemic by WHO since March 11, 2020; **antiviral vaccine**, a biological preventive preparation strategy to train the immune systems to fight against viral pathogens; **vaccine formulations**, biomaterial engineering strategies aiming to boost the efficacy or reduce side effects of vaccines through co-delivering adjuvants, targeting immune cells, or protecting vaccines activities; **vaccine delivery devices**, tools for administering the vaccine formulations, and they can be used by medical workers or the patients themselves; **pattern recognition receptors (PRRs)**, proteins that can recognize pathogenic molecules as part of innate immunity in response to virus infection, including identifying pathogen-associated molecular patterns from a virus; **adjuvants**, supplementary agents that could be co-delivered with vaccines to boost the immune response, minimize the dosage of antigens, and maintain longer immunity; **lymph nodes**, round or bean-shaped clusters of cells containing abundant immune cells that can initiate the robust immune response toward viral infections

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