Review:

Nanomaterial for Adjuvants Vaccine: Practical Applications and Prospects

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Abstract: *Vaccines contain adjuvants to strengthen the immune responses of the receiver against pathogen infection or malignancy. A new generation of adjuvants is being developed to give more robust antigen-specific responses, specific types of immune responses, and a high margin of safety. By changing the physical and chemical properties of nanomaterials, it is possible to make antigen-delivery systems with high bioavailability, controlled and sustained release patterns, and the ability to target and image. Nanomaterials can modulate the immune system so that cellular and humoral immune responses more closely resemble those desired. The use of nanoparticles as adjuvants is believed to significantly improve the immunological outcomes of vaccination because of the combination of their immunomodulatory and delivery effects. In this review, we discuss the recent developments in new adjuvants using nanomaterials. Based on three main vaccines, the subunit, DNA, and RNA vaccines, the possible ways that nanomaterials change the immune responses caused by vaccines, such as a charge on the surface or a change to the surface, and how they affect the immunological results have been studied. This study aims to provide succinct information on the use of nanomaterials for COVID-19 vaccines and possible new applications.*

Keywords: *nanomaterials; adjuvants vaccine; subunit vaccine; DNA vaccine; RNA vaccine; Covid-19 vaccine*

■ INTRODUCTION

The issues for vaccination are similar across many diseases that exist today, making nanomaterials a special challenge to solve some of these challenges [1]. One major issue is that the immunological response to infection does not usually lead to protective immunity,

unlike other vaccine-preventable diseases like measles or chickenpox, which typically do [2-3]. Effective disease vaccine techniques need to elicit immune reactions that are distinct from those seen during a real infection [4]. The fact that nanomaterials are made in a lab, that their structures are clear and can be changed, and that engineering design principles are becoming more obvious to make such vaccines. Nanomaterials' synthetic makeup may also have benefits in this way [5-6]. Additionally, the versatility and adaptability of vaccines made from synthetic and nanomaterials have benefits.

Since the last decade of the 20th century, nanotechnology has been a field that is fast developing [5,7-8]. To influence the performance of nanomaterials in biomedical applications, substantial advancements have been made in their nanoscale design and manipulation [9-11]. The parameters of high bioavailability, sustained, and regulated release patterns, imaging, targeting, and other factors can be met by the delivery of a variety of substances, including chemical medicines, proteins, and vaccinations. Nanomaterial-based delivery of antigens can be preserved from deterioration, released sustainably, and more effectively absorbed by antigen-presenting cells (APCs). The intrinsic governing action of nanoparticles in humoral and cellular immunological reactions has also been demonstrated in many investigations [12]. As a result, integrating nanoparticles' transport and immunomodulatory activities in adjuvant vaccine applications will significantly improve the immunological results of vaccination.

Adjuvant vaccines have been frequently used to boost the intensity of a defense mechanism induced by vaccination against an antigen. A vaccine's adjuvant can boost, direct, and speed the defense mechanism to the most potent form of each infection or cancer. As the importance of adjuvants in vaccines becomes more well recognized, the demand for novel adjuvants is urgent to address unmet clinical needs [13]. A new generation's expectations of vaccination adjuvants are centered on the development of complex and widening immune responses while maintaining safety, as well as improved T-cell responses of desired types [14]. The rational design and fabrication of new adjuvants that have the requisite efficacy and safety are becoming conceivable with the expanding developments in material science and nanotechnology [15-16].

In this review, we discuss recent developments in the creation of cutting-edge vaccination adjuvants thanks to nanotechnology. We will talk about the potential ways in which nanomaterials affect immune reactions to various vaccinations, including subunit, DNA, and RNA vaccines, and the immunological effects that arise. This review seeks to offer concise information to encourage fresh perspectives for the creation of innovative vaccination adjuvants.

■ FUNDAMENTAL OF NANOMATERIALS FOR ADJUVANTS VACCINE

Adjuvants are added to vaccines to boost the immunological responses specific to the recipient against a pathogen infection or malignancy. To address the need for stronger antigen-specific reactions, immune responses of particular types, and a high safety margin, a new generation of adjuvants is being created. The building blocks of specifically created nanoparticles work as a multipurpose integration stage for appropriate adjuvant activities. By changing the physical and chemical properties of nanomaterials, it is possible to create antigen delivery systems with long-lasting and controlled release patterns, bioavailability, imaging, and targeting. Also, the immune-regulating properties of certain nanomaterials can be used to boost and shape cellular and humoral immune responses (Fig. 1) in helpful ways. It is believed that the combination of nanomaterials' immunomodulatory and delivery functions as adjuvants greatly enhances the immunological consequences of vaccination [15].

While mRNA vaccinations do not integrate and do not run the risk of insertional mutagenesis, DNA vaccines are more stable than mRNA vaccines. Additionally, mRNA's stability, immunogenicity, and half-life can all be adjusted through recognized changes. By transporting the vaccine to the proper cellular populations and subcellular regions, nanotechnology-based techniques provide answers to the difficulty of delivery. Even though synthetic carriers like cationic liposomes and polymeric

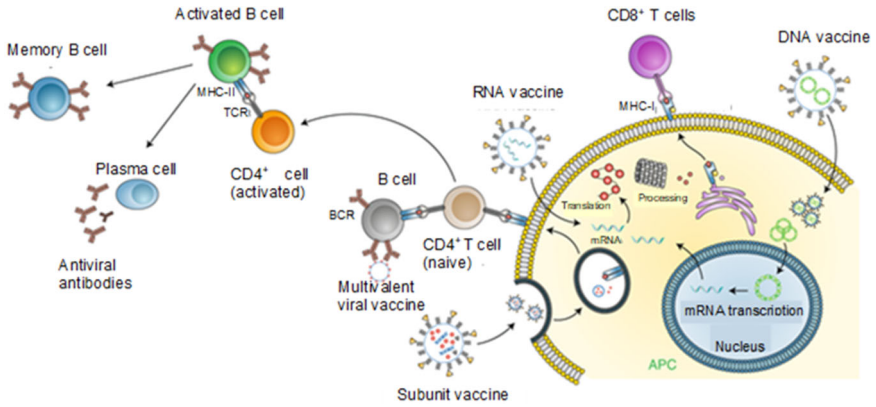


Fig 1. Key phases in the production of vaccines based on nanoparticles and immunological responses. The APC processes the antigenic cargo, and MHC-I and MHC-II present epitopes, resulting in the creation of $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells necessary for the production of antiviral antibodies. Reproduced with permission from [17]. Copyright 2020, Nature

nanoparticles (NPs) have been used to cross cell membranes in DNA vaccinations, targeted formulations may help the plasmid DNA move into the nucleus. To improve the distribution and stability of mRNA-based vaccines, nanotechnology platforms such as liposomes, cationic nano-emulsions, dendrimers, or polysaccharide NPs have been used [17].

One of the most important medical treatments in the struggle against infectious diseases is vaccination. Smallpox, diphtheria, hepatitis, malaria, and influenza are only a few of the infectious illnesses whose global transmission has been eradicated thanks to vaccinations. In general, vaccines should deliver antigens and modulate immunity. Adjuvants have a firm ground in the fight against the virus. In addition to adjuvants' ongoing use, NPs have emerged as promising adjuvant candidates because of their distinct qualities. Numerous common NPs [18], including lipids, gold [19], polymers [7], and silica [20-21], have been examined concerning their potential use as adjuvants in antiviral vaccines (Fig. 2). The difficulty of creating secure and efficient vaccinations, however, continues to be a barrier to treating many more infectious diseases. Although adjuvants come in a vast range of structural variations, they all serve the same purpose in vaccine constructs: to strengthen and extend an immune response. However, the possibility of negative consequences resulting from adjuvant use must be recognized and carefully managed.

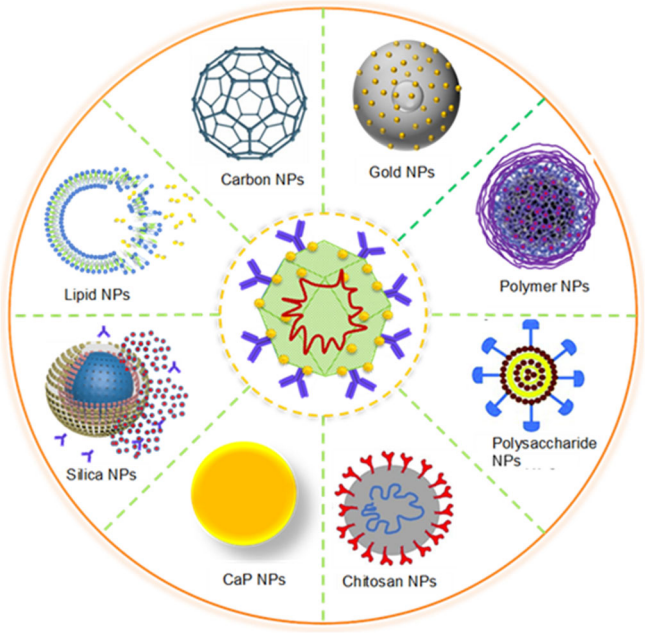


Fig 2. Several common nanoparticles could be used as vaccine adjuvants to shield people against viral diseases

Adjuvants provide greater options for enhancing vaccine development, and the significance of immune stimulants promotes the creation of new and more potent adjuvants [22-24].

Adjuvants are chemicals that, by simulating the actual illness, improve the immune response to vaccination. The immunizations aid in improved functioning and provide more durable protection [25]. The following are some popular adjuvants used in U.S.-

licensed vaccines. Aluminum salts adjuvants are the most frequently used ones in vaccinations for humans. For more than 70 years, they have been used without risk in vaccinations against illnesses including diphtheria, tetanus, hepatitis, and HPV. By creating a depot at the injection site and turning on antigen-presenting cells, they boost the immune system.

AS01 B is a brand-new adjuvant that combines two ingredients: QS-21, a natural substance derived from the Chilean soapbark tree, and monophosphoryl lipid A (MPL), a derivative of a bacterial cell wall component. This adjuvant is used in the zoster vaccination (Shingrix) to protect elderly persons from developing shingles. AS04, another adjuvant that combines MPL and aluminum salt, is included in the Cervarix HPV vaccination to protect against cervical cancer and other HPV-related illnesses. Matrix-M TM formulation contains saponins and was made from the bark of the *Quillaja saponaria* tree. By creating NPs containing antigens and activating dendritic cells and T cells, it promotes both humoral and cellular immunity.

■ NANOMATERIAL FOR ADJUVANTS VACCINE

To create an effective defense against the antigen, a vaccination involves exposing the immune system to an antigen derived from a disease agent [26-27]. A potent protective immune response happens when the vaccinated person contact with a dangerous microbial agent [28-30]. Any vaccine's ideal qualities should be long-lasting immunity, absence of autoimmunity or hypersensitivity, simplicity of delivery, and easy storage. In addition, secure vaccine ingredients should be used, and the vaccine itself should not cause the disease condition [31-32].

Nanomaterial for Subunit Vaccine

The antigen in subunit vaccinations is purified rather than employing entire bacteria. Purified antigens, which travel via various carriers, can be toxoids, subcellular fragments, or surface molecules [33-34]. Depending on the antigen utilized, immune responses to subunit vaccination vary. Polysaccharide antigens typically cause a T-cell-independent response, while protein antigens typically trigger an adaptive immune response that is T-cell dependent [35-36]. Receivers' safety is a benefit of subunit vaccinations; however, subunit antigens have a low level of immunogenicity. By concentrating their distribution on lymph nodes and successfully exposing immune cells to antigens, nanoparticular delivery devices have been used to increase the immunocompetence of subunit vaccines [37]. Additionally, adding the right molecular adjuvants may make the antigens more potent at triggering an immune response [25,37-38].

Research efforts to create subunit vaccines that can produce a more widespread protective response by targeting specific parts of the virus have been motivated by the prospect of new influenza viruses. Subunit vaccines are safer even if they are less immunogenic than injections of live, attenuated, or inactivated entire cells. Thus, as we move closer to the era of modern immunizations, the demand for cutting-edge adjuvants that boost immunogenicity is on the rise. Additionally, the surface distribution, targeting, and display of the selected antigens by expert antigen-presenting cells are essential for the design and development of vaccines. Utilizing NPs is one way to boost immunogenicity since antigen-presenting cells can easily recognize them. They can act as carriers of the selected antigens as well as immunopotentiators [39].

Lam et al. [40] described a subunit vaccination that is based on the simultaneous delivery of the SARS-CoV-2 spike protein and the CpG adjuvant. The adjuvant and the antigen were encapsulated with a unique artificial cell membrane (ACM) and polymersomes to increase the immunogenicity of our formulation. ACM polymersomes are nanoscale vesicles that self-assemble and are constructed of 1,2-dioleoyl-3-trimethylammonium-propane and poly(butadiene)-b-poly. Dendritic cells of DC1 and DC2, which are essential for regulating the adaptive immune response, efficiently absorb ACM polymersomes as delivery vehicles. Strong, long-lasting neutralizing antibody titers are produced in C57BL/6 mice after receiving the formulation in two doses (Fig. 3). T helper type 1 cytokine is also produced by $CD4^+$ and $CD8^+$ T cells, and it has a working memory [40].

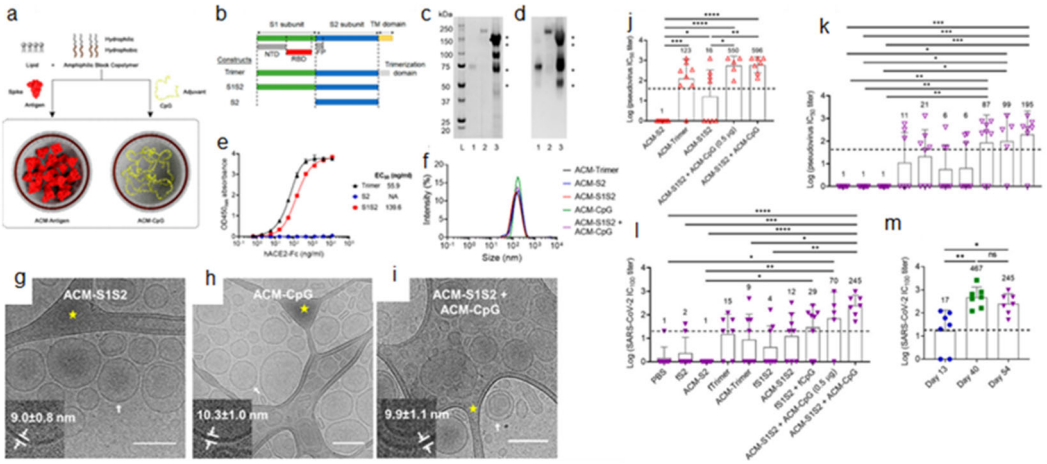


Fig 3. (a) ACM vaccine preparation schematic illustration, (b) a schematic illustrating the many forms of the spike protein; N-terminal region domain that binds to receptors, or transmembrane fusion peptide; (c) the total protein stain SYPRO Ruby, Precision Plus Protein Standards Lane L; (d) Western blot displaying S1S2 bands reacting to antibodies; I Trimer, S2, and S1S2 protein ACE2-binding curves. (f) Analysis of ACM-trimer, ACM-S1S2, ACM-S2, and ACM-CpG using dynamic light scattering. (g–i) ACM-CpG, ACM-S1s2, and a combination of ACM-S1S2 and ACM-CpG are shown in cryo-EM images to show the vesicular architecture. Lacy carbon can be found where a yellow star is present. (j) Day 28 sera from five important mice groups, following immunization with ACM-S1S2 + ACM-CpG, ACM-S1S2, ACM-Trimer, ACM-S2. These sera reflect the time point of peak response, 54th day IC_{50} pseudovirus neutralizing titers. (l) On day 54, IC_{100} live SARS-CoV-2 neutralizing titers. (m) Neutralizing titer kinetics in mice treated with ACM-CpG and ACM-S1S2. Reproduced with permission from [40]. Copyright 2021, ACS

Following vaccination, long-lasting protective immune responses are anticipated. The majority of vaccines cannot produce effective immunity on their own. To produce increased immune responses, adjuvants, and vaccinations must be administered together. *Mycobacterium bovis* antigens are assembled with biocompatible poly(4-vinylpyridine) as a template and an iron/molybdenum oxide cluster (2.5 nm) via molecular hydrogen bonding. At the molecular level, after inoculation in mic, "$Mo_{72}Fe_{30}$" and the antigens have been integrated, and their complete exposure to body fluid enhances both humoral and cellular immune responses to the vaccines. After two weeks, the anti-inflammatory factor IL-10 steadily rises, and by the fifth week, it returns to normal levels (Fig. 4). The balance between anti-inflammatory factors and pro-inflammatory cytokines indicates that the immune system may be activated by the antigens carried by supra-particles in the early stages of infection and release immediate inflammatory mediators for host defense and inhibitors of inflammatory mediators for immune defense [41].

Low efficacy exists for the dengue vaccination currently available, and there is a high risk of side effects, so other strategies are needed to provide a safer and more effective vaccination against the virus. As a dengue subunit vaccine, Quach et al. [42] provide a hybrid mixture of different-sized AuNPs and domain III of envelope glycoprotein generated from serotype 2 of dengue virus (EDIII). The ability of gold NPs (AuNPs) with EDIII functionality (AuNP-E) to stimulate neutralizing antibodies in BALB/c mice is assessed. According to the data, AuNP-E caused a significant number of antibodies, which is what neutralizes the dengue virus in a serotype-specific manner (Fig. 5). More significantly, the amount of antibodies is influenced by the size of AuNPs and AuNP-E, suggesting that these factors might be changed to modify the antibody level [42].

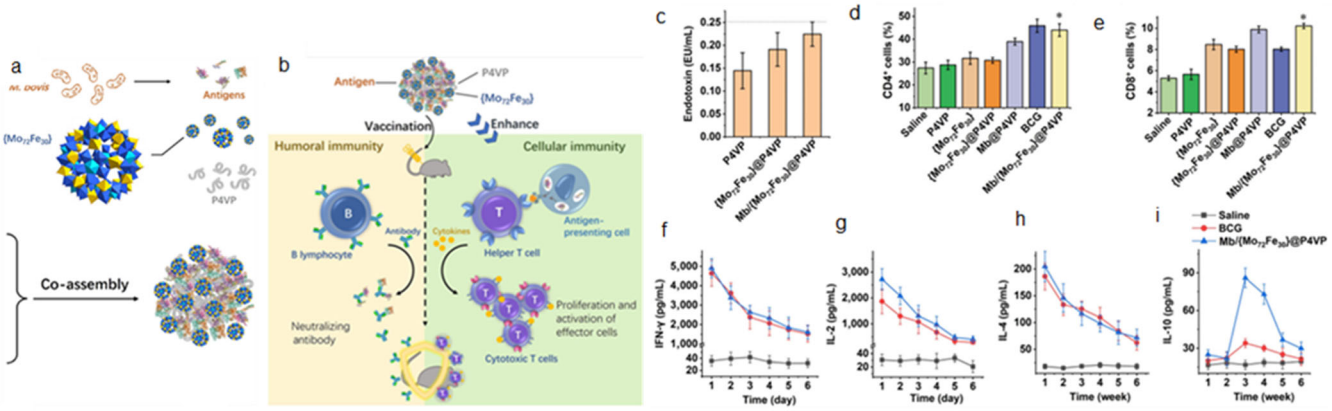


Fig 4. (a) The structural analysis and design of the Mb/$Mo_{72}Fe_{30}$@P4VP supra-particle assemblies, illustration of a co-assembling Mb/$Mo_{72}Fe_{30}$@P4VP schematic (a) Mb/$Mo_{72}Fe_{30}$@P4VP SPAs improve immune stimulation. (b) Schematic illustration of Mb/$Mo_{72}Fe_{30}$@P4VP SPAs' enhanced immune stimulation. (c) Using a chromogenic limulus amebocyte lysate endotoxin assay kit, endotoxin levels of P4VP, $Mo_{72}Fe_{30}$@P4VP, and Mb/$Mo_{72}Fe_{30}$@P4VP were determined. Count rates of (d) CD4+ and (e) CD8+ cells in the spleen of Balb/c mice one week after inoculation as determined by flow cytometry. Mb/$Mo_{72}Fe_{30}$@P4VP induces cytokine release SPAs include IFN-, gIL-2, hIL-4, and iIL-10. The ELISA method was used to determine the serum cytokines. Reproduced with permission from [41]. Copyright 2022, Springer

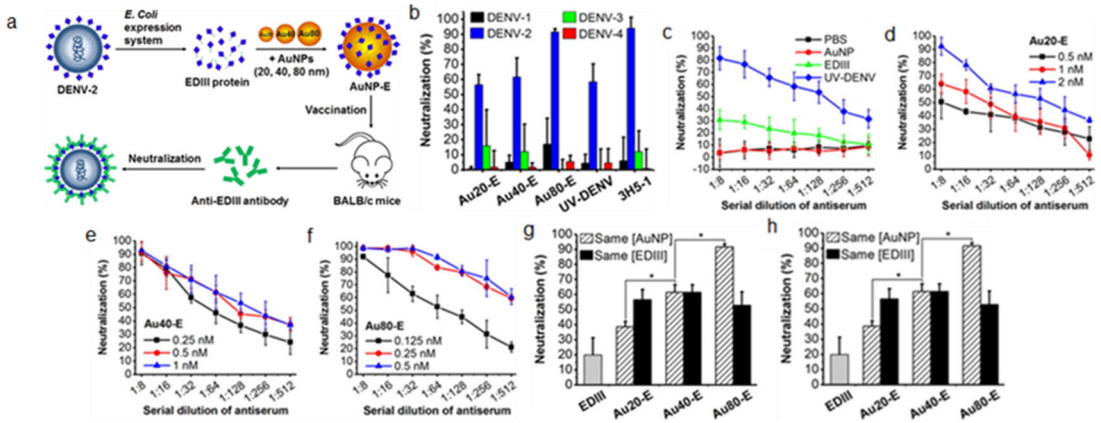


Fig 5. (a) DENV-2-derived purified envelope glycoprotein domain III (EDIII) created a protein corona around three different-sized AuNPs: Au20, Au40, and Au80. The development of anti-EDIII antibodies that neutralize DENV-2 was then induced in BALB/c mice using these complexes (AuNP-E). (b) PRNT study of mouse antisera to four different DENV serotypes, blood drawn at week 5 following the initial immunization was diluted 64 times before being used to generate the antisera from mice inoculated with AuNP-E. Mouses that had received different concentrations of (c) controls (AuNPs, UV-DEN, EDIII, and PBS), (d) Au20-E, (e) Au40-E, and (f) Au80-E were used to produce the antisera. (g) A comparison of the neutralizing abilities of antisera isolated from mice given the same quantity of EDIII protein and AuNPs cores at the same dilution in all three sizes; (h) A comparison of the neutralizing properties of antisera collected from different mouse groups against the easily available DENV-2 antibody 3H5-1. Reproduced with permission from [42]. Copyright 2018, Elsevier

Nanomaterial for mRNA Vaccine

The COVID-19 pandemic has thrust mRNA vaccines to the forefront of the biotechnology and pharmaceutical industries [43-44]. These results appeared barely 10 months after the SARS-CoV-2 sequence was made accessible to the public, which indicates that the

vaccine's development was completed more quickly than anticipated [45-46]. This accomplishment is a tribute to the biotech and pharmaceutical industries' capacity to address a pressing and unmet worldwide need, as well as to mRNA's intrinsic powers as a medicinal modality - in this case, a preventative vaccine [26,47]. There have recently been many great studies of mRNA delivery methods for vaccines and treatments that are older than COVID-19 [48-49].

Yin et al. [50] described an injectable hydrogel made of graphene oxide (GO) and polyethyleneimine (PEI) after subcutaneous injection (at least 30 d), which can produce mRNA (model antigen, ovalbumin), adjuvants (R848), and adjuvant-loaded nanovaccines (Fig. 6). The dispersed nanovaccines are delivered specifically to lymph nodes and shield mRNA against deterioration. Data indicate that with just one treatment, this transformable hydrogel can greatly boost antigen-specific $CD8^+$ T-cells and consequently suppress tumor growth. In the interim, this hydrogel can cause the serum to produce an antigen-specific antibody, which in turn stops metastasis from occurring [50].

As a potential vaccination, Zhang et al. [51] created an mRNA contained within lipid nanoparticles (mRNA-LNP) addressing the SARS-CoV-2 receptor binding domain (RBD) (called ARCoV) (Fig. 7). In non-human primates, such as mice, intramuscular administration of ARCoV mRNA-LNP induced strong anti-SARS-CoV-2 neutralizing antibodies and a Th1-biased cellular response. A SARS-CoV-2 mouse-adapted strain could not be overcome in mice after two doses of the ARCoV vaccine were administered. A liquid formulation of ARCoV is also produced, and it can be kept at RT for at least a week [51].

Dong et al. [52] found that GO NPs with polyethyleneimine functionalization were very good at stimulating the immune system and loading a lot of antigens [52]. Using a simple electrostatic adsorption

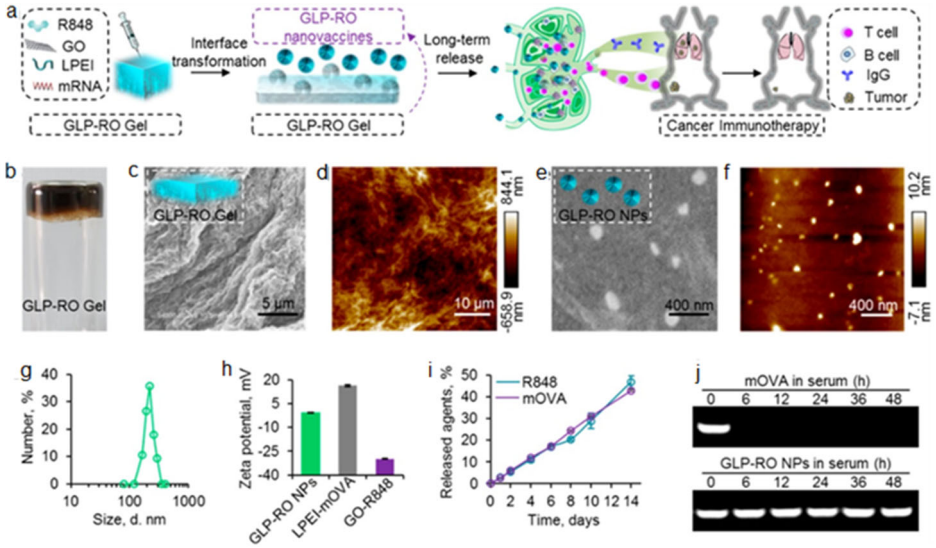


Fig 6. (a) Schematic representation of transformable hydrogel and its descriptions through electrostatic contact and stacking, GO and LPEI are created in the injectable hydrogel that will include mOVA, and hydrophobic adjuvants (R848). This GLP-RO Gel can constantly release nano-vaccines to elicit an immunological response to aid in the treatment of cancer (O for mOVA, R for R848). (b) A standard image of the GLP-RO Gel, GLP-RO Gel's layered structure, and rough surface is depicted in (c) and (d) SEM and AFM pictures, respectively. Pictures of the released GLP-RO NPs in (e) SEM and (f) AFM. (g) GLP-RO NP size distribution as calculated by DLS. (h) Zeta potential of GO-R848 and the GLP-RO NPs, LPEI-mOVA, R848, and mOVA were released from GLP-RO Gel *in vitro*, and the profiles released are identical. (j) Free and encapsulated mOVA's stability over time in GLP-RO NPs in serum at 37 °C. Reproduced with permission from [50]. Copyright 2021, ACS

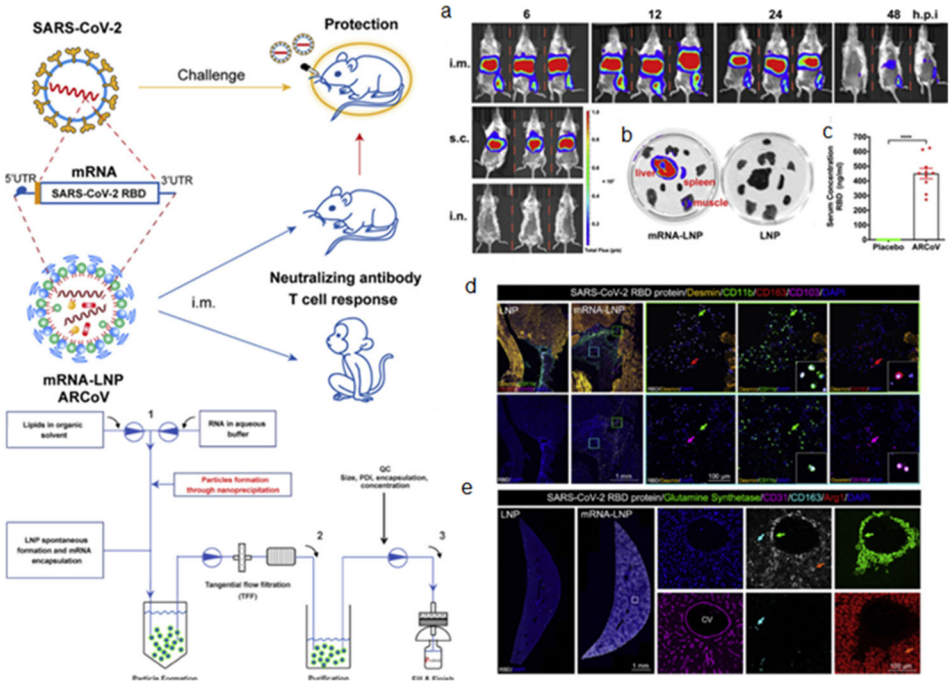


Fig 7. LNPs that self-assemble and contain mRNA are the products of this technique. The solution was concentrated, and ethanol was eliminated using tangential flow filtration. ARCoV mRNA-LNP formulation is delivered (a) by *in vivo* BLI of mRNA-LNP in mice. After injecting 10 g of FLuc-encoding reporter mRNA-LNP in different ways, the IVIS spectrum was used to take pictures of female BALB/c mice at the times given. (b) Mouse reporter mRNA-LNP tissue distribution, using empty LNP as a control. (c) Mouse expression of the RBD encoded by mRNA 6 h after the inoculation, the serum RBD concentration was determined by ELISA. (d) Analysis of LNP-delivered mRNA expression by multiplex immunostaining in mouse muscle tissues 10 ng of the ARCoV mRNA-LNP vaccine was administered to female BALB/c mice, with empty LNP serving as the control. Six h following the injection, the muscle around the injection location was taken and stained using multiplex immunofluorescent techniques for the SARS-CoV-2 RBD (white) and additional cell markers like CD103 (magenta), Desmin (gold), CD163 (red), and CD11b (green). (e) Mouse liver expression of mRNA supplied by LNP, SARS-CoV-2 RBD, and numerous cell markers for glutamine synthetase (green), CD163 (cyan), CD31 (magenta), and Arg1 was detected in liver tissue obtained 6 h after injection (red). On the right, magnified views of the white-boxed areas are displayed. Reproduced with permission from [51]. Copyright 2020, Cellpress

method, influenza hemagglutinin (HA) was added to GO NPs while keeping their structure and antigenicity (Fig. 8). The GO NPs that were made helped antigens get inside the cell boosted the production of inflammatory cytokines and helped JAWS II dendritic cells mature. When mice were immunized intravenously with GO NPs formulations instead of soluble HA, the immune responses at mucosal surfaces and systemic sites were much stronger and more cross-reactive. The GO NPs greatly increased cellular immune responses and antigen-specific humoral responses in the absence of any additional adjuvant, equivalent to the well-known powerful mucosal immunomodulator CpG. Strong immune responses provided defense against threats from heterologous and homologous viruses. The role of CpG, when incorporated, may be hidden by the solid self-adjuvant impact of GO NPs. GO NPs can be produced into powerful intranasal influenza vaccinations that offer comprehensive protection in the absence of already approved mucosal adjuvants [52].

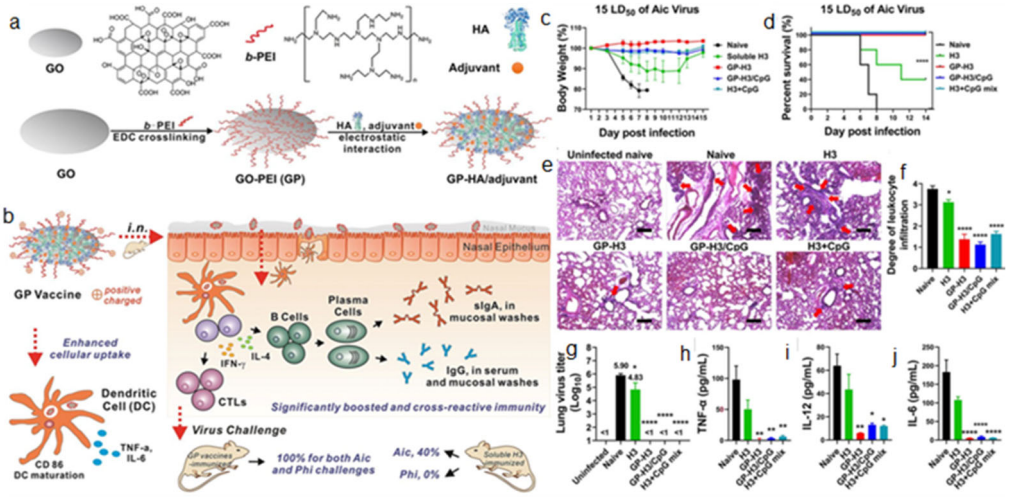


Fig 8. The creation and operation of influenza GO NPs are illustrated schematically. GO and GO-HA/adjuvant NPs preparation (a) Effects of GO NPs vaccinations on boosting immunity (b) GO NPs vaccinations increased the release of pro-inflammatory cytokines and helped DC cells take up the vaccine better. After being given influenza GP nanoparticles through an IV, immune protection against homologous and heterologous influenza virus challenges was greatly improved and widespread. (c) Mice's mortality and (d) morbidity following challenge (e) Analysis of histological pathology using H&E staining, as a negative control, a mouse lung section that wasn't infected was employed. Leukocyte infiltration is indicated by red arrows in the pictures. Each group is represented by an image. (200 nm scale bars.) (f) A bar graph displaying the leukocyte infiltration scores and the measurement of mouse lung virus titers is in (g). TNF-, IL-12, and IL-6 levels in the BALF of infected mice are assessed in (h–j). Reproduced with permission from [52]. Copyright 2021, PNAS

Nanomaterial for DNA Vaccine

DNA vaccine, a novel type of vaccination that has recently come into existence, has many distinct advantages over conventional vaccines [53]. First off, DNA vaccination is a significantly less expensive and riskier method because it merely consists of the DNA sequence, which host cells absorb and transform into antigens [54]. Second, because the immune responses are targeted specifically at the chosen antigens, the DNA vaccine is extremely focused [55-56]. Additionally, the DNA vaccine is very successful because it expresses antigens *in situ* to simulate the ongoing infection and prime both B- and T-cell responses [57-58]. Additionally, DNA vaccines offer a quick response platform that can quickly produce a protective vaccine [47,59-60].

Mesoporous silica NPs (MSNs) are frequently used for gene transport or as adjuvants to boost immune responses, although DNA vaccines are hardly ever reported to contain them. The MSNs-based DNA vaccine developed by Song et al. [61] is presented. It uses rambutan-like MSNs, both adjuvants and gene vectors. DNA vaccination based on MSNs induces a superior immune response because of increased antigen expression and better dendritic cell maturation. For mice given MSN-based vaccinations, increased antigen-specific IgG antibody and IFN^{-} cytokine production, amplified $CD8^{+}$ T cells are seen (Fig. 9). This paper presents a potent DNA vaccine with exceptional immune responses, offering helpful recommendations for the development of efficient nanomaterial-based vaccinations [61].

Oncogene-based cancer vaccines have demonstrated considerable promise in preclinical research. The inadequate immunogenicity of DNA vaccines due to low cellular internalization and dendritic cell (DCs) activation limits their effectiveness. Although stable, calcium phosphate (CP) NPs are biodegradable carriers because of their high DNA loading capacity and

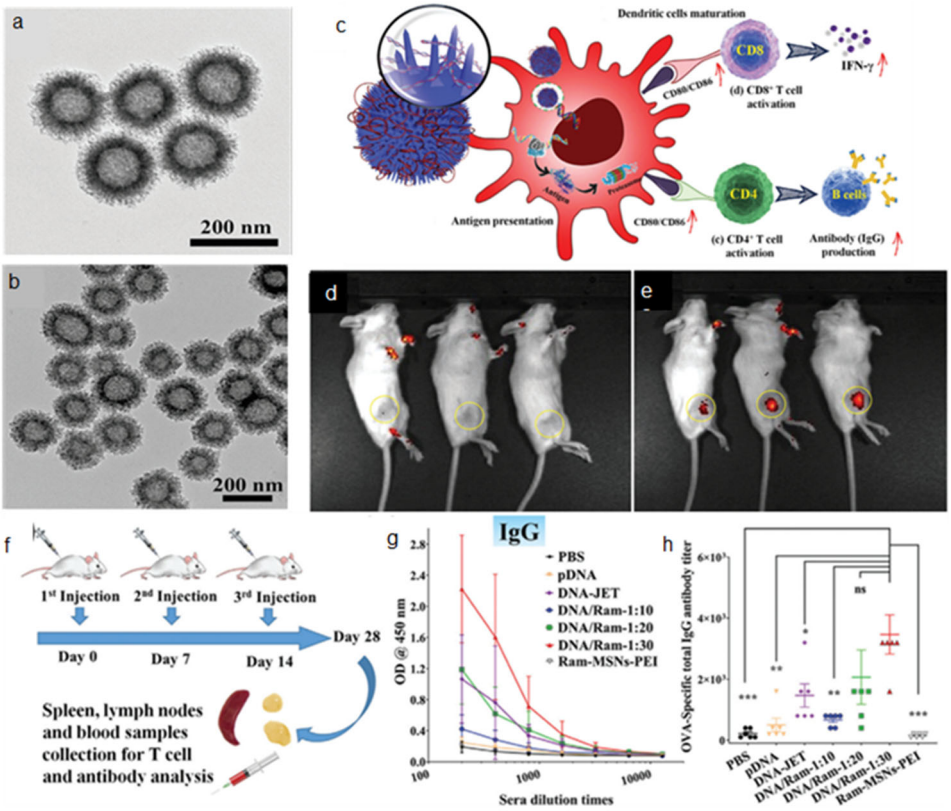


Fig 9. (a-c) TEM photos of Ram-MSNs and Ram-MSNs-PEI, as well as a schematic illustration of the immunological responses that the DNA vaccine based on Ram-MSNs-PEI induces. Fluorescent imaging of mice receiving pDNA-m cherry administration diagrammatic depiction of the vaccine research design, DNA-JET (d), (e) DNA/Ram at 1:30 I, proliferation of splenocytes without (grey bar) and after OVA stimulation (black bar). (f) OVA-specific IgG antibody analysis by ELISA using serial serum dilutions, (g) and (h) corresponding antibody titer analysis. Reproduced with permission from [61]. Copyright 2019, Wiley

low toxicity. The ATP-modified CP NPs attached to the DNA vaccine and used ATP as a dual-functional agent, stabilizing CP and serving as an immunological adjuvant. The DNA vaccine's cellular uptake and transfection efficiency were boosted by ACP NP, and it also demonstrated the ability to activate DCs, which are essential for preparing T-cells for immunotherapy against cancer. As a result, mice given the ACP-DNA vaccine developed higher levels of antigen-specific antibodies and showed stronger tumor growth inhibition. For cancer DNA vaccines, these one-step synthesized ACP NPs serve as an effective nano-adjuvant and nano-delivery system [54].

For the delivery of DNA vaccines, Lu et al. [62] created the NPs-in-microsphere (NIM) hybrid, which combines a sustained-release microsphere with a nanoscale polymer/DNA polyplex. The solid-in-oil-in-water (S/O/W) emulsion was used to create PLGA microspheres from the nano-sized cores, which were made of polyethylene glycol-graft-polyethyleneimine (PEG-g-PEI)/DNA polyplexes. To make DNA soluble and stable in organic solvents and prevent DNA inactivation at the aqueous-organic interface during encapsulation, the PEG block was utilized as a stabilizing excipient (Fig. 10). The efficiency of DNA encapsulation in NIMs was greatly increased by the design of DNA in solid form. In a physiological context, this novel formulation had a burst release that was less than 15% and a sustained release that was nearly zeroth-order kinetic. The microspheres also exhibited pH sensitivity and decomposed more quickly in lysosomal compartments, which increased the intracellular release

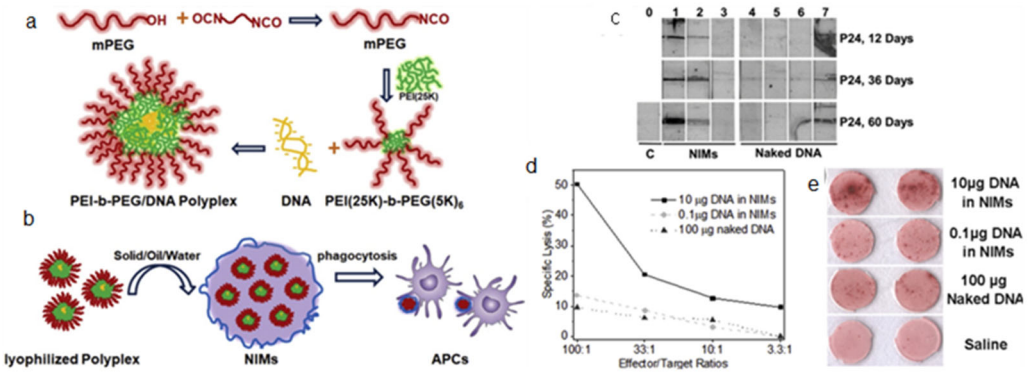


Fig 10. Diagrammatic depictions of S/O/W microencapsulation techniques for NIMs with micronized DNA are provided. S/O/W NIMs were developed, which could size exclude and passively "target" phagocytic cells. (a) PEG-g-PEI attached DNA to generate condensed PEG-g-PEI/DNA polyplexes; (c) Analysis of mice's serum antibody responses to HIV-1 p24 antigens using Western blotting. Mice's sera were collected on 12, 36, and 60 d after injection and subjected to a dilution of 1:40 for analysis. 1, 2, and 3 NIMs (10, 1, 0.1 g), naked DNA (10, 1, 0.1, 100 g), 4, 5, 6, 7, and 0 as the negative control were used. Reproduced with permission from [62]. Copyright 2020, Elsevier

kinetics. Finally, mice developed unique humoral and cellular immune responses after receiving low doses of intramuscular injections of NIMs expressing HIV proteins [62].

■ NANOMATERIAL FOR SUBUNIT, DNA, AND RNA COVID-19 VACCINE

Millions of people have been infected by the COVID-19 epidemic, which is still going strong due to its high prevalence, lengthy incubation period, and lack of effective treatments or vaccines [63-64]. The most promising method of preventing new virus strains is vaccination [65-66]. Since NPs are excellent for antigen transport, as adjuvants, and as viral structural mimics, current vaccine design benefits from the use of nanotechnology [17]. In actuality, subunit, RNA, and DNA vaccines are used to introduce vaccine candidates into clinical trials. One of these is the delivery of vaccine mRNA via lipid NPs, which has a lot of potential (Fig. 11). A successful manufacturing vaccine platform must enable quick discovery, scalability, and worldwide dissemination to remove pandemics in the present and the future [17,67].

After moving through their early safety and immunogenicity investigations, these are companies—CanSino, Modera, BioNTech, Sinovac, Sinopharm, Zydus Cadila, Inovio, Novavax, Anhui Zhifei Longcom, Vaccine,

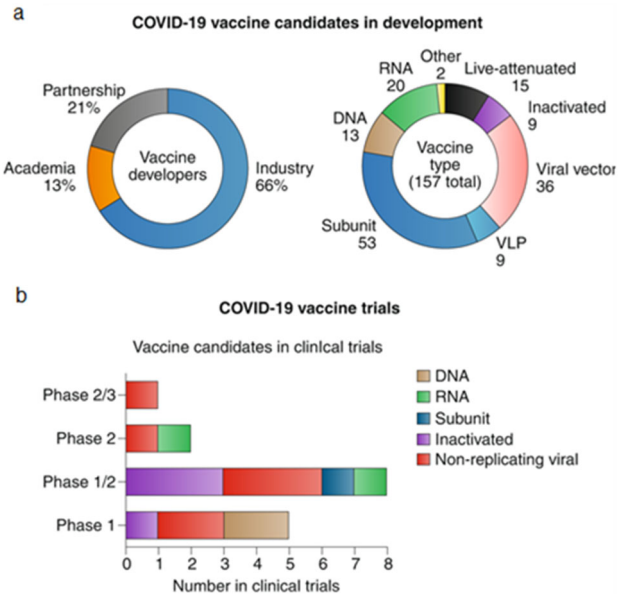


Fig 11. (a) Since June 1, 2020, academic research facilities and industry have been working to produce 157 vaccine candidates (and partnerships thereof). 16 vaccine candidates for COVID-19 have entered clinical testing, including viral vectors, virus-like particles (VLPs), RNA, and DNA vaccines, along with live-attenuated vaccines, inactivated vaccines, and subunit immunization vaccines. Reproduced with permission from [17]. Copyright 2020, Nature

and the University of Oxford. Many nations are having difficulty containing SARS-CoV-2 despite testing,

tracking, and social isolation. Only when herd immunity develops, as a result of an efficient vaccination or if the population has already been exposed and is resistant to reinfection, will COVID-19 be suppressible. Until there is a successful vaccination, there is almost little hope of a return to pre-COVID-19 social behavior [66,68]. Antiviral drugs and other sorts of drugs that prevent viral replication may be delivered via nanotechnology. Nanotechnology may provide useful suggestions for use in the battle against viral infection at different stages of viral infection. Approaches based on nanotechnology may be useful in the fight against SARS-CoV-2 infection. The advancement of SARS-CoV-2 therapy and vaccine manufacture in terms of effectiveness and safety may be greatly aided by nanoparticles. The efficiency and speed of vaccine development have both been accelerated by nanocarriers. As a consequence, it is crucial to enhance research into NPs as nano-delivery systems and nanotherapeutics in viral infection, as well as the creation of fresh, efficient approaches, in order to prevent the spread of SARS-CoV-2 [64,69-70].

Zhao et al. [59] reported the creation of special liposome-polymer hybrid NPs (pSFV-MEG/LNPs) with mPEG-PLGA (core) and a hydrophilic lecithin/PEG-DSPE-Mal 2000 (shell) to administer a multi-epitope self-replication DNA vaccine. The pSFV-MEG/LNPs and high encapsulation effectiveness of 87.60% as compared to PBS induced strong humoral and cellular immune responses (Fig. 12). Additionally, pSFV-MEG/LNPs had immune responses that were 1.58 and 1.05 times stronger than those of pSFV-MEG [59].

Lipid squalene NP, so-called SQ@NP adjuvanted vaccines for COVID-19, were reported by Ho et al. [71] to modulate the immune response and improve vaccine effectiveness. SQ@NP and the SARS-CoV-2 spike (S) subunit protein were intramuscularly injected into mice.

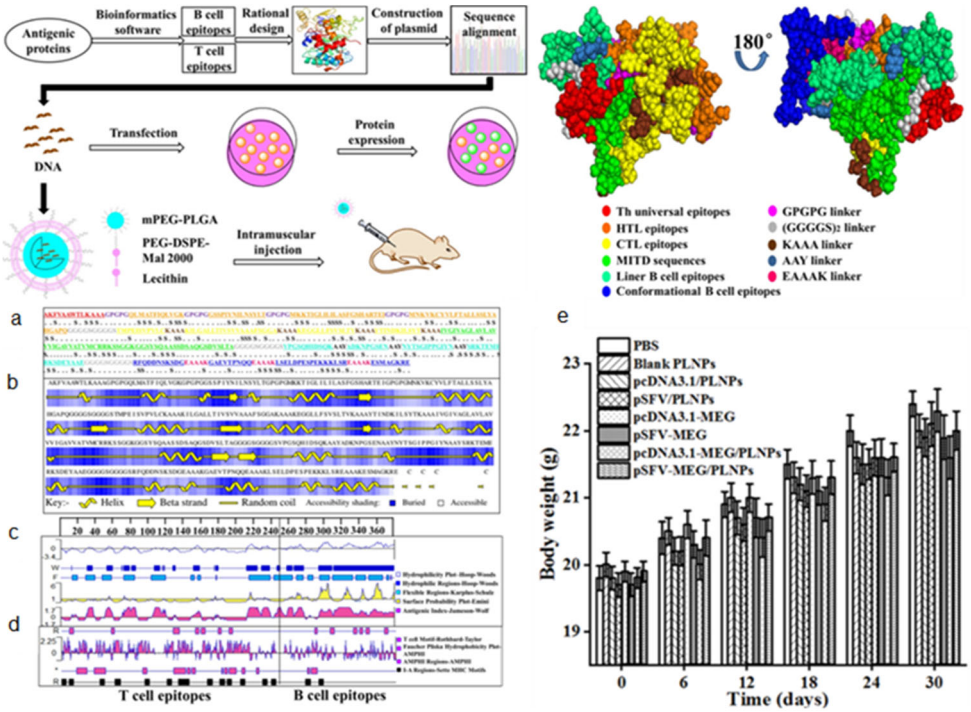


Fig 12. I-TASSER, an online program, simulates and analyzes MEG's three-dimensional structure, and evaluates MEG's immunogenicity. (a) PAProC software's prediction of the proteasome cleavage sites in MEG; (b) the examination of MEG's secondary structure; and (c) MEG's B- and T-cell epitopes' hydrophilicity, flexibility, surface accessibility, and antigen index were assessed. (d) A review of MEG's T-cell epitopes was conducted. body weight variations and HE staining of the mice's major organs, (e) The mice's body weight was recorded every three days. Reproduced with permission from [59]. Copyright 2021, Elsevier

A single dose of SQ@NP-adjuvanted S-protein vaccine can elicit antigen-specific IgG and protective antibodies comparable to those elicited by ELISA and virus-neutralizing assays. Groups of animals receiving adjuvanted vaccination showed anamnestic reactions when the animals received a boosting vaccine injection (Fig. 13). The release of cytokines, including interferon (IFN), interleukin (IL)-5, and IL-10 in splenocytes was also significantly elevated after the adjuvant. After adjuvant the S-protein vaccine with SQ@NP, the production of cytokines such as IFN, IL-5, and IL-10 in splenocytes was also noticeably increased; however, this was not the case with the immunization adjuvanted with traditional aluminum mineral salts. In the S-protein vaccine with SQ@NP, this was not the case with the immunization adjuvanted with conventional aluminum mineral salts. The SQ@NP-adjuvanted vaccine was very well tolerated after being injected intramuscularly in mice, according to a histological study of the injection sites [71].

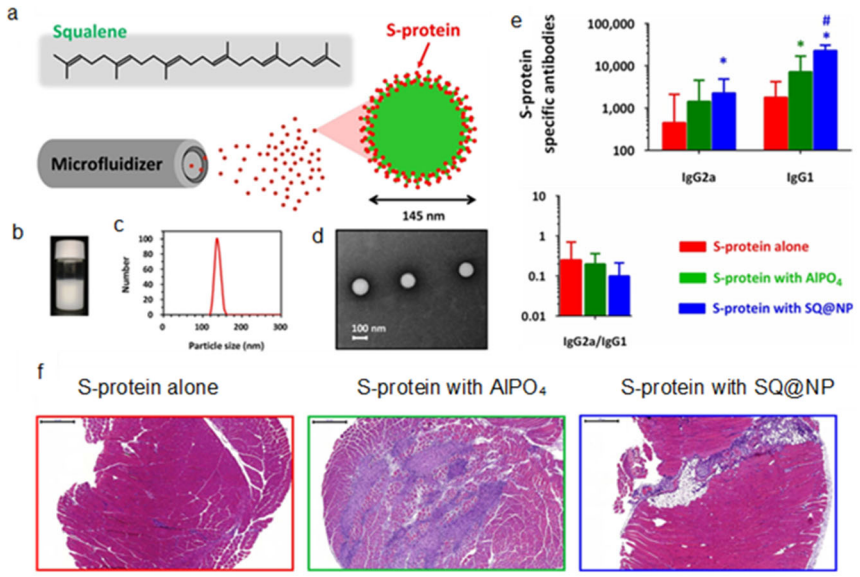
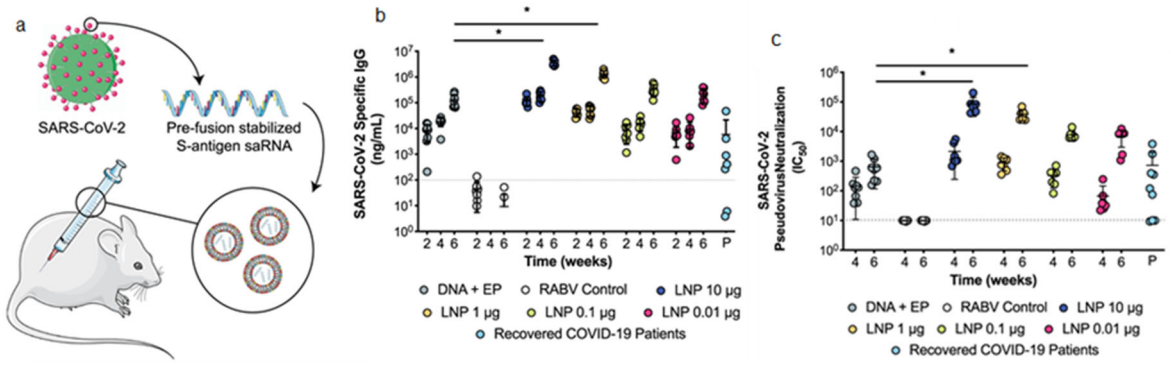


Fig 13. (a) A diagram of the high-shear micro fluidization technique's lipid squalene NPs, (b) visual aspects, (c) particle size distribution, and (d) TEM images, using DLS, the particle size was determined. (e) Two intramuscular doses of SARS-CoV-2 S-protein alone or one intramuscular dose of SARS-CoV-2 S-protein that had been adjuvanted with $AlPO_4$ or SQ@NP were administered to BALB/c mice at week 12 to generate an antigen-specific IgG subclass response. (f) Illustrations of representative tissue sections stained with hematoxylin and eosin at the injection site one week after mice were boosted with SARS-CoV-2 S-protein alone or in combination with $AlPO_4$ or SQ@NP for histological analysis. Reproduced with permission from [71]. Copyright 2021, Elsevier



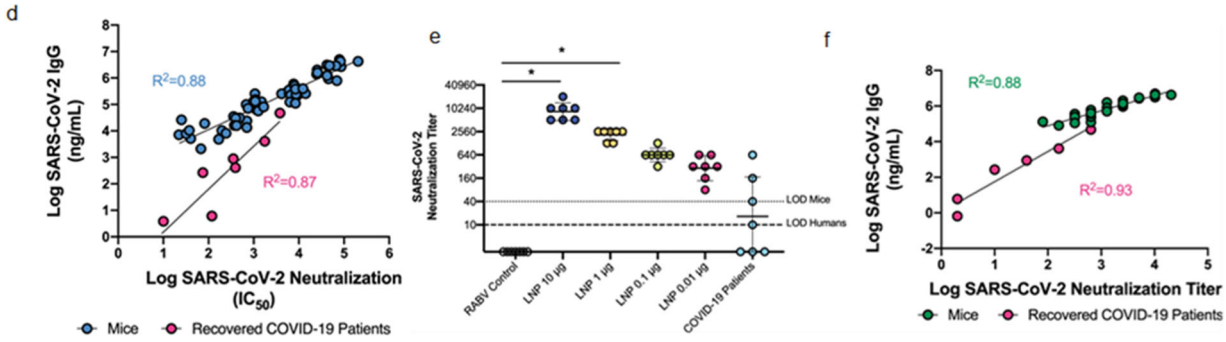


Fig 14. (a) Vaccination of BALB/c mice with saRNA that encodes a pre-fusion stabilized spike protein in LNP is shown in the diagram. (b) study of COVID-19 recovered patients and investigation of SARS-CoV-2 specific IgG responses in mice given dosages of LNP-formulated saRNA ranging from 0.01 to 10 g of saRNA. (c) analysis of SARS-CoV-2 pseudotyped virus neutralization of sera; (d) correlation between SARS-CoV-2-specific IgG and SARS-CoV-2 neutralization IC_{50} in recovered COVID-19 patients and vaccine-treated mice (n = 7 physiologically independent animals). (e) Serum from BALB/c mice that received vaccinations with dosages of saRNA made by LNP ranging from 0.01 to 10 g and n = 7 physiologically independent animals; (f) a correlation between animals that had received the vaccine and SARS-CoV-2-specific IgG and SARS-CoV-2 wild-type virus neutralization titers. Reproduced with permission from [45]. Copyright 2021, Nature

MCkay proposed a vaccine consisting of a self-amplifying RNA expressing the SARS-CoV-2 spike protein enclosed in a LNP. They find that mouse sera have exceptionally dose-dependent SARS-CoV-2 specific antibody titers and that both a fake virus and the real virus are effectively neutralized. The authors discover that the neutralization is greater in magnitude than in recovered COVID-19 patients and is proportional to the amount of a particular IgG (Fig. 14). When mice are immunized with saRNA LNP, the immune response is Th1-biased and no antibody-dependent enhancement (ADE) is seen. Finally, after re-stimulating cells with SARS-CoV-2 peptides, the authors notice strong cellular responses, as indicated by IFN production [45,72].

■ CONCLUSION

The subunit vaccine is still being developed and tested due to additional difficulties, such as unexpectedly large reactions and complex and expensive synthesis. Although subunit, DNA and RNA vaccination can address the majority of the drawbacks of conventional methods, two major technological obstacles are preventing the commercialization of vaccines: i) a lack of vaccines or techniques to pinpoint the RNA/DNA-encoding antigens that can best elicit an immune response; and ii) a lack of an effective means to trigger a potent, stable, and long-lasting immune response. Despite this, the COVID-19 pandemic has spread worldwide; these vaccines have been growing quickly. In the creation of novel vaccines, choosing the proper adjuvant to support the antigen and enhance immune responses is crucial. This is because the immune system's response to a specific vaccine and adjuvant is highly dependent on the specific situation at hand, and no adjuvant is suited for the antigens in every scenario. It's important to consider the affordability and safety of adjuvants. The impact of each of these elements on vaccine adjuvant efficiency is possible.

Considering that the development of adjuvants uses a combination of immunostimulants and nanoparticle delivery technologies to generate better immune activation. However, the drawback of conventional adjuvants is their insufficient capacity for immunological activation. Because of this, novel adjuvants are desperately needed to make up for the drawbacks of traditional adjuvants. The novel adjuvant has to provide a way for good biosafety. The introduction of novel adjuvants is, however, constrained by the expense and length of preclinical research and clinical testing. Research projects with clinical

significance may involve the performance optimization and formulation improvement of conventional adjuvants, including surface modification, granulation, and combination with other adjuvants that increase their immunological activity. In addition, the current manufacturing processes, equipment, and technologies for nanomaterials that make it relatively simple to adapt to the development of new vaccines need to be developed and studied in more depth.

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■ AUTHOR CONTRIBUTIONS

Vy Anh Tran did the supervision, conceptualization, methodology, such as writing - the original draft, review, editing, and validation, and project administration, such as resources and funding acquisition. Vien Vo, Vinh Quang Dang, Ta Ngoc Don, and Van Dat Doan did the methodology, such as validation and investigation. Giang Ngoc Linh Vo did the methodology, such as software and investigation. Van Thuan Le did the conceptualization and methodology, such as formal analysis and validation.

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