Zhang *et al. Zoonoses* (2023) 3:47
DOI 10.15212/ZOONOSES-2023-0018

REVIEW ARTICLE

Nanoparticle-Based Vaccines against Zoonotic Viruses: A Review

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Abstract

Vaccines are the most promising tools for maintaining public health. Most emerging human infectious diseases are caused by viruses originating from an animal reservoir via zoonotic transmission. Therefore, zoonotic virus spillover and spread in humans have become global health threats. Nanoparticle-based vaccines are ideal for antigen delivery, as adjuvants, and as viral structure mimics. Nanoparticles benefit vaccine design and are utilized to protect the antigen cargo, and increase the immunogenicity and efficacy. Therefore, nanoparticle vaccines are a novel method of immunization by which optimal immune responses are elicited. Herein we review current approaches in the development of nanoparticle vaccines and highlight the role of nanoparticle vaccines against zoonotic viral diseases.

Keywords: zoonotic virus, vaccine, nanoparticles

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Received: May 15 2023
Revised: August 5 2023
Accepted: September 18 2023
Published Online: December 7 2023

INTRODUCTION

Vaccines have a pivotal role in public health by reducing infection and transmission of a variety of diseases worldwide [1]. Vaccination currently prevents 3.5–5 million deaths annually from infectious diseases, such as diphtheria, influenza, and measles [2]. In addition, vaccines have been crucial in eradicating smallpox and rinderpest, and are now close to eradicating polio. Moreover, vaccines have a significant economic impact by reducing disease-related costs and hospitalizations exceeding $500 billion on a global scale [3].

Zoonotic pathogens, such as Ebola virus, Henipavirus, avian influenza virus, and most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), pose a substantial threat to global public health [4]. Combatting outbreaks depends on the rapid development of novel vaccines that have not been typically manufactured with traditional vaccine platforms. The use of nanoparticles as platforms displaying viral antigenic structures, therefore, has facilitated an alternative approach to traditional vaccines [5].

The challenges associated with zoonotic viruses have stimulated enormous interest in the development of novel nanoparticle-based vaccines. In this review we discuss current application of nanoparticle vaccine formulations against zoonotic viruses. We also highlight a promising way forward for using nanoparticle vaccines as safe, efficacious, and affordable vaccine candidates.

VACCINE IMMUNOLOGY

Vaccination is one of the most efficient and cost-effective methods by which to combat zoonotic viral infections in humans and animals. Vaccines function

by serving in part or entirely as a viral agent mimic, thus triggering the immune system.

By mimicking virus infections, vaccine components are first recognized by a series of sensors that activate the innate immune system. For example, nucleic acid sequences and viral secondary structures are detected by toll-like receptors (TLRs) [6]. These sensors then trigger the downstream innate immune response, which is characterized by inflammation, cytokine production, immune cell recruitment, and phagocytic cell activation. The innate immune response in turn neutralizes and kills virus-infected target cells. Furthermore, antigen-presenting cells (APCs) are recruited to sites of infection where APCs take up antigens and transport antigens to secondary lymphoid organs, such as lymph nodes and the spleen. T and B cells within the secondary lymphoid organs are stimulated by the APCs, which gives rise to adaptive immunity.

Within secondary lymphoid organs, APCs drive the activation and differentiation of T cells to acquire complete effector properties. APCs stimulate naïve virus-specific $CD4^+$ T cells, which then differentiate into armed effector cells. APCs expand $CD4^+$ Th cells, which guide the differentiation of B cells that ultimately produce virus-specific antibodies. Moreover, with the help of $CD4^+$ Th cells, activated APCs promote the proliferation of naïve $CD8^+$ T cells to generate cytotoxic T lymphocytes (CTLs) against specific viral agents [7]. Eventually, these T and B cells adopt memory phenotypes and function, which endow these cells with the ability to expand and re-activate in response to the next viral encounter [8].

NANOPARTICLE-BASED VACCINE PLATFORMS

In the past decade the use of nanoparticles in the development of novel nanoparticle vaccine candidates has increased exponentially. Briefly, nanoparticle vaccines are generated by encapsulating antigens within the nanoparticles or by conjugating the nanoparticle surface with viral antigens. Various nanoparticles are currently utilized as carriers of antigens. Polymeric nanoparticles, virus-like particles (VLPs), liposomes, and self-assembled protein nanoparticles (SAPNs) are examples of antigen carriers (Fig 1). The benefits of these nanoparticles mainly rely on an ability to protect antigens from proteolysis, improve bioavailability, and maintain long-term antigen release (Table 1).

Nanoparticles are used as a flexible system to deliver multiple antigens or antigen/adjuvant complexes, which enhance antigen uptake and concurrent activation of APCs. For example, poly(lactic-co-glycolic acid) [PLGA] nanoparticles, 300 nm in size, lead to greater activation of dendritic cells (DCs) and stronger antigen-specific T cell responses in immunized animals compared to exogenous soluble antigens and larger microparticles (>1000 nm) [9]. In addition to effective antigen delivery, nanoparticles also

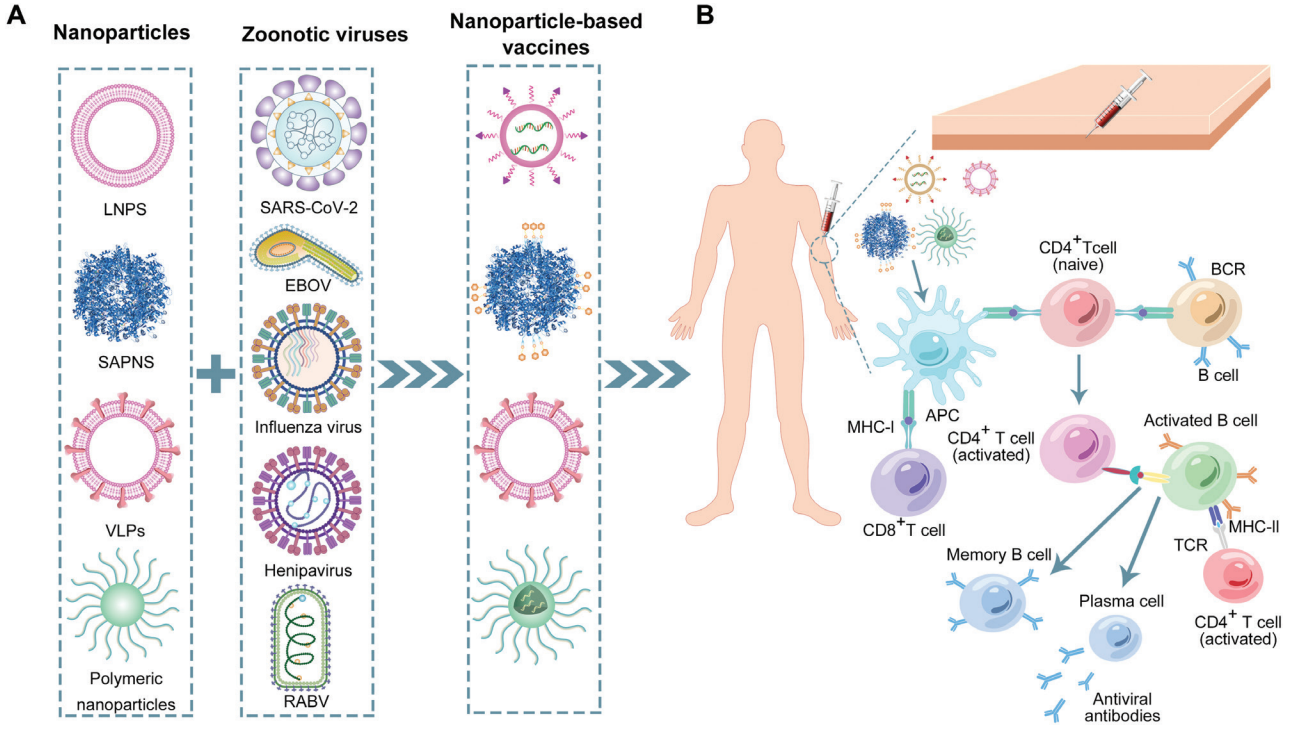

FIGURE 1 | Nanoparticle-based vaccine platform against zoonotic viruses. (A) Schematic view of different nanoparticles used as antigen carriers for vaccination against zoonotic viruses. (B) Nanoparticle vaccines were administered and processed by local cells or APCs. The antigenic cargo was then presented by MHC-I and MHC-II, leading to $CD8^+$ cytotoxic T cells or $CD4^+$ T helper cell activation and cell-mediated immunity. Further activation of B cells mediated humoral immunity. MHC: major histocompatibility complex. TCR: T cell receptor. BCR: B cell receptor.

TABLE 1 | Formulations and properties of nanoparticle-based vaccines.

Platforms	VLPs	SAPNs	LNPs	PLGA
Size (nm)	20-200	12-70	~100	~300
Formulations	VLPs are multiprotein nanoparticles that resemble the structures of viruses and are devoid of any viral genes.	SAPNs are nanosized particles generated from the polymerization of identically folded monomer subunits which are derived from oligomerization domains and antigens joined by a short linker.	LNPs for the encapsulation of nucleic acids for production of DNA/mRNA vaccines usually consist of four main components: an ionizable lipid; a sterol; a polyethylene glycol lipid (PEG lipid); and a neutral phospholipid.	PLGA nanoparticles is a copolymer of polylactides and polyglycolides and their hydrolysis leads to metabolite monomers, lactic acid, and glycolic acid.
Advantages	1. Resemble the actual structure of a virus, which elicits a strong humoral and cellular immune response 2. Cannot be amplified in the body and are thus safe 3. Do not need freezing conditions	1. Self-assembly enables retention of multiple antigenic units within a single assembly structure 2. Their sizes enhance lymph node trafficking and delivery of antigenic portions 3. Do not need freezing conditions	1. LNPs are easier to manufacture and are less immunogenic 2. Can carry larger payloads 3. Their ability to co-deliver both antigen and adjuvant to the same antigen presenting cell	1. PLGA nanoparticles have high biodegradability and biocompatibility 2. Can protect the antigens from degradation and clearance 3. Allow for co-encapsulation of antigens and adjuvants 4. Can be targeted to antigen presenting cells

have a role as an immunostimulant to enhance immunity. For example, aluminum oxyhydroxide nanoparticles, PLGA, and polystyrene nanoparticles have been shown to induce NLRP3-associated inflammasome activation, which results in production of interleukins (ILs) as downstream signaling events [10,11].

VLPs

VLPs are multiprotein nanoparticles that are assembled from virus-derived structural proteins and are devoid of any viral genes. VLPs often imitate the organization and conformation of authentic viruses, which are naturally optimized for interaction with the immune system [5]. The nanoparticle size and highly repetitive structure suggest that VLPs tend to induce potent innate and adaptive immune responses, even in the absence of an adjuvant [12]. In addition, VLPs contain functional viral proteins responsible for virus entry into the host cell, which ensures tissue-specific targeting and evokes immunity by VLPs.

VLPs are derived from a variety of viruses with sizes most ranging from 20–200 nm. It has been shown that structural proteins, such as virus capsids, envelopes, and sometimes core viral proteins, have been used to produce VLPs. VLPs can be generated using recombinant viral proteins that are obtained from several expression systems, including prokaryotic cells, insect cells, mammalian cells, yeast, plants, and cell-free systems [13].

VLPs present antigenic epitopes in the required orientation, which mimics the overall structure of the corresponding native viruses. Therefore, this highly repetitive display of antigens leads to B cell receptor crosslinking and activation. Like native viruses, VLPs are phagocytosed and processed in antigen-presenting DCs, then are transported to lymph nodes for T cell priming and induction of cell-mediated immunity. Therefore, VLPs have been shown to be highly immunogenic and have recently attracted attention due to their use as novel vaccines. Some VLP vaccines have been licensed and commercialized. Prophylactic human vaccines based on VLPs that have been licensed for use protect against hepatitis B virus and human papilloma virus infections [12].

SAPNs

SAPNs are nanosized particles generated from the polymerization of identically folded protein monomers, approximately 12-70 nm in size [14]. Generally, each SAPN monomer subunit is derived from oligomerization domains and antigens joined by a short linker. At the terminus of these protein chains, antigen epitopes can be conveniently displayed on the surface or encapsulated into the core of the nanoparticles. Therefore, monomers spontaneously self-assemble into well-ordered three-dimensional nanoparticles consisting of multiple copies of the antigens.

Nanoparticles can serve two distinct functions through encapsulation and conjugation with antigens: delivery platforms; and adjuvants. SAPNs are much better than soluble antigens in inducing an immune response. The high density and structurally ordered antigenic array in the germinal centers can engage multiple B cell receptors, which transforms resting B cells by delivering strong survival and proliferation signals. Other processes account for promotion of long-lived plasma cell differentiation and a stronger interaction with follicular Th cells, leading to higher levels and a longer duration of immunity. Some approved conventional vaccines mount antigen-specific humoral immune responses but poor cellular immune responses. Thus, conventional vaccines are administered in combination with adjuvants to enhance cell-mediated immunity. In contrast, SAPNs activate immune responses without conventional adjuvants. For example, it was demonstrated that protective T cell responses can be induced via APC interactions without the use of an

adjuvant using SAPNs, such as P22 nanoparticles. Two antigenic proteins of respiratory syncytial virus (RSV [matrix and matrix 2 proteins]) were co-encapsulated within P22. The resulting P22-M/M2 nanoparticle containing two antigens generated potent $CD8^+$ and $CD4^+$ T cell responses and decreased lung viral titers following RSV challenge [15].

A ferritin nanoparticle is a spherical shell of protein that self-assembles from 24 equivalent polypeptide subunits with remarkable biocompatibilities, and thermal and chemical stabilities. Hence, ferritin nanoparticles are well-suited to carry and expose immunogens for vaccine design [16]. It has been shown that ferritin effectively displays the trimeric hemagglutinin protein of influenza virus [17] and the glycoprotein 350 receptor-binding domain of the envelope protein from Epstein-Barr virus [18]. These nanoparticle vaccines efficiently induce neutralizing antibodies in mice and non-human primates. Receptor-binding domain (RBD) or spike protein-based vaccine candidates against SARS-CoV-2 using self-assembling ferritin nanoparticles have been reported [19,20]. SARS-CoV-2 spike ferritin nanoparticle vaccines elicit potent $CD4^+$ T cell helper responses and neutralizing antibodies against SARS-CoV-2 wild-type and mutant strains, as well as SARS-CoV-1.

Our group devised a novel nanoparticle vaccine (designated as 3M2e-rHF) by displaying the influenza virus 3 sequential repeats of M2e (3M2e) on the surface of self-assembled human ferritin heavy chain (rHF) nanoparticles [21] (Fig 2). Indeed, 3M2e-rHF nanoparticles are capable of driving potent immune responses in animals when intranasally vaccinated in the absence of an adjuvant. Moreover, 3M2e-rHF nanoparticles confer complete protection against lethal dose challenges of homo-subtype H1N1 and hetero-subtype H9N2 viruses. We further developed an intranasal nanoparticle vaccine (designated as HMNF) that displays three highly conserved influenza epitopes (HA A α-helix, M2e, and NA HCA-2) on the surface of ferritin nanoparticles. The nanoparticle vaccine, HMNF, induces highly potent and long-lasting immunity and confers broad protection against the lethal challenge of divergent influenza A and B viruses [22]. In a corollary study we constructed a ferritin-based self-assembling nanoparticle vaccine that provided complete protection against Zika virus (ZIKV) infection. The ZIKV envelope protein domain III (zEDIII) was displayed on the surface of a ferritin cage to form the zEDIII-rHF nanoparticle. The zEDIII-rHF nanoparticle vaccine showed sufficient protective efficacy without antibody-dependent enhancement (ADE) and provided a safe and potent vaccine candidate against ZIKV [23].

Recently, non-natural self-assembling proteins based on cutting-edge technologies, such as artificial intelligence (AI) design, have been widely reported [24]. The extensive data collected in recent decades on protein sequence, structure, and function have enabled the emergence of AI-based nanoparticle vaccine design approaches [25]. For example, instead of relying on molecules that naturally form nanoparticles, Marcandalli et al. [26,27] reported the computational methods for designing novel self-assembling proteins with atomic-level accuracy. Marcandalli et al. [26,27] showed that among computationally designed two-component protein nanomaterials, I53-50 is capable of scaffolding and stabilizing DS-Cav1, a prefusion-stabilized RSV F glycoprotein [28]. I53-50 is an artificial 2-component nanoparticle comprised of 12 I53-50B pentamers and 20 I53-50A trimers with strict icosahedral symmetry [29]. The resulting nanoparticle immunogens enhance DS-Cav1 immunogenicity and trigger potent RSV-neutralizing antibody responses. In addition, antigens, including human immunodeficiency virus envelope glycoprotein [30] and the SARS-CoV-2 receptor binding domain [31], have also been displayed on the surface of computationally designed SAPNs, resulting in potent neutralization and broad protection.

Lipid nanoparticles (LNPs)

LNPs are artificial delivery vehicles, approximately 100 nm in diameter, with at least 1 lipid bilayer. LNPs

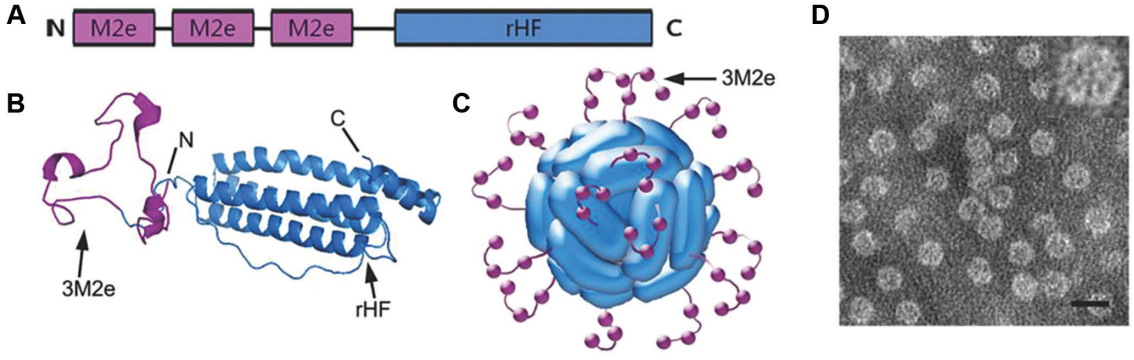

FIGURE 2 | Construction and characterization of 3M2e-rHF nanoparticles. (A) The design of the 3M2e-rHF fusion protein was as follows: three sequential repeats of M2e peptides were fused to the N-terminus of human ferritin heavy chain (rHF). (B) 3D illustration of the structure of a 3M2e-rHF monomer. (C) Diagrammatic scheme of a 3M2e-rHF nanoparticle. (D) Transmission electron microscopy image of 3M2e-rHF cages. Scale bar = 20 nm. Adapted with permission Qi et al. 2018; copyright 2018 Wiley.

typically consist of four major elements: an ionizable lipid; cholesterol; PEG-lipid; and a helper lipid, such as distearoylphosphatidylcholine. LNPs encapsulate multiple cargo molecules with hydrophilic and hydrophobic properties in aqueous and non-aqueous phases of their vesicles [32]. The liposomal formulations of negatively charged nucleic acids are prepared through a rapid co-precipitation method and a self-assembly process at low pH, with cationic lipids and nucleic acids interspersed throughout the bilayer [33].

LNPs have been shown to protect nucleic acids against degradation and allow long-term delivery of nucleic acids. Moreover, LNPs improve the release efficacy of mRNA and DNA by rationally designing and modulating lipids to boost cellular uptake, as well as improve endosomal escape capabilities after endocytosis [34]. A dozen cationic liposome formulations, especially those based on 1,2-dioleoyl-3-trimethylammonium propane, have been proposed with the potential to act as safe and effective vaccine adjuvants [35]. LNPs have also been shown to affect the immunostimulatory activity of mRNA vaccines and act as an adjuvant component of modified mRNA vaccines. Mechanistically, the capacity of the LNP–mRNA nanoparticle formulations to induce robust Tfh and GC B cell responses is mainly dependent on the presence of the ionizable lipid component and induction of the cytokine, IL-6 [36].

LNPs have been shown to function as a RNA or DNA vaccine delivery system. Until now, nearly all SARS-CoV-2 mRNA vaccines receiving clinical approval employ LNPs. LNPs offer multiple advantages for mRNA vaccine delivery, including ease of production, modularity, and biocompatibility to large mRNA payload capacity. The nucleoside-modified mRNA vaccines (BNT162b2 and mRNA-1273) generally exceed the titers from COVID-19 convalescent plasma samples [20]. Moreover, both BNT162b2 and mRNA-1273 induce remarkable S-specific $CD4^+$ T and $CD8^+$ T cell responses [37]. With respect to DNA vaccines, LNPs have been used to formulate pDNAs encoding *Leishmania major* cysteine proteinase (cp). Mice vaccinated with LNPs-pcDNA-cpa/b/c exhibit development of significantly higher protective Th1 immune responses [38].

Polymeric nanoparticles

Polymeric nanoparticles can be generated via the polymerization of monomeric units or preformed polymer chains. The former bottom-up methods involved synthesizing polymeric nanoparticles through particle nucleation during polymerization reactions of monomeric/oligomeric precursors. In contrast, the latter top-down processing relied on reduction of macroscopic materials, such as preformed polymers in solutions into polymer nanoparticles.

Overall, polymeric nanoparticles have numerous superior properties, including high biocompatibility and stability, high antigen encapsulation, controlled release of antigens, and intracellular persistence in APCs [39]. For example, poly(lactic-co-glycolic acid) [PLGA] is a biocompatible and biodegradable polymer that is used in medical applications. PLGA nanoparticles can be prepared to encapsulate antigens and release the antigens in a sustained manner under physiologic conditions, which is essential for mucosal vaccination [40]. It has been shown that oral administration of PLGA nanoparticles confers a simple, non-invasive vaccination strategy that is effective against viral infections occurring via rectal or vaginal mucosa invasion [41]. Moreover, PLGA nanoparticles promote antigen recognition and uptake by APCs and facilitate antigen processing for MHC presentation [42]. Immunization with PLGA nanoparticle-encapsulated inactivated/killed porcine reproductive and respiratory syndrome virus antigen (NPs-KAg) promoted the activation of natural killer and T-cells. NPs-KAg vaccine also induced a higher frequency of $CD8^+$ T cells and greater secretion of interferon compared to control KAg-vaccinated animals [43].

NANOPARTICLE VACCINES AGAINST ZOONOTIC VIRAL INFECTIONS

Zoonotic viruses have caused the majority of severe epidemics and pose the greatest threat of a worldwide calamity due to the high rate of transmission, rapid evolution, and lack of therapeutic choices. Given deforestation, agricultural intensification, and high human-wildlife contact, the rate of zoonotic viral spillover and disease emergence is increasing [44]. There are > 250 known zoonotic viruses, among which several viral agents, including Ebola virus, SARS-CoV-2, Henipavirus, and avian influenza virus, could cause severe diseases in people [45]. Nanoparticle-based immunization strategies hold promise as ideal vaccines against these zoonotic viral infections.

SARS-CoV-2

The SARS-CoV-2 pandemic and its unprecedented global social and economic disruptive impact has highlighted the transmission of a highly pathogenic zoonotic virus into the human population from other species [46]. SARS-CoV-2 rapidly spread among the human population after a likely spillover from bats or unknown intermediate hosts [47]. As of March 2023, > 676 million confirmed cases have been reported in > 200 countries, leading to > 6.8 million deaths.

The BNT162b2 (Pfizer-BioNTech, Kronach, Germany) and mRNA-1273 (Moderna, Inc., Cambridge, MA, USA) are LNP-encapsulated, mRNA-based vaccines that encode the prefusion stabilized full-length spike protein of SARS-CoV-2. The first vaccine given emergency use authorization from the Food and Drug Administration, BNT162b2, had 95% efficacy [48]. The second mRNA-LNP vaccine, mRNA-1273, had 94.1% efficacy [49]. Both the BNT162b2 and mRNA-1273 vaccines begin to protect recipients as early as 10 days after the first dose, with maximum protection after a second dose. Until now, the mRNA-LNP vaccines have contributed

significantly to mitigating the negative impact of the SARS-CoV-2 pandemic. Indeed, mRNA-LNP technology has the potential to change vaccine design for future viral outbreaks [50].

SAPNs displaying a high density of antigen on a repetitive array promote various immunologic processes, such as B cell activation, transport to lymph nodes, and retention on follicular DCs. For example, nanoparticle vaccines were developed by covalently coupling the RBD and/or heptad repeat (HR) subunits of SARS-CoV-2 to the surface of self-assembled 24-mer ferritin. RBD and RBD-HR nanoparticle vaccines triggered more robust neutralizing antibodies and cell-mediated immunity compared to monomer vaccines. Nanoparticle vaccines have been shown to significantly decrease lung virus loads and protect against SARS-CoV-2 infection in animals [51].

Two-component 120-subunit icosahedral nanoparticles (I53-50) have been used to present stabilized prefusion SARS-CoV-2 S proteins. The SARS-CoV-2 S-I53-50 nanoparticle vaccine induces strong humoral responses in mice, rabbits, and cynomolgus macaques. The vaccine-induced immunity protects macaques from a high-dose challenge, resulting in decreased viral loads in the upper and lower respiratory tracts and fewer lung lesions when compared to non-vaccinated animals [52]. Two-component protein nanoparticles (I53-50) multivalently display different SARS-CoV-2 RBD epitopes (Fig 3) and induce potent and protective neutralizing antibody responses in mice. Antibodies elicited by the nanoparticle vaccine target multiple distinct RBD epitopes, indicating that the two-component protein nanoparticles may not be easily affected by escape mutations [31].

SARS-CoV-2 VLP nanoparticles that incorporate the four structural proteins of the virus were generated using a mammalian expression system [53]. VLPs expressing the stabilized prefusion spike antigen adsorbed with alum adjuvant elicited high titer anti-S, anti-RBD, anti-N IgG, as well as multifunctional Th1-biased T-cell responses. High-dose vaccine reduced both acute lung injury and virus load upon challenge. Another study showed that a novel VLP-based vaccine candidate against SARS-CoV-2 was developed by displaying viral receptor-binding motif (RBM) on the surface of cucumber-mosaic VLPs [54]. It has been demonstrated that the vaccine candidate, mCuMVTT-RBM, is highly immunogenic and can efficiently induce high levels of viral neutralizing antibody response. The induced antibodies also cross-recognize mutant RBD variants of concern.

Zoonotic influenza viruses

Influenza is an acute respiratory illness caused by influenza viruses (IVs). There are four genera of IVs based on genetic variations of the nucleoprotein gene: influenza A viruses (IAVs); influenza B viruses (IBVs); influenza C viruses (ICVs); and influenza D viruses (IDVs). IAV and IBV are both responsible for severe epidemics in the human population. Human infections with ICV usually cause relatively mild diseases without reported epidemics. IDV mostly infects cattle and does not cause clinical disease in humans [55]. IVs are responsible for an estimated 290,000–650,000 deaths each year despite the availability of an inactivated vaccine [56]. The H3, H5, H7, and H9 subtypes of IAV, such as the swine H3N2 variant and avian H5N1 and H7N9 viruses, have enormous potential for zoonotic diseases because of the ability to infect avian and swine, from which they can be spread to humans and initiate a pandemic [57].

Avian-origin H7N9 VLPs have been generated from the full-length HA, NA, and matrix M1 genes in insect Sf9 cells and mammalian HEK293 suspension cells. Purified

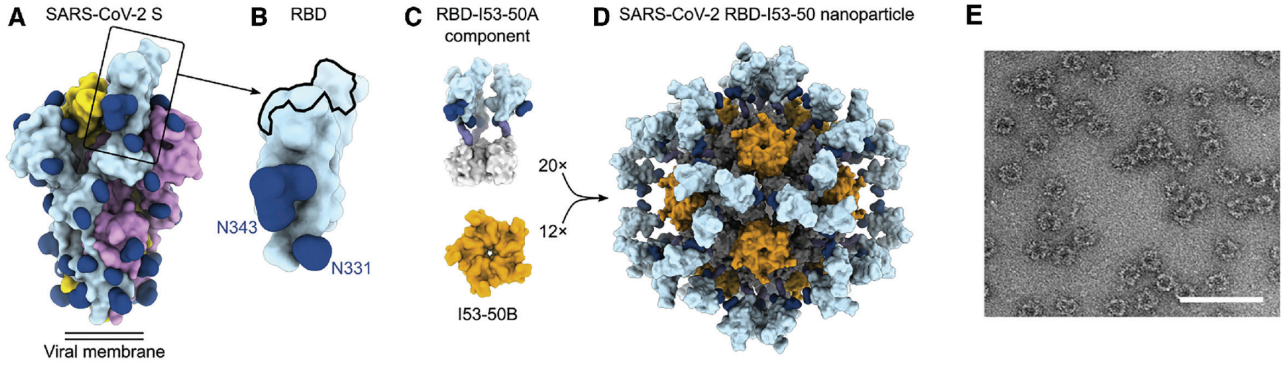

FIGURE 3 | Design and *in vitro* assembly of SARS-CoV-2 RBD nanoparticle immunogens. (A) Molecular surface representation of the SARS-CoV-2 S-2P trimer in the prefusion conformation (PDB 6VYB). Each protomer is distinctly colored. N-linked glycans are rendered dark blue (the glycan at position N343 was modeled based on PDB 6WPS). A single open RBD is boxed. (B) Molecular surface representation of the SARS-CoV-2 S RBD, including the N-linked glycans at positions 331 and 343. The ACE2 receptor-binding site or RBM is indicated with a black outline. (C) Structural models of the trimeric RBD-I53-50A (RBD in light blue and I53-50A in light gray) and pentameric I53-50B (orange) components. Upon mixing *in vitro*, 20 trimeric and 12 pentameric components assembled to form nanoparticle immunogens with icosahedral symmetry. Each nanoparticle displays 60 copies of the RBD. (D) Structural model of the RBD-12GS-I53-50 nanoparticle immunogen. (E) Representative electron micrograph of negatively stained RBD-12GS-I53-50 nanoparticles. Scale bars, 100 nm. Adapted with permission from Walls et al. 2020; copyright 2020 Elsevier.

recombinant H7N9 VLPs are similar to the influenza virion in morphology and induce high-titer neutralizing antibodies. After challenge, VLP vaccine elicits a robust humoral response and results in protective responses with a significant reduction in clinical symptoms among animals [58]. In a phase I clinical trial involving the experimental H7N9 VLP vaccine, participants received two identical doses. High titers of N9 neuraminidase-inhibiting antibodies were observed in up to 71.9% of recipients of the vaccine without adjuvant. The vaccines under investigation could be available for human use within 3 months after the HA and NA sequences had been obtained [59].

Influenza vaccines that confer broad and long-lasting protection against divergent viral strains and subtypes would have a significant impact on global health. Computationally designed two-component nanoparticles were constructed from multiple copies of two distinct protein building blocks to generate nanoparticle immunogens that co-displayed diverse influenza HA trimmers [60]. Nanoparticle immunogens that co-displayed the four haemagglutinins of commercial quadrivalent influenza vaccines induced antibody responses against vaccine-matched strains. The induced immunity was comparable to or better than licensed quadrivalent influenza vaccines in animals. The nanoparticle immunogens simultaneously induced a wide range of protective antibody responses to heterologous zoonotic H5 and H7 by targeting the conserved haemagglutinin stem epitope.

The highly conserved influenza virus hemagglutinin (HA) stalk is a potential target for a universal influenza virus vaccine against zoonotic subtypes. Nucleoside-modified mRNA encoding full-length influenza virus HA was formulated into LNPs [61]. It was shown that HA mRNA-LNPs induce effective antibody responses targeting the conserved HA stalk domain. The HA stalk-specific antibody response is relevant to protection from homologous H1, but also heterosubtypic H5 influenza virus infection in mice. Another study used nucleoside-modified mRNA vaccine encapsulated by LNPs to deliver a combination of conserved influenza virus antigens (hemagglutinin stalk, neuraminidase, matrix-2 ion channel, and nucleoprotein) [62]. This mRNA-LNP vaccine triggers strong immune responses with substantial potency and offers protection against heterologous and heterosubtypic strains, such as avian H5N8 and H9N2, in mice.

Ebola virus

Ebola virus (EBOV), a type of the *Ebolavirus* genus in the *Filoviridae* family, causes a zoonotic disease referred to as viral hemorrhagic fever with a high mortality rate [63]. Ebola virus disease is prevalent in some countries in West and Central Africa. Between 2014 and 2016, West Africa experienced the most severe Ebola outbreak to date that claimed a death toll of 11,325 [64].

EBOV GP is a trimer of GP1-GP2 heterodimers responsible for virus entry into host cells. EBOV GP can be recognized by neutralizing antibodies and is the major target for vaccine design. The well-designed GP trimers have been displayed on re-engineered protein nanoparticles, which encapsulate a layer of locking domains and a cluster of helper T-cell epitopes. GP trimers and nanoparticles trigger cross-ebolavirus neutralizing antibodies in mice and rabbits. This study reported on multilayered SAPNs for the development of a vaccine that is applicable to other filoviruses [65].

Ebola DNA formulation is incorporated into PLGA—poly-l-lysine/poly-γ-glutamic acid (PLGA-PLL/γPGA) nanoparticles delivered using a microneedle (MN) patch. Preparation with PLGA-PLL/γPGA nanoparticles enhances stability on Ebola DNA, and the DNA vaccine can be produced at low cost. The Ebola DNA formulation and PLGA-PLL/γPGA nanoparticle combination and administration using MN patches elicited potent immune responses *in vivo*. The preclinical data indicated the safety and immunogenicity of this nanoparticle vaccine [66].

LNPs-encapsulated, non-replicating EBOV mRNA vaccines encoding full-length GP have been developed and examined in a guinea pig model [67]. This mRNA-based non-replicating EBOV vaccine not only induced virus-specific IgG and neutralizing antibody responses, but also successfully protected guinea pigs from EBOV disease. Self-amplifying RNAs (saRNAs) encoding EBOV GP, NP, or a combination of both can also be delivered by an LNP formulation [68]. Vaccination of mice with LNP-formulated GP and NP saRNAs induced antigen-specific immune responses. Moreover, cell-mediated immunity, such as the $CD4^+$ T cell response against GP and the $CD8^+$ T cell response against NP can be detected using ELISpot assay. The NP and GP saRNA combination protected mice against lethal EBOV infection.

Henipavirus

Hendra virus (HeV) and Nipah virus (NiV) are zoonotic viruses and members of the genus *Henipavirus*, family *Paramyxoviridae* which cause lethal respiratory illness and encephalitis in humans [69,70]. The mortality rates of Henipavirus-mediated disease up to 60% and 90% for HeV and NiV, respectively. NiV and HeV infect various kinds of wild animals and livestock as well as humans. The main natural reservoir of Henipavirus is the Pterosaur bat, which does not exhibit clinical disease [71]. Human-to-human dissemination is frequently reported, most commonly between patients and hospital staff or family caregivers. Currently, there are no licensed vaccines or antiviral therapeutics available for Henipavirus infections [72].

The efficacy of a single-dose of an LNP-encapsulated mRNA vaccine encoding the te soluble Hendra virus glycoprotein (sHeVG mRNA LNP) against NiV challenge was evaluated in Syrian hamsters. The sHeVG mRNA LNP vaccine protected up to 70% of animals from lethal NiV challenge, even though animals had suboptimal immune responses before challenge. This study evaluated

the mRNA LNP platform in hamsters for the first time and demonstrated partial protective efficacy. This study supports the further development of this mRNA LNP vaccine platform and provides a basis for optimizing mRNA vaccines against NiV and other highly pathogenic viruses [73].

To focus on how to quickly provide optimized Henipavirus immunogens, mRNA encoding pre-fusion/glycoprotein (pre-F/G) chimera formulated in LNPs (mRNA-LNPs) was evaluated. Compared with glycoprotein alone, this mRNA vaccine triggered a strong neutralizing antibody response and a superior NiV-specific $CD4^+$ T follicular helper cell and other effector T cell response. Combining a rational immunogen design with the mRNA vaccine platform promoted the selection of an F/G chimeric candidate for clinical development. This research showed how the structure-guided antigen design can be combined with the mRNA-LNP vaccine platform to achieve a rapid response to the threat of a future Henipavirus pandemic [74].

Rabies virus

Rabies virus (RABV) is a member of the genus *Lyssavirus* within the *Rhabdoviridae* family. RABV is the causative agent of a lethal zoonotic disease which lead to > 60,000 human deaths annually [75]. RABV has a 12-kb genome encoding 5 viral proteins: nucleocapsid protein (N); phosphoprotein (P); matrix protein (M); glycoprotein (G); and polymerase protein (L). The RABV G protein is responsible for attachment and penetration into the host cell and is the main antigen that gives rise to and reacts with neutralizing antibody.

RABV vaccines are among the oldest antiviral interventions. Current regimens of RABV vaccines are costly and require multiple doses to achieve high neutralizing titers [76]. Given that rabies continues to be a problem in large parts of the world, novel innovations are needed to control rabies disease. A proof-of-concept of RABV-G mRNA for human vaccines in clinical trial mRNA was demonstrated with the cationic protein protamine [77]. Further preclinical studies have shown that formulation of the mRNA in LNPs protects the mRNA from degradation and enhances the immune response in animal models. It was found that a new type of mRNA-LNP formulation containing the RABV-G mRNA antigen elicits immune responses in non-human primates comparable to those induced by licensed vaccines [78]. In an ongoing phase I clinical study, the safety and immunogenic potential of this novel vaccine candidate was further evaluated in adult volunteers. RABV-G mRNA in LNPs was highly immunogenic and two low doses (1 or 2 μg) of mRNA-LNP induced immunity acceptable by WHO standards [79].

CONCLUSION REMARKS

Quick emergence of zoonotic diseases, such as EBOV and SARS-CoV-2, poses a challenge for traditional vaccines, such as live attenuated viral vaccines and inactivated/killed viral vaccines. Traditional vaccine manufacturing is time-consuming. The development pathways of traditional vaccines, such as isolation and culturing of virus, developing seed virus, inactivation or recombination, testing, scaling up, and mass production, may take on average > 10 years [80]. In comparison, nanoparticle vaccine platforms do not need isolation and culturing of virus and mainly rely on rapid antigen design and ease of manufacture [81]. By applying nanoparticle-based technologies, such as mRNA LNP or VLP, we can prepare for zoonotic viral diseases by investing in innovative rapid response platforms, which could move from sequences to clinical trials within weeks rather than months or years. For example, two SARS-CoV-2 vaccines have been rapidly developed using mRNA technology (Pfizer–BioNTech and Moderna), both of which were safe and highly effective.

Of note, nanoparticle vaccines have aroused great interest and various nanoparticles have been developed and employed as delivery carriers or immune modulators. Encapsulating antigens in nanoparticles can prevent degradation and improve stability. Multivalent display of antigens on the surface of individual nanoparticles allows B-cell receptor cross-linking, thereby enhancing immune responses. In addition, nanoparticles loaded together with antigens and immune stimulants can target draining lymph nodes and maintain slow antigen release over a period of time.

Nevertheless, the application of nanoparticle vaccines is still at an early phase of development and many challenges remain to be solved. It is difficult to reproducibly synthesize stable nanoparticles with a consistent and ideal performance. There is a lack of basic data regarding the *in vivo* behavior of nanoparticle vaccines, which can play a role as either delivery system for antigens and/or as an immunostimulant adjuvant. We still do not have a fundamental understanding of how the chemical and physical characteristics of nanoparticles affect their biodistribution and uptake. Therefore, the interaction of nanoparticles with the immune system has not been established. The preparation methods of nanoparticle vaccines should be improved to be more suitable for scale-up production and low-cost storage in the future. Rational design of optimally stabilized nanoparticle vaccine formulations during transport and storage could lower the cost and facilitate more rapid worldwide supply of nanoparticle vaccines. In addition, nanoparticle vaccines that deliver antigens to specific APCs has become an attractive strategy not only to increase selectivity but also to minimize side effects [82]. Through surface modification, structural design, and composition modulation, nanoparticles can be tailored to target immune cells and reduce the accumulation of cargo in non-targeted organs, further enhancing immune efficacy and safety [83].

Although the current nanoparticle vaccines still need improvement, the nanoparticle vaccines hold great potential in optimizing vaccines against infectious diseases.

Thus, the development of safe and effective nanoparticle vaccines with simple structure and ease of manufacture is a promising method to curb zoonotic viral diseases in the future.

ACKNOWLEDGMENTS

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDB29050201); Key Research and Development Program, Ministry of Science and Technology of China (No. 2022YFC2303502); National Natural Science Foundation of China (No. 31925025, 22293033, and 32271459).

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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