Review Article

A Pervasive Review on New Advancements of Nano Vaccines on Covid-19 Pandamic

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ABSTRACT

The infection that causes COVID-19 may be a pathogen referred to as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and is believed to possess originated from China's Wuhan Province. The rapid spread of coronavirus disease 2019 (COVID-19) has become a worldwide concern, with the planet Health Organization (WHO) declaring it an epidemic on March, 2020. To enter the cells, SARS-CoV-2 S requires angiotensin-converting enzyme 2 (ACE2). Many existing vaccines have drawbacks like insufficient system stimulation, in vivo instability, high toxicity, the need for a chilly chain, and multiple administration. A nanotechnology is an efficient tool for addressing these issues. A successful vaccine against SARS-CoV-2 infection is predicted to stimulate innate and adaptive immune responses and protects against severe sorts of coronavirus disease 2019 (COVID-19). Different strategies are introduced because the go after an efficient and safe vaccination has begun. Currently, the foremost common vaccine types studied in clinical trials include viral vector-based vaccinations, genetic vaccines, attenuated vaccines, and protein-based vaccines. during this review, we cover the foremost promising anti-COVID-19 vaccine clinical trials also as various vaccination strategies to shed more light on the continued clinical trials. it's also discussed how nanotechnology is often wont to better understand the pathology of the present pandemic, also as how our understanding of SARS-CoV-2 cellular uptake and toxicity can influence future nanotoxicological considerations and nanomedicine design of safe yet effective nanomaterials.

Keywords: Nano vaccine, COVID 19, strategies, clinical trial, drawbacks.

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INTRODUCTION

The novel coronavirus which is now officially declared as severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2, is considered to be the main causative agent of that COVID-19 disease. The Wuhan city which is located in China was the first epicenter of this disease, but now more than 8.3 million cases have been reported. The particular word "coronavirus" is given because the club-shaped protein spikes are present on their upper surface when viewed under a special type of microscope which is known as transmission electron microscope (TEM). According to the genetic information, the virus is divided into four categories Alpha, Beta, Gamma, and Delta [26] of which the main causative agent behind all the COVID-19 pandemic situations belongs to the beta class [9].

Two types of regional epidemics are a middle east respiratory syndrome known as MERS and a severe acute respiratory syndrome known as SARS in 2012 to 2015 and 2001 to 2003, respectively. [6]

The three months from the date of 30th January 2020, when the first COVID-19 case was recorded officially in India, we have found that there were over thousand and thousand deaths happened from that disease, which is now across 537 in the rest of South Asia. In this pandemic situation, India logged a huge amount of COVID-19 deaths than Ireland. Statistically found that from May 10, 2020, India had passed 2,000 death cases and therefore India was starting lockdown restrictions because it touched up to 180,000 death cases by the middle of June. [1]

The direct transmission mode of this virus is happening via (1) aerosols, which is formed via dental procedures and surgical procedures or it can be transmitted in the form of respiratory droplet; (2) mother to her child (3) human secretions and discharge and body fluids, for example, urine, feces, semen, tears, and saliva. This virus is most commonly spread via respiratory droplets which formed while sneezing, talking, coughing of an infected person to other normal people. The risk of transmission will increase if a virus-infected person is present within a 1-meter diameter of another person. On the other way, Indirect transmission occurs via (1) surfaces of furniture and fixtures present within the immediate using environment of infected patients and (2) objects which are used by the infected person. [24]

Epidemiological study shows that the symptoms of this disease are cough, fever, dyspnoea, accounting for 82%, 83%, and 31% of the COVID-19 patients respectively.

Generally, the incubation period in COVID-19 is quick, it may vary from 5–6 days or 2–11 days in co-vid infections.[21]

The main symptoms of covid-19 patients are fatigue, chills, fever, loss of appetite and protracted cough in the lungs, etc. Some features of infection of SARS-CoV-2 are anosmia, loss of smell, and loss of taste, these symptoms are found in approximately 64% of cases within the infected patients. When this viral infection spreads to the lower respiratory tract then it becomes a precursor of severe disease is unclear; then pneumonia with the characteristic of pulmonary ground-glass changes its opacity on the chest CT scans. Some other features of severe disease are respiratory compromise, renal damage, Blood clotting, and cardiovascular collapse. [2]

Based on their genetic relationship, genetic information, and genomic structures, this virus can be divided into four genera. They are referred to as Alpha, Beta, Gamma, and Delta. Among the seven recognized human coronavirus which is founded or reported, the HCoV-NL63 and the HCoV-229E belong from the Alpha genera, and the other rest of the five types belong from Beta genera. [26]

Structure of corona virus

The basic structure of coronavirus is designed with long RNA polymers which are tightly packed into the center part of the particle because it is a single-stranded RNA virus, and then it is surrounded by a protective capsid. This capsid has a lattice of repeated protein molecules, that's why they are called capsid proteins or coats. These repeated proteins are called nucleocapsid (N). Then the core of the coronavirus particle is further surrounded by another outer membrane envelope. This membrane envelope is made of lipids where proteins are inserted or embedded. The virus was last assembled in these membranes which are derived from the cells, but they are modified to contain specific viral proteins, which are the spike (S), membrane (M), and envelope (E) proteins. [9]

First, (i) the S protein is also known as Spike glycoprotein that helps in the attachment of the virus to host cells by membrane fusion method, so they encourage the entry of corona virus into the host cells. These S proteins are clove-shaped and are type-I transmembrane proteins. They have 3 segments: one is a large ectodomain, another is a single-pass transmembrane, and the last one is an intracellular tail. now the ectodomain part of the S proteins contains the S1 subunit (which containing a receptor-binding domain also known as RBD), and the membrane-fusion S2 subunit. [34]

Now, the S2 domain also has two heptad repeats (HR1 and HR2) and enriched alpha-helices, which are featured as typical fusion proteins [26].

(ii) the abundant protein is called M protein (membrane). the purpose of this protein is to maintain the membrane integrity of that virus [9].

The M protein is a small type of transmembrane protein (25–30 kDa). they contain three transmembrane sections, one N-terminal ectodomain, and one C-terminal endodomain, which helps in defining the shape of the virion [26].

(iii) the E protein is called an envelope. it is the smallest protein among them and plays a very important structural role, it helps in assembly and budding in the virus. This E-protein is a short type of protein that comprises 76-109 amino acids, because of its small size its ranging between 8.4 and 12 kDa. It also contains 40 loops and 35 α-helices. The protein contains a large part of hydrophobic transmembrane made of 25 amino acids with a long c terminal domain and has a short length hydrophilic terminus containing 7–12 amino acids. The hydrophobic region helps in the gathering of viral genomic information[34].

According to the cell entry mechanism of Coronavirus it is attached to cellular attachment factors and bind with specific cellular receptors such as angiotensin-converting enzyme 2 (ACE2). this ACE2 receptor is mostly originating in the human epithelia of the lung and small intestine [17].

So, this virus attaches with ACE2 and host factors which are the cell surface serine protease TMPRSS2, endorse the fusion method by combined at the cellular membrane [46].

Previously known that the S protein or Spike has a receptor-binding domain (RBD) that intermediates direct interact with that cellular receptor such as angiotensin-converting enzyme 2 (ACE2) after binding with that cellular receptor the S1/S2 polybasic cleavage site is cleaved with the help of cellular cathepsin L and the host factor transmembrane protease serine 2 (TMPRSS2). So, after that TMPRSS2 helps in the viral entry at the human plasma membrane surface whereas in another site cathepsin L also activates the Spikes in cellular endosomes and it compensates with the cell for the viral entry into cells that are absent TMPRSS2 [21].

After that, the next step in the coronavirus lifecycle is the translation of the replication of the gene from the viral gRNA. That viral replicase gene encodes two large ORFs, rep1a and rep1b. Two co-terminal polyproteins, one is pp1a and another one pp1ab are expressed by those ORFs, rep1a and rep1b. the replicase polyproteins cut by some proteases, which are encoded by coronaviruses. To produce an environment for promoting RNA synthesis by the replicase–transcriptase complex (RTC), this (RTC) is created by the assembling of the NSPS. This procedure leads to RNA replication and transcription of the viral RNAs[15].

Corona viruses can quickly replicate their viral gRNA to create another RNA copy those are united into newly shaped fresh viral particles. Corona viruses have one of the significantly large RNA genomes edged by 5′ and 3′ untranslated regions. This particular RNA genome holds cis-acting structures that help in RNA synthesis [46].

When the viral RNA is entered into the human cytoplasm and translation started then ORF1a and ORF1ab produce

the pp1a polyprotein and pp1ab polyprotein. This leads to producing 16 non-structural proteins by the viral proteases (encoded by ORF1a). after construction of the RNA replicase–transcriptase complex (RTC), this RTC produce full-length (−) RNA copies, which then offer templates to produce full-length (+) RNA genomes by the use of rough endoplasmic reticulum (ER)-derived membranes. Then after transcription, these RNA genomes also produce another subset of sub-genomic RNAs. this viral RNA then encoding all accessory and structural proteins for the viral particle. In the end, all translated proteins and genomic RNA are collected into some envelope in the ER–Golgi intermediate compartment. At last, these envelopes are later released by exocytosis [3].

Conventional approaches to treat Covid-19

One of the most important drugs to treat covid-19 is Hydroxychloroquine sulfate and chloroquine phosphate. these two drugs are anti-malaria drugs. In the mammalian cell, culture studies show that they revealed to change in the acidic conditions of organelles. They are very much used in inhibiting the terminal glycosylation of ACE2 against the corona virus, which leads to stop the fusion of the corona virus with the cell membrane and stop the infection [17].

Not only it inhibits the glycosyl-transferases of the corona virus but also modifies the viral post-translational part of some other viral families [37].

Hydroxychloroquine was a primarily effective solution to covid infection, but the safety of the drug was stressed.

Remdesivir is also an important drug, which acts as an inhibitor of the RNA-dependent, RNA polymerase. In vitro, it shows the inhibitory activity against SARS and the MERS. For patients who have mild to moderately severe COVID-19 infection and no need for respiratory support, remdesivir cannot show significant benefit and its use is not suggested by doctors. patients who have a high risk of hyperinflammation and who need supplemental oxygen and who are diagnosed in the initial stage in less than 10 days, redeliver is very useful for them because it shortens the time to recovery of those patients and reduces the risk of further infection [28].

If we compare between the Remdesivir and the Chloroquine, Remdesivir found post entry blockage of viral infection with concentration at 50% of EC50=0.77 µM whereas Chloroquine was showed with concentration at 50% of EC50=1.13 µM. Remdesivir Cytotoxic concentration at 50% of CC50>100 µM and Chloroquine cytotoxic concentration at 50% of CC50>100 µM and an effective concentration at 90% EC90 of 6.90 µM. Chloroquine shows efficiency at entry or post-entry level, but remdesivir is shown efficiency only post entry-level [37].

Similarly, favipiravir is moderately popular for covid-19. it is also inhibiting RNA polymerase because it is a guanine analog. Generally, it is used for influenza treatment. After many clinical trials, the effectiveness against COVID-19 is found. Not only by drugs but a vast range of vaccines are proposed against covid-19 [17].

Testing for the Virus

Corona virus is a single strand RNA virus, and thus all existing RNA detection is the best detecting method to detect this virus. so, the reverse transcriptase method can transcribe this viral genome into a DNA complement. the ideal corona virus test is amplifying the DNA templates by PCR, and then proceed the other real-time versions of such tests [34].

First, the test sample is submitted to CDC for testing then the result is confirmed with a TaqMan real-time RT-PCR assay. The requirement of this RT-PCR is three different probe and primer sets. First collect multiple primers and probe from the different sections of the corona virus viral genome, which can be get from other human and animal coronaviruses discriminately with a possible detection limit. The real-time RT-PCR assay is more complex than a conventional RT-PCR assay or culture isolation. So, it's the acceptable assay technique to detect covid during a clinical sample [13].

reverse transcriptase real-time PCR (RT-PCR) involves most of the testing to detect active COVID-19 infections, but there are also different molecular technologies. Two different molecular technologies like loop-mediated isothermal amplification or CRISPR-mediated detection also uses in some cases, but the application of these tests is often finished with previously developed tests that already detect viral agents. rapid antigen detection tests also use to detect the infection, but rapid antigen detection tests are less sensitive, and also less utilized as compared with RT-PCR. there are many drawbacks during these methods, so RT-PCR is sort of the only method among them [44].

Why choose nano in the COVID -19 PANDEMIC?

The idea of using nanotechnology is that viruses like COVID-19 work on a similar scale as nanomaterials. Nanomedicine has emerged as a powerful platform that has led to the development of novel materials and devices with a wide range of applications, especially in imaging, diagnostics, and therapy, which contributed to the early detection and treatment of diseases. The promise of nanotechnology is lying in the potential of manipulating matter on the small scale used either by nature or by humans. This opens new vistas to work at the scale of the cells and microbe-like viruses. [41]

Nanotechnology is defined as the development and use of materials and devices with at least one dimension of fewer than 100 nanometers. Nanomedicine is the application of nanotechnology that involves the use of nanoparticles for disease diagnosis, treatment, control, and prevention. Nanocarriers or nanoparticles are used to deliver drugs to target sites to deliver nano-therapeutic molecules or nano vaccines. [8]

Nano-sensors are used for viral detection and monitoring. Nano-based viral receptor blockage:

- Inhibition of viral attachment
- Inhibition of viral entry and infection
- Host cell protection

Nanotechnology could help the fight against COVID-19 through different approaches, such as avoiding viral contamination and spray by [8]

- Design of infection-safe personal protective equipment (PPE) to enhance the safety of healthcare workers and development of effective antiviral disinfectants and surface coatings, which can inactivate the virus and prevent its spread.
- Design of highly specific and sensitive nano-based sensors to quickly identify the infection or immunological response.
- Development of new drugs, with enhanced activity, decreased toxicity, and sustained release, as well as tissue target, for example - to the lungs.
- Development of a nano-based vaccination to boost humoral and cellular immune responses.

Conventional vaccines

Conventional vaccines usually consist of disease-causing pathogens or antigens that function by mimicking the infectious agent to stimulate the host's immune response. The pathogens can be modified as replicating or non-replicating agents, but in either case, the structure of cell membranes or proteins of the pathogens remains intact to be able to interact with the immune system. [46]

A range of stimuli-responsive properties such as genetic, molecular, enzymatic, targeting, optical, or chemical could be incorporated into nanomaterials, and nanomaterials can be engineered to have some of or all these properties in one single device. Those smart materials have the potential to provide highly active and specific therapies with minimal side effects and maximum therapeutic outcome. [31]

Design materials to overcome the most common delivery obstacles, which are often associated with circulation time and stability as well as tissue extravasation and cell internalization. This will be essential to find viral particles efficiently and target them for destruction by developing nano vaccines involved in host cell protection and immune and immunity response and/or anti-viral nano agents, involved in inhibiting viral attachment, cell entry, and systemic infection. [21]

For the treatment COVID-19, vaccine applicants include different kinds of vaccines. they're inactivated vaccines, live-attenuated vaccines, subunit vaccines, for virus-like particles (VLPs), and last DNA and RNA vaccines. Nowadays almost 16 vaccine candidates are tried for clinical testing[35,45].

Nano vaccine

Nanotechnology is popularly increasing as a strong device by the use of Nano vaccine for solving these kinds of infection, which are spreading from viruses because nano vaccines are new kind of vaccines are incorporated nanoparticles as carriers or adjuvants [19].

This inert nanocarrier creates a strong shield after one dose, that's why they're very useful for various numbers applications. because of the small size of nanoparticles, it gets easy admission to tumors, which shows much potential within the treatment of cancer therapy. Nano-sized particles also use for vaccine construction for the treatment of varied viral agents [20].

Nano vaccines can encourage not only quick but also long-lasting humoral and cellular protection. It is often administered through multiple delivery routes so easily, like oral, intravenous, intranasal, transdermal, etc. it can also use to specialize in the crossing of the blood-brain barrier. the foremost mechanism of Nano vaccines is to clone the size and shape of pathogens to endorse easy approval by immune cells. The nano vaccine is dependable on nanoparticles, which successively depends upon the particle shape, size, surface charge, and hydrophobicity, so as that they're going to control and alter the self-adjuvating effects, release kinetics, also controls multiple delivery routes [31].

In nano-vaccines the size of nano particle is that the foremost vital thing, it determines the internalization and biodistribution and internalization. Particles that range between 20–100 nm size can penetrate directly into the lymphatic system within a few hours from the time of administration, but if the particle size is between 200–500 nm then it must be affected by antigen-presenting cells and reach late to the lymphatic system [2].

Nanoparticles are very helpful as delivery systems because it facilitates antigen uptake and processing by antigen-presenting cells. However, now a day's scientists were capable of decorating self-assembling protein Nanoparticles (1c-SApNPs) with the spike's protein of corona virus. These new Nano vaccines are great samples of how can incorporate Nanotechnology and that enhance the therapeutic effect of COVID-19 vaccines. Hence, an oral multi-modal NANO vaccine is often used for specific targeted delivery into the tract by the use of a man-made mRNA, to increase the immunostimulatory activity of the vaccine, so simply adding antibodies or some small molecules that might help in inhibiting the interaction between ACE2 and corona virus. [11]

Advantages of using nano vaccine [19]

- Better stability in blood flow to increase the shelf life in the blood
- Enhanced immune system stimulation
- No need for booster doses
- No need to maintain the cold chain

- Ability to create active targeting

Nanoparticles can interact with immune machinery, inducing cellular and humoral immunological responses. Studies have shown that nanoparticles that range in size between 20 and 200 nm are preferentially internalized by endocytosis into APCs (resulting in the T cell response), while large particles (0.5-5µm) are usually internalized by phagocytosis (inducing the humoral immune response). [8] Vaccines using NP systems (NPb-Vs) can be delivered in two ways: [53]

1. Antigen or RNA/DNA is encapsulated in a nanocarrier.

2. Attaching antigens on the nanocarrier surface, to be exposed to the environment.

Novel NPs can improve the performance in the treatment of respiratory diseases through different mechanisms of action which are: [48]

(i) The development of polymers with faster mucus penetration and do not remain stuck, overcoming this barrier

(ii) The creation of biodegradable NPs with the stability to overcome the cell membrane and act in the lung with minimal levels of toxicity, causing no lesions during treatment

(iii) Modification of the chemical structure of NPs by adding surface capping agents such as polyethylene glycol (PEG)

Two types of vaccines based on nanoparticles: [43]

i) Self-assembly nanoparticles- representing modified Adenoviruses.

Delivers the target gene (cell generate protein needed to trigger an immune response).

ii) Synthesized polysaccharide nanoparticles- target protein molecules.

Nanocarriers/NPs-based delivery system [8]

- Protect nano vaccines from premature degradation
- Increase stability
- Have excellent adjuvant properties
- Help in the targeted/controlled delivery of immunogens to APCs (Antigen-presenting cells)

Nanomedicine Approach for COVID-19 Therapeutics

(Rational Selection of Drug-Nanocarrier Combination)

Nanocarriers can load and distribute a wide range of active moieties, including antivirals, biologics, and nucleic acids. The commercial success of nanomedicine against the SARS-CoV-2 virus depends on connecting the right therapeutic candidate to the right nanocarrier for a given clinical state. Smart nanocarrier designs, such as the prodrug strategy, are also required for the nano-delivery of biologics.[9]

Table 1: Characteristics of nanocarriers as nano vaccines [20]

Nanocarriers	Source	Size (nm)	Immune response
Spore	Bacterial	Various	Humoral-cellular
Proteosome	Membrane protein-based	20-80	Humoral-cellular
Exosome	Cellular	50-100	Cellular
Liposome	Lipid based	Various	Humoral-cellular
Virosome	Liposome + viral envelope proteins	Various	Humoral-cellular
Super-fluid	Biodegradable polymer	25-250	Humoral-cellular
Nanobead	Inert nanomaterial	40	Humoral-cellular
Virus-like particles	Viral	Various	Humoral-cellular
Phage	Bacterial	Various	Humoral-cellular

Acute respiratory distress syndromes (ARDS) are distressing diseases characterized by severe pulmonary inflammation and hypoxemic conditions, which can result in severe illness and death. The most important therapeutic approaches have been focused on a variety of ways for effectively inhibiting severe inflammations or managing the subsequent physiological imbalance, which triggers respiratory lapse. [38]

ARDS patients are now treated with a variety of treatment approaches:

- ❖ Anti-inflammatory agents - corticosteroids, pharmaconutrients, antioxidants, anti-proteases, and ketoconazole
- ❖ Ventilator agents - neuromuscular blockers, β2 agonists, and surfactants
- ❖ Diuretics
- ❖ Anti-coagulants
- ❖ Vasodilators a
- ❖ Others.

The general treatments for ARDS and blocking IL-6, IL-1, and TNF in cytokine storms may be useful for COVID-19-induced ARDS [8]. Apart from these approaches, various emerging therapies have also been reported, which include anti-inflammatory agents (statins, insulin, macrolides, MMPs, aspirin, vitamin D, anti-interleukin 8), cellular therapies (stem cells, growth factors, colony-stimulating factors) angiotensin-converting enzyme (ACE) inhibitors.

Additionally, there are some emerging treatments for COVID-19, for example, [25,26]

- Small-molecule drug-based therapies
- Immune regulation therapy, and interferon utilizing
- NK cell therapy
- Exosomes
- Pluripotent stem cells (iPSCs)

Strategies for Nanomedicine Approach for COVID-19 Therapeutics

a. Nanocarrier Selection to Bypass the Conventional Limitations of a Drug Candidate

Nanocarriers are used to prevent the systemic immunotoxicity of protein-based drugs and promote immuno-oncology therapeutics. E.g. Lipid nanoparticles (LNPs), polymeric nanoparticles were developed with a high auristatin payload.

Chemically Alter/ (Re) engineer Drugs.

Drug molecules are altered to improve their compatibility with a particular class or type of nanocarriers, rendering this a more generic approach for drug candidates with similar physicochemical properties. Use of cholesterol-modified hydroxychloroquine (Chol-HCQ) loaded liposomes that lowered the dose and toxicity of hydroxychloroquine and also inhibited the proliferation of rat lung fibroblasts, thereby, reducing pulmonary fibrosis. This strategy can be adopted to have dual benefits in SAR-COV-2 patients, which show viral load and pulmonary fibrosis.

Nanomedicine for Combination Drug Therapeutics

Combination drug therapy is another possibility for treatment of COVID-19, offering several advantages such as lower dosages of the individual drugs causing fewer side effects, achieving multiple and complimenting therapeutic targets, and reducing the likelihood of resistance development. [9]

Multidrug-loaded (anti-retroviral, latency reactivating agents, and drug abuse antagonist) PEGylated-magneto-liposomal nano formulations have shown *in vitro* and *in vivo* BBB transmigration with significant anti-HIV activity in primary CNS cells. This multifunctional nano-therapeutic strategy can be applied to target SARS-COV-2 that has migrated to the CNS. [19,29]

Plan to design nonvaccine for COVID-19

One of the bigger challenges in the COVID-19 vaccine research is to identify approaches that stimulate both the T cell and B cell immunity against this virus. Another challenge is the necessity of accelerating the development of precise “next-generation” vaccine strategies that may also address specific population subgroups or individuals with compromised immunity. [13]

The nano vaccine strategy also requires a strong focus on the cellular presentation of the selected antigen, along with the selection of appropriate nanocarrier/ nanomaterial to induce complimenting immuno-modulatory effects. The following section highlights the rational design of nanocarrier-based vaccines with two strategies

Antigen-Dependent Nanocarrier Selection

Loading antigens inside or on the surface of nanocarriers is dependent on several factors including the antigen’s physicochemical characteristics, biological stability, target sites, and required immunogen release rate. The physical adsorption of antigens on nanoparticles is based on their surface charge and non-covalent hydrophobic interactions. Antigens with an amphoteric nature are most suitable for adsorption or surface immobilization on nanocarriers such as chitosan and dextran sulfate-based polymeric nanoparticles, inorganic nanoparticles (such as AuNPs), and carbon nanotubes. [9]

Antigen release in such cases is predesigned based on the properties of the biological environment like pH, ionic strength, temperature, *etc.* Encapsulation and matrix entrapment of the antigens within a nanocarrier is another technique used to prevent its biological degradation. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles are ideal for encapsulating antigens and provide controlled or extended biological release. These nanoparticles are effective preclinically in carrying antigens such as HBsAg, malaria antigens tetanus toxoid, *Listeria monocytogenes* antigens, and *Bacillus anthracis* spores, generating prolonged cellular and humoral immune response. [9]

The mRNA-based COVID-19 vaccine is already under clinical trial employing LNPs as a carrier. Naked mRNAs are sensitive to the degradation by extracellular RNases, thus formulating its delivery vehicle is essential. LNPs are virus-sized (80–200 nm) particles synthesized by the self-assembly of an ionizable cationic lipid. They possess the ability to deliver mRNA efficiently into the cytoplasm. An ongoing clinical trial (NCT04336410) is using a DNA plasmid encoding SARS-CoV-2 S-protein as a vaccine candidate for intradermally administration using an electroporation device (CELLECTRA 2000). [9]

Vaccine Adjuvant Nanoparticles

Vaccine adjuvants nanoparticles (VANs) are considered to improve the overall efficacy and safety of the generated immune response. Vaccine adjuvants are critical to reducing the required antigen dose (dose-sparing), permitting the production of more units and making it available to the larger population. Informing specific immune cells to mount a protective immune response against a specific antigen is the basic mechanism of VANs designed to improve efficacy (by serving as immunity promoting cues, also called as “danger signals”). In the case of a virus, these danger signals are characterized as PAMPs and damage-associated molecular patterns (DAMPs) derived from the same virus. PAMPs and DAMPs are recognized by specific receptors called pattern recognition receptors (PRRs). An example of such receptors is Toll-like receptors which are expressed by immune cells to

upregulate robust T and B cell priming by releasing inflammatory cytokines. VANs can either act as a nanocarrier for molecular adjuvants or have an inherent physicochemical property to stimulate pro- or anti-immunity pathways. VANs are designed to tackle the limitations related to the conventional delivery of molecular vaccine adjuvants such as rapid bloodstream clearance, systemic distribution, and lack of immune cell targeting as well as lack of antigen-adjuvant co-localization. VANs are an established strategy to achieve a significantly high-dose-sparing effect, whereas DCs targeting VANs may enhance its adjuvanticity. VANs (including PLGA, AuNPs) are also employed to co-deliver self-antigens or immunoregulatory drugs as adjuvants to induce antigen-specific peripheral tolerance of auto-reactive T cells and block any serious autoimmune response. Nanoparticles because of their intrinsic adjuvanticity (by activating complement system, inducing autophagy and activation of inflammasome) are also considered as VANs. [9]

Scope of miscellaneous nanotechnology approaches

Advanced nanomaterial and biomimetic approaches represent good potential usage in a COVID-19-like outbreak. *Functionalized carbon quantum dots (CQDs)* to inhibit the human coronavirus (HCoV-229E) infection. CQDs of different sizes (<10 nm), surface potential (−7.9 to −39.2 mV), and functionalities were explored as inhibitors of Huh-7 cells (host cell) infection by HCoV-229E, and they showed a concentration-dependent virus inactivation. *Boronic acid-modified CQDs* showed the maximum efficacy with an EC_{50} value of 5.2 ± 0.7 µg/mL, illustrating the significance of boronic acid functionality to inhibit the early stage interaction of viral S-protein receptor with the host cell membrane. [19]

Where are we at nano vaccines?

The use of nanoparticles in vaccine formulations could fulfill three different purposes: [21]

- Enhanced antigen stability by protecting them from premature degradation by proteolytic enzymes
- Enhanced immunogenicity as an immuno-stimulant adjuvant to provoke an immune response
- Targeted delivery where nanoparticles are used as delivery systems to facilitate antigen uptake and processing by antigen-presenting cells.

Types of Nano Vaccines

Biomimetic nano vaccines

Biomimetic vaccines contain biomimetic vehicles that are loaded with target antigens. Various types of biomimetic nano vaccines such as lipid nanoparticles, protein nanoparticles, and virus-like particles can be produced with the aid of biotechnology, nanotechnology, or both. [36]

Protein sub-unit vaccine

Based on the synthetic peptides or recombinant antigenic proteins, which are necessary for invigorating long-lasting protective and/or therapeutic immune response. [25]

Subunit vaccines are composed of purified antigens instead of whole microorganisms, and different carriers serve as transporter for those antigens. In the anti-SARS-CoV-2 subunit vaccines, the antigens are represented by viral proteins, peptides, or nanoparticles. Because of the relatively low immunogenicity of the subunit vaccines, adjuvants are required to create a stronger immune response. Subunit vaccines provide a high level of safety.[41]

Advax-SM (clinical trial #NCT04453852) is an adjuvant composed of polysaccharide delta-inulin and CpG oligodeoxynucleotide (CpG ODN). CpG ODN is a TLR 9 agonist with T-helper 1 skewing properties. The granulocyte-macrophage colony-stimulating factor is a pro-inflammatory cytokine that may also serve as an adjuvant (#NCT03305341 and #NCT04386252). [31]

The University of Queensland in collaboration with GSK and Dynavax is developing stabilized pre-fusion recombinant protein subunit vaccine against SARS-CoV-2 using its Molecular Clamp technology that locks the SARS-CoV-2 specific protein in the three-dimensional shape with the ability to develop a humoral immune response against appropriate viral epitopes. Novavax has developed a protein subunit vaccine, NVX-CoV2373 by combining its nanoparticle technology. [30]

The S protein of the SARS-CoV-2 is the most suitable antigen to induce the neutralizing antibodies against the pathogen. The S Protein consists of two subunits. The S1 subunit has the NTD, RBD, and RBM domains while the S2 subunit comprises FP, HR 1, &2. The virus enters into the cell via endocytosis by utilizing the S-Protein mediated binding to the hACE2 receptor. Therefore, the S-Protein and its antigenic fragments are the prime targets for the institution of the subunit vaccine. [25]

The S glycoprotein is a dynamic protein, possessing two conformational states i.e. pre-fusion and post-fusion state. Therefore, the antigen must maintain its surface chemistry and profile of the original pre-fusion spike protein to preserve the epitopes for igniting good quality antibody responses. Moreover, the means to target the masked RBM as an antigen will enhance the neutralizing antibody response and improve the overall efficacy of the vaccine.[25]

NVX-CoV2373 (Novavax, Inc. Emergent BioSolutions)

NVX-CoV2373 is a nano-particle-based immunogenic vaccine that is based upon the recombinant expression of the stable pre-fusion, coronavirus S-Protein. The University of Queensland in collaboration with GSK and Dynavax is developing stabilized pre-fusion recombinant protein subunit vaccine against SARS-CoV-2 using its Molecular Clamp technology that locks the SARS-CoV-2 specific protein in the three-dimensional shape with the ability to develop a humoral immune response against

appropriate viral epitopes. The company plans to use the Matrix-M adjuvant to enhance the immune response against SARS-CoV-2 spike protein by the induction of high levels of neutralizing antibodies.

Molecular Clamp Stabilized spike protein vaccine candidate

It is being developed by the University of Queensland in collaboration with GSK and Dynavax. The University will have access to vaccine adjuvant platform technology (AS03 Adjuvant system), which is believed to strengthen the vaccine response and minimize the amount of vaccine required per dose. The University is developing a stabilized pre-fusion, recombinant viral protein sub-unit vaccine which is based upon the Molecular Clamp technology. This technology has been proved to induce the production of neutralizing antibodies. [25]

Triple Antigen Vaccine (Premas Biotech, India)

It is a multi-antigenic VLP vaccine prototype wherein the recombinant spike, membrane, and envelope protein of SARS-CoV-2 have been co-expressed in an engineered *Saccharomyces cerevisiae* expression platform (D-Crypt™). The proteins then undergo self-assembly as the VLP. The TEM and allied analytical data simultaneously furnished the biophysical characterization of the VLP. This prototype has the potential to enter the pre-clinical trials as a vaccine candidate after further research and development.

PittCoVacc (University of Pittsburgh)

It is a Micro-Needle Array (MNA) based recombinant SARS-CoV-2 vaccine that involves the administration of rSARS-CoV-2 S1 and rSARS-CoV-2-S1fRS09 (recombinant immunogens). The immunogenicity of the vaccine was maintained even after the sterilization using gamma radiation. The statistically significant titers of antibodies at the early stages and also before boosting, support the feasibility of the MNA-SARS-CoV-2 vaccine. [25]

NP-Based Vaccines - Virus-like particles (VLPs)

Nano-sized VLPs, which have the characteristic function of the virus, have the advantage of being better delivered through the lymph and capillaries than other small vaccines. It has the effect of reducing the systemic inflammatory response, and similar to viruses, has the advantage of being able to very easily enter cells. Therefore, the synthesized complex recognized by the T cell receptor increases the vaccine's immunogenicity and efficacy, thereby ensuring patient safety. 293 Nano-sized VLPs entering the host cell are directly involved in B cell activation and boosting the immune system.292,295 Indeed, the characteristics of these synthetic nano-sized VLPs are the principle of developing vaccine platforms.296–298 Nano-sized VLPs have also been reported to overcome viruses by increasing the immune response effectively in animal experiments. VLPs are structurally identical to the virus particles but lack a viral genome. Therefore, VLPs are termed as non-replicating and non-pathogenic viral capsid protein-based vaccines. VLPs can be either enveloped or non-enveloped. Enveloped VLPs contain viral proteins on their outer lipid membrane formed through budding from respective host cells, whereas non-enveloped VLPs lack lipid layers and are composed of one or more viral structural proteins. VLPs can be decorated on their surface to display target antigen using recombinant fusion protein expression or chemical cross-linking with bi-functional linkers. VLPs also allow encapsulation of target antigenic gene or peptide fragment for the immune cell presentation and activation. VLPs can be loaded with single or multiple capsid proteins and can be formulated in different diameters ranging from 20 to 200 nm. VLPs hold all the conformational epitopes to the native virus to allow repetitive antigen presentation.

Several VLP nano vaccines have been tested preclinically against β-coronaviruses.

MERS-CoV VLP vaccine was prepared by assembling viral S protein on the surface of nanoparticles (100-200 nm) secreted from the BM5 insect cells that co-express viral E and N proteins using mechanical extrusion. The resulting VLPs express all the viral components, S, E, and N proteins, and can bind human dipeptidyl peptidase 4 (DPP4) which is the target receptor for the MERS-CoV host entry.

A recombinant SARS-CoV VLP nano vaccine containing both the SARS-CoV S and influenza M1 proteins produced using the baculovirus Sf6 insect cell expression system was shown to elicit an immune response in mice with a reduction in lung virus titers to below detectable levels when administered intramuscularly or intranasally.

Virus mimicking particles have garnered great attention for vaccine development because they can trigger a strong immune response. Because the particles resemble the size, form, and structure of viruses' shells, they mimic the essential viral features suitable for vaccination. Another advantage of these viral particles is that they are non-infectious as they lack nucleic acids. But the eminent disadvantage of this technology is associated with the manufacturing challenges of vaccines as the virus-like particles are produced in heterologous host systems.[33]

Although many virus-like particles-based vaccines against viral infections have been undergoing clinical trials, only a few of them have been licensed and commercialized. [46]

Engerix-B (GlaxoSmithKline) was the first virus-like particles vaccine developed for hepatitis B virus (HBV) which was licensed in 1986. Similarly, Cervarix (GlaxoSmithKline) was licensed in 2009 for the prevention of human papillomavirus (HPV), and Hecolin (Xiamen Innovax) to protect from hepatitis E virus was licensed in 2012. No doubt, coronavirus-like particles with targeted structural proteins could be a potential vaccine candidate for COVID-19 control. The assembly of viral membrane proteins into particles has been well studied.

In a study, a recombinant host cell was made to express coronavirus membrane proteins which assembled into

particles and released from the cell. The released particles were spherical and identical to corona virions in size (100 nm) and shape. The study also revealed that the small envelope protein (E) and the membrane glycoprotein (M) were required for particle formation while the spike protein (S) and the nucleocapsid protein (N) were dispensable. The results were consistent with another study which demonstrated that the viral membrane proteins E and M when expressed together in eukaryotic cells, assembled into coronavirus-like particles.

Similarly, coronavirus-like particles that express one or more structural proteins of SARS CoV were produced using a heterologous expression system. In this study, a recombinant virus was used to express a high level of S, E, and M proteins simultaneously which self-assemble to produce and release the coronavirus-like particles. [6]

Nucleic acids vaccines

Currently, vaccines using nucleic acids (DNA or RNA), based on the nanotechnology approach, are aiming to help fight against coronavirus. The nucleic acids are often encapsulated in a lipid coat to insert into human cells. Following the internalization in the cells, the nucleic acids are converted into the targeted virus proteins, mostly spike proteins. These foreign proteins induce danger signals to the immune system and trigger antigen-presenting cells to engulf them. Then these engulfing cells display the foreign proteins to T cells and B cells to activate the short and long-term immunity. Nucleic acids vaccines are safe and less reactogenic. Due to the advancement of nanotechnology, these vaccines are easy to develop and free from complicated processes of manufacturing. Many biotech companies are well known for their expertise in the targeted delivery of mRNA therapeutics using multiple mRNA delivery formulations. Depending upon the therapeutic application and route of delivery, various formulations can be designed as follows:

Lipid Nanoparticles (LNPs)

The formulation is made by encapsulating mRNAs in LNPs. LNPs can be modified externally with various proteins or chemicals for targeted delivery. LNPs can transfer the encapsulated mRNAs inside the human cells to express the target antigens. [26]

Lipoplexes

The formulation is a complex of embedded mRNA in a lipid bilayer. The size and charge of the lipoplexes can be modified by changing the ratio of negatively charged mRNA and cationic lipids. The lipoplexes are served to deliver mRNA to target cells such as dendritic cells in immune compartments for antigen presentation to activate the immune system.

Polyplexes

The formulation is a polymer complex of nuclei acids. The polyplexes can be formed from a wide variety of cationic polymers and different forms of nuclei acids. The polyplexes form nanoparticles of various sizes and shapes with possible surface modifications. Therefore, the polyplexes have diverse applications in drug, gene, and vaccine delivery. [46]

Often, nucleic acid-based vaccines use LNPs to avoid the degradation of nucleic acids when administered in the body.

mRNA Vaccine

mRNA is an emerging, non-infectious, and non-integrating platform with almost no potential risk of insertional mutagenesis. Currently, the non-replicating RNA and the virus-derived self-replicating RNAs are being studied. The immunogenicity of the mRNA can be minimized, and alterations can be made to increase the stability of these vaccines. The stabilization is further achieved by various transport systems (such as lipid nanoparticles, nano-emulsions, and cationic peptides) or methods enabling facilitated transfection (gene gun and electroporation).[9]

The mRNA-based nano vaccines have benefits over other technologies which include short development time, simple manufacturing and purification processes regardless of the antigen, and most importantly, mimic natural infection to promote potent cellular and humoral immunity by eliciting CD4 and CD8 T cell responses. Multiple mRNAs can be combined into a single vaccine to deliver mRNA transcripts of interest into the host cell cytosol, which allows the encoding of one or more antigen(s). [30]

Mainly two types of mRNA constructs have been evaluated: self-amplifying mRNA and non-replicating mRNA.

mRNA-1273 (Moderna TX, Inc)

It is a vaccine composed of synthetic mRNA encapsulated in Lipid nanoparticle (LNP) which codes for the full-length, pre-fusion stabilized spike protein (S) of SARS-CoV-2. It has the potential to elicit a highly S-protein-specific antiviral response. [25]

BNT162b1 (BioNTech| FosunPharma| Pfizer)

BNT162b1 is a codon-optimized mRNA vaccine that encodes for the trimerized SARS-CoV-2 RBD, a critical target of the virus nAb. The vaccine portrays increased immunogenicity due to the addition of the T4 fibrin-derived fold on the trimerization domain to the RBD antigen. The mRNA is encapsulated in 80 nm ionizable cationic lipid nanoparticles, which ensures its efficient delivery. [25]

DNA Vaccines

The most revolutionary approach to vaccination is the introduction of the DNA vaccine which encodes for the antigen and an adjuvant that induces the adaptive immune response.

The transfected cells express the transgene which provides a steady supply of the transgene-specific proteins which is quite similar to the live virus. Furthermore, the antigenic material is endocytosed by the immature Dendritic Cells which ultimately present the antigen to the CD4+ and CD8+ T cells in association with MHC 2 and MHC 1 antigens on the cell surface hence stimulating effective humoral as well as cell-mediated immune responses. [25]

DNA vaccines deliver coronavirus genes to the human cells. The vaccination principle depends on the DNA translocation into the cell nucleus where the transcription of the antigen is initiated and followed by a translation. DNA vaccines frequently use plasmids as vectors. Depending on the route of vaccine administration (intramuscular, intradermal, and subcutaneous), either myocytes or keratinocytes are addressed. [42]

DNA vaccines are a harmless complement to conventional live- and inactivated-virus vaccines. DNA vaccines are generally safe and stable compared to conventional vaccines because the vectors used are non-replicating and express only the antigen of interest. Therefore, unlike viral vector vaccines, they are not able to revert to the disease-causing form. DNA vaccines also lack vector-induced immunity which allows their use with other vaccines in the same individual. In short time intervals, DNA vaccines are produced in bulk quantities. [25]

INO-4800 (Inovio Pharmaceuticals)

It is a prophylactic DNA vaccine against SARS-CoV-2. It uses a codon-optimized S protein sequence of SARS-CoV-2 to which an IgE leader sequence is affixed.

5.4 Viral Vectored vaccines

A vaccine based on viral vectors is a promising prophylactic solution against a pathogen. These vaccines are highly specific in delivering the genes to the target cells, highly efficient in gene transduction, and efficiently induce the immune response. They offer a long term and high level of antigenic protein expression and therefore, have a great potential for prophylactic use as these vaccines trigger and prime the cytotoxic T cells (CTL) which ultimately leads to the elimination of the virus-infected cellsViral vector-based vaccines (VBVs) are constructed by engineering a viral vector to carry coronavirus genes and slowly replicate in the host cells. The replication leads to the production of coronavirus proteins and subsequent immune system activation. Potential viral vectors include a broad spectrum of both DNA and RNA viruses. [22]

These viral vectors can be constructed as replicating or non-replicating vectors In SARS-CoV-2 vaccine development, the most commonly used vectors are the adenoviral vectors, such as ChAdOx (#NCT04536051 and #NCT04516746), adenovirus type 5 (#NCT04564716, #NCT04540419, and #NCT04526990), and adenovirus type 26 (#NCT04564716 and #NCT04505722). All these vaccines are currently being evaluated in phase III clinical trials.

Existing viral vectors can be divided into two categories: replicating (replication-competent) and non-replicating (replication-deficient).[25] The replicating viral vector produces infectious viruses capable of infecting target cells to produce viral antigens. AD26 associated vaccine against SARS-CoV-2 showed complete protection in rhesus macaques after infection. Thereafter, a series of AD26 vectors encoding different SARS-CoV-2 spike protein epitopes were developed with encouraging outcomes. Recently, Russia approved a COVID-19 vaccine Sputnik V exhibiting two different adenovirus vectors (rAd26 and rAd5), both carrying the gene for SARS-CoV-2 S glycoprotein (rAd26-S and rAd5-S). [25]

Developed by Gamaleya Center in Russia, the two-vector vaccine, as the name suggests, uses two different vectors (Ad5 and Ad26). Vector development involves the use of S-protein mRNA to generate the complementary DNA, followed by insertion of this S-protein encoding DNA into adenoviral vectors, Ad26 and Ad5. Ad26 vector encoding S-protein was administered as the first vaccination followed by a booster dose of Ad5 vector encoding the same S-protein 21 days later. Inside the recipient, these vectors generate the S-proteins, which upon entering the circulation induce protective immunity. The illustration is prepared in-house and schematic ideas and technical details were followed as presented in the previously published report. [25]

Ad5-nCoV (CanSino Biologics Inc | Beijing Institute of Biotechnology)

It is a recombinant, replication-defective adenovirus type-5 vector (Ad5) expressing the recombinant spike protein of SARS-CoV-2. It was prepared by cloning an optimized full-length gene of the S Protein along with the plasminogen activator signal peptide gene in the Ad5 vector devoid of E1 and E3 genes. The vaccine was constructed using the Admax system from the Microbix Biosystem.

Coroflu (University of Wisconsin-Madison | FluGen | Bharat Biotech)

M2SR, a self-limiting version of the influenza virus, is modified by the insertion of the SARS-CoV-2 gene sequence of the spike protein. Furthermore, the vaccine expresses the hemagglutinin protein of the influenza virus, thereby inducing an immune response against both viruses. The M2SR is self-limiting and does not undergo replication as it lacks the M2 gene. It can enter into the cell, thereby inducing immunity against the virus. It shall be administered intra-nasally, mimicking the natural route of viral infection. This route activates several modes of the immune system and has higher immunogenicity as compared to intramuscular injections. [25]

LV-SMENP-DC (Shenzhen Geno-Immune Medical Institute)

The LV-SMENP-DC vaccine is prepared by engineering the dendritic cells (DC) with the lentiviral vector expressing the conserved domains of the SARS-CoV-2 structural proteins

and the protease using the SMENP minigenes. The subcutaneous inoculation of the vaccine presents the antigens on antigen-presenting cells (APCs) that ultimately activate the Cytotoxic T cells and generate the immune response. [25]

ChAdOx1 (University of Oxford)

ChAdOx1 recombinant adenovirus vaccine was developed using codon-optimized S glycoprotein and synthesized with the tissue plasminogen activator (tPA) leader sequence at 5' end. The sequence of SARS-CoV-2 coding for amino acids (2 to 1273) and the tPA leader and was propagated in the shuttle plasmid.

The Adenovirus vector genome is constructed in the Bacterial Artificial Chromosome by inserting the SARS-CoV-2 S gene into the E1 locus of ChAdOx1 adenovirus genome.[15]

Live Attenuated Vaccines

These use live viruses to elicit protective immune responses. Live vaccine raises the possibility of viral virulence reversal. Therefore, live virus vaccines need to be developed by altering the viral genome and selecting non-pathogenic mutant strains incapable of causing disease. Live virus vaccines can also be produced by viral attenuation. These are viruses weakened in their pathogenicity but can elicit antiviral immune responses without causing disease.

DelNS1-SARS-CoV2-RBD (University of Hong Kong)

This LAV is an influenza-based vaccine strain with a deletion in the NS1 gene. It is re-organized to express the RBD domain of SARS-CoV-2 spike protein on its surface and, is cultivated in the chick embryo and/or Madin Darby Canine Kidney Cells (MDCK) cells. It is potentially more immunogenic than the wild type influenza virus and can be administered as a nasal spray

Inactivated vaccines

These viruses are inactivated by radiation, heat, and chemicals such as binary ethyleneimine and formalin. This leads to an inability to cause illness. However, inactivated viruses maintain the ability to induce host immunity that recognizes and destroys pathogens. This vaccine type contains viral particles from inactivated virus and hence do not develop pathogenicity. The inactivated vaccine developed against poliovirus is an example, where the virus is inactivated by formaldehyde treatment. Nine SARS-CoV-2 vaccines are being developed using this technology. Sinovac Biotech is developing inactivated vaccine PiCoVacc which is now known as CoronaVac against SARS-CoV-2 and this candidate induces broadly neutralizing antibodies against ten different viral strains in multiple species that include primates. [35]

Inactivated vaccines are based on presenting the form of the pathogen with a loss of disease-producing capacity. The virus cultivation occurs in cell lines that represent a substrate for the production of large quantities of antigen. Virus multiplication is often followed by purification and concentration before the vaccine inactivation. Formaldehyde and beta-propiolactone are used in the majority of licensed human antiviral vaccines to inactivate the virus. Multiple doses or adjuvants are required to achieve sufficient efficacy of inactivated vaccines. To date, 4 inactivated vaccines have reached the phase III clinical trials and are currently under evaluation (#NCT04510207, #NCT04508075, and #NCT04456595). [22,23]

Whole inactivated vaccine

Whole inactivated vaccines are composed of chemically or radiationally inactivated virions. They contain a full repertoire of immunogenic components of the original virus, and compared with attenuated viruses, they carry no risk of viral reactivation if properly inactivated. Although safer than live attenuated vaccines, the immunogenic epitopes of inactivated viruses may be structurally deformed during the inactivation process, which can undermine the protection they may provide. Moreover, both SARS-CoV and MERS-CoV whole inactivated vaccines have been reported to induce eosinophil-related lung pathology. These disadvantages make whole-inactivated vaccines a less attractive strategy for coronavirus vaccine development. Currently, there are 7 whole inactivated COVID-19 vaccines in clinical trials. [26]

Whole Inactivated Virus Vaccine of SARS-CoV induces eosinophilic pro-inflammatory pulmonary response. Immunization with SARS-CoV virus-like particle (VLP) vaccine leads to eosinophilic immunopathology in the lung after viral challenge. They found that appropriate adjuvants, such as Toll-like receptor agonist and delta-inulin polysaccharide, can increase serum neutralizing antibody titers and reduce lung eosinophilic immunopathology. Their results provide a promising strategy to deal with the T2-skewed immune response induced by some CoV vaccines. [26]

Others

The revelation of the structure and genome of the SARS-CoV-2 has led to the rapid development of various vaccine candidates with potential immunogenicity but also adverse reactogenicities. The task of vaccine development is long and cumbersome which requires evaluation in some long-lasting clinical trials.

Various Biotech ventures are using different technologies for the development of their vaccine candidates; British and American Tobacco Company (BAT) recently unfolded the COVID-19 vaccine using their new, and fast-growing tobacco plant technology, while Tianjin University has developed an oral vaccine that has successfully employed *Saccharomyces cerevisiae* to carry the S protein. The GRAS (Generally Regarded as Safe) status of the yeast provides high scalability, robustness, and cost-effective production of cosmic dosages required to fight off this pandemic. Furthermore, *in silico* studies, using various databases like VaxiJen, have revealed that the epitope

sequences WTAGAAAYY and YDPLQPEL can be employed for the formulation of epitope-based peptide vaccines.

Self-Assembling Vaccine (HaloVax)

The vaccine uses a heat shock protein (hsp) to activate the immune system. It is composed of a fusion protein sandwiched between an hsp and Avidin. Biotinylated immunogenic peptides are also incorporated to customize the vaccine. [25]

Safety and Hazards of Nano Vaccine

The accumulation of therapeutics at nontargeted or undesired locations is one of the most difficult problems in the treatment of COVID-19. By using active targeting of nano-vehicles to guide therapeutics to the targeted site of action, this may be greatly reduced. It is also feasible to target intracellular and cellular sites, as well as specific organs, such as cathepsin binding sites, ACE2 expressing cells, and viral S protein domains, all of which are involved in SARS-CoV-2 pathogenesis. The controlled and sustained release of drugs from nano vehicles can improve patient compliance, reduce adverse effects, reduce dose quantity and frequency, and lower the danger of viral rebound during viral infection therapies. [42]

What are the risks associated with nanotechnology?

Nanotechnology is potentially hazardous for three reasons: [5,7,16]

1. Nanoparticles (NPs) may cause lung damage: ultra-fine particles.
2. Carbon nanotubes inhaled can suppress the immune system by altering the activity of T- cells, a kind of immune cell that helps the body fight infections.
3. Brain damage - Discrete nanometer-sized particles were deposited in the nasal cavity (in this case in rats), completely bypassing the blood-brain barrier and traveling up the olfactory nerves straight into the brain.

Overall, the importance of nanotechnology and nanoscience cannot be overstated, yet these promising materials have the potential to create serious problems in the lungs and respiratory systems.

Cell toxicity, fibrosis, inflammation, oxidative stress, genotoxicity, and *immunotoxicity* are the six primary pathobiological factors that must be considered when employing nanoparticles or related methods to treat present and future coronavirus infections.

Despite extensive research on nanotechnology-based methods (polymeric, inorganic self-assembling materials, PBNPs, and nano vaccine) to combat COVID-19, there have been significant disadvantages, including cost, time, and possible cell toxicity of these nanoparticles. [42]

The oxidative stress typically arises due to disparity amongst the reactive oxygen species (ROS) production and the capability of a biological system to voluntarily eradicate the reactive moieties. Sometimes, it could be caused directly due to the generation of ROS within the cell or indirectly disturbing the mitochondria respiration, or reduce the antioxidant moieties inside the cell environment. The hindrance of oxidative stress might act as a significant phase in initiating few harmful pathobiological activities within the cellular micro-environment. Moreover, the effect of NPS over the oxidative stress conditions in the animal models or at the cellular level is usually considered as a common endpoint study to detect the toxicity profile of NPs. Both in vivo and in vitro studies play a crucial role in understanding the mechanisms of NPs causing oxidative stress. For example, studies have shown significant accumulation of titanium dioxide NPs (TiO2-NPs) in the lungs of mice after a 90-day successive intra-tracheal administration of TiO2 NPs. The TiO2-NPs expressively enhanced the accumulation of ROS level, inflammation, lipid peroxidation level, and also reduced the antioxidants competency in the lungs. The NPS could produce ROS followed by oxidation of antioxidant moieties, and thus could influence the respiratory system and associated pathobiological activities including pulmonary inflammation and genotoxicity. [38]

In some cases, it has been observed that the NPs, administered through the nasal route, caused chronic or acute inflammation-mediated processes such as the inclusion of inflammatory cells and proclamation of cytokines. In one of the studies, it was noticed that the direct administration of graphene oxide (GO) solution into the lungs of C57BL/6 mice caused extreme pulmonary inflammation with alveolar exudate. The NPs are involved in triggering few pro-inflammatory pathways, including mitogen-activated protein (MAP) kinases. The NPs-treated cells have shown an increased level of AP-1 (activator protein-1) transcription factors and NF-κB (nuclear factor kappa enhancer of triggered B cells), thus affecting the DNA transcription, production of cytokines, and survival of cells. [38]

Genotoxicity, either primary or secondary, is a major concern when using NPs to deliver drugs. The genotoxic moieties or NPs influence directly by attaching to DNA structures or constituents of cellular division such as the microtubule spindle or centromere. Carbon nanotubes (CNTs) have made direct contact with DNA assemblies. This indicated that CNTs might be genotoxic in vivo (in animal models) or at the cellular level. According to studies, inhaling multi-walled CNTs induced genotoxicity by generating chronic inflammation, which resulted in persistent oxidative stress. [38]

Fibrosis is an indication of an accumulation of inhaled NPs in the lungs, and it can lead to unusual forms of pulmonary inflammation including eosinophilia. In one study, single-walled CNTs inhaled into the lungs of C57BL/6 male mice produced multifocal granulomatous pneumonia and fibrosis.

There are immuno-pathological problems linked with the SARS-CoV vaccines that need to be addressed and improved. [26]

- The production of *antibody-dependent enhancement (ADE)* effect, which is generally produced by vaccine-induced inadequate antibodies that aid viral entrance into the host cell, is one of the side effects**.** Design vaccines that only include significant neutralizing epitopes, such as the S1 subunit or the RBD domain of the S protein, to address the ADE issue. This approach can decrease the ADE impact by reducing the induction of non-neutralizing antibodies by CoV vaccines.
- Vaccine-induced eosinophilic immunopathology, which is an undesirable T2-skewed immunological response generated by vaccination, is another potential adverse effect. [26]

These particles are toxic at the molecular, cellular, and tissue levels. [43]

- ✓ At the tissue level: inflammation - damage may occur.
- ✓ At the cellular level: reactive oxygen species - disruption of compartments.
- ✓ At the molecular level: conformational change, loos of function, and aggregation.

Nanomedicine toxicity has been examined numerous times. Carbon-based nanoparticles, for example, have been shown to have a variety of laboratory and clinical toxicities, and carbon nanotubes have been shown to cause mesothelioma. Surprisingly, the toxicity was ascribed to its form rather than the elements themselves.[43]

The biocompatibility of drugs is also determined by the size of nanoparticles. Au nanoparticles, for example, were toxic at 1.4 nm but not at 15 nm. Ag nanoparticles have also been proven to have toxic effects. Because iron can accumulate in the immune system, the majority of the toxins identified in NMPs were related to iron nanoparticle toxicity. [11]

This toxicity has been reported to be reduced in a variety of ways. The addition of hydroxyl groups to gadolinium fullerene particles, for example, can prevent the production of reactive oxygen species (ROS). Iron oxide nanoparticles with polymer coatings can also improve cell viability significantly. Selective targeting and preservation of healthy organs are the most important steps in reducing their side effects. Surface modification is another option [51].

Nanotechnology-based ideas to combat COVID-19 in the future:

- ✓ The potential of nanoparticles to circumvent the traditional restrictions associated with antiviral drugs is used in the design and development of successful COVID-19 treatment.
- ✓ Nano-vehicles are being used in combination therapeutics.
- ✓ Decorating specific (nano) targeting moieties on the surface of nanomaterials allows for active targeting.
- ✓ Rapid detection with nano-biosensors.
- ✓ Surface coatings having antiviral properties created by incorporating nanoparticles into the polymer matrix.
- ✓ Nanomaterials' capacity to act as disinfectants.
- ✓ Developing vaccines with nanomaterials and chemically altering/reengineering medicines. [42]

Nano vaccine's Drawbacks

1. Due to the restrictions of nanoparticles in vaccine manufacture, the toxicity, and difficulty in presenting naive antigens are often mentioned [28]. The manufacturing of nanoparticles is hard to process [35]. One of the harder processes is scaling up nano polymers [18]. However, with the introduction of the latest techniques like scaled-up methodology for spray drying, certain scale-up difficulties are overcome, yet during a one amongst one in every of one among the foremost significant issues facing nano vaccines are scaling up in a sterile environment [39].
2. Nanoparticles' small size allows them quick access to numerous biological tissues and organs, which could be a double-edged sword [36]. Intradermal injections of the nano vaccine can cause dermatological problems; oral administration can cause gastrointestinal problems, and nasal delivery might cause respiratory difficulties [44]. Cardiovascular problems also can be caused by the parenteral method [37]. Furthermore, if these nanomaterials are ready to transit the barrier, they'll induce brain injury [27]. The aggregation of nanoparticles could trigger vascular thrombosis, consistent with a rat study [41].
3. On the opposite side, the utilization of nanoparticles in vaccines might cause nanoparticle accumulation within the cell, which may cause problems, particularly in long-term exposures [18]. Quantum dots, for instance, are shown to remain within the body for up to 2 years during a rat research [40].
4. Despite the very fact that ISCOMS are utilized during a sort of vaccination, the utilization of saponin-based adjuvants in humans has been restricted thanks to their toxicity [20]. The usage of nanoparticles in humans remains a source of worry [22].
5. Nano vaccines may create additional toxicity and other risks of their own, which might need to be addressed concurrently with nano vaccine development, influencing their design and testing [29].
6. Biopersistance is often a double-edged sword because a vaccine must work for an extended time before

booster injections are required, but persistent adverse effects are unacceptable [23].

7. Nanoparticle adjuvants for vaccines must be created to reinforce vaccine absorption and immunogenicity, but they need to also biodegrade as quickly as feasible, whereas conventional vaccines don't require biodegradation [28].
8. Because the system operates at the nanoscale, therapeutic enhancement frequently veers perilously on the brink of unintended toxicity; many nanoparticles are easily picked up by phagocytes, which can or might not be what the designer intended, counting on the condition being vaccinated against it [37].
9. An autoimmune response against the system is going to be triggered if nanoparticles are mistakenly identified as foreign, leading to harmful effects and even death [40].
10. Nanoparticles also interact with other critical immune mechanisms, like the complement system, whose activation by nanoparticles might be deleterious, like in tumor locations, where complement activation is understood to market tumors [29].
11. Some challenges with risk management are unique to developing countries:
    a) Traditional markets are being displaced [28].
    b) Foreign values are being imposed [28].
    c) Concerns that technology advancements are going to be unrelated to development goals [28].
    d) Inadequate resources for establishing, monitoring, and enforcing safety rules [28].
12. If a household can afford the medications to successfully treat a disease after it strikes, seeking out vaccination against sickness might not be considered a priority 40.
13. Eliminating the disparity in nano vaccine can't be as simple as offering resources to everyone without also considering how local people understand and use these services 39.
14. Because certain possible hazards are yet unknown, the subsequent are the first concerns of nano vaccine technologies:
    a) Variations in toxicology/biocompatibility [25].
    b) Reproducibility of nano formulations on a broad scale [25].
    c) Toxicological concerns, notably long-term organ accumulation [15].
15. Nanoparticle toxicity is difficult to quantify, especially when trying to quickly screen an outsized number of nano formulations for vaccines or other medications [38].
16. Many of the negative impacts of nanoparticles on humans are thought to be the consequence of long-term, low-level exposure, which is difficult to quantify and requires extensive testing [37].
17. Researchers are performing on developing greater throughput assays for chemical fingerprints that would indicate long-term issues with nano vaccines [44].
18. However, obtaining accurate chemical markers is difficult, and our knowledge of the long-term effects of nanoparticle exposure is comparatively limited 29.
19. Research into their function and therefore the particular mechanisms of nano vaccine are limited [40].
20. Nanomedicine toxicity, scaling-up processes, and a scarcity of regulatory requirements are all potential hindrances within the development of nano vaccines[38].
21. Nanoscale size is often a double-edged sword since preclinical and clinical studies on nano vaccines revealed dose-dependent acute and chronic toxicities, also as preferential bioaccumulation counting on the route of administration [36].
22. Only a couple of sorts of nanoparticles, like inorganic nanoparticles, have inherent toxicity after prolonged exposure (e.g., metallic nanoparticles) [37].
23. ISCOMS are employed during a sort of animal vaccinations, but their use in humans was forbidden thanks to the toxicity of saponin-based adjuvants. As a result, there are still worries about the utilization of nanoparticles in vaccines [31].
24. Scaling up is another key issue that has been somewhat mitigated by technical developments, but scaling up during a sterile environment remains a considerable obstacle [18].
25. a previous assessment of autoimmune adverse effects caused by nano vaccine-induced immune activation is required. Overactivation of antigen-presenting cells by nano vaccine is often harmful because it can cause dendritic cell mortality [40].
26. The increased complexity, manufacturing cost, and commercialization hurdles related to nano vaccine treatment necessitate solutions to minimize these restrictions [28].
27. Multiple loading of diverse components like antigens and adjuvants during a single nanoplatform is hard and demanding [44].
28. Thanks to their stability and cost-effective manufacture in an efficient manner from batch to batch, scaling up nano vaccines is additionally an enormous difficulty [27].
29. Because inhalation is that the commonest channel of access for humans, the method of nanoparticle aggregation in inhaled air within the context of nano vaccine is often detrimental [28].
30. When the dimensions of a chemical are reduced to the nanoscale level, the surface-to-volume ratio skyrockets, leading to more molecules of the chemical being present on the surface, increasing the intrinsic toxicity [37].

31. In comparison to barium sulfate when dosed at mass burden in milligrams, titanium dioxide caused more severe lung inflammation and particle lymph gland burden in trials of low toxicity particles [18].
32. Lung irritation could also be a side effect of inhaled nanoparticles about nano vaccine [18]
33. Several hypotheses are offered to elucidate the negative health impacts of nanoparticles, including: -

Particle characteristics

- the necessity of a broad area for interactions with cells and tissues [37].
- The creation of complexes with biomolecules [40].
- In comparison to greater particles, a better level of radical species is formed [13].
- Increased oxidative stress induction [40].
- Cellular DNA damage is induced [23].
- Lipid peroxidation induces oxidative stress [29].
- Distribution: -
- Size-dependent deposition properties [26].
- Uptake by respiratory epithelial cells [28].
- Increased interstitial space access [39].
- circulation access [44]
- Organ system effects including effects on immune & inflammatory systems: -
- Reduced macrophage function, reduced particle phagocytosis, reduced macrophage mobility, and cytoskeletal dysfunction [10].
- Increased pro-inflammatory activity and production of cytokines and other mediators [45].
- Negative effects on cardiac functions and vascular homeostasis [18].

34. Metals that are not particularly dangerous on their own might be poisonous when breathed as nanoparticles [36].
35. The nerve olfactory is another possible pathway for inhaled nanoparticles to enter the body; nanoparticles may pass the mucosa inside the nose then attend the brain via the olfactory nerve [27]. within the case of poisonous nanoparticles in nano vaccine, this might be damaging to the human body [37].
36. tract disorders and other contaminants can alter the pulmonary inflammation and oxidative stress caused by nanoparticles within the elderly [29].
37. Nanoparticle toxicity is decided by their chemical makeup, also because the chemical makeup of any substances adsorbed onto their surfaces 28. If the chemical composition of nanoparticle surfaces isn't carefully adjusted, they will be harmful to human health [44].
38. The repetition of the assembly of water-soluble nano-size formulations, also because of the combination and discovery of immunological responses, issues during this nanotechnology that require to be addressed through follow-up clinical trials [26].
39. Nano vaccines may cause toxicity if they're not cleared for an extended time [23].
40. Other big hindrances are unchanging production, transit, and storage [29].
41. Nanoparticles have the power to pass the barrier, which may be a significant disadvantage because nanoparticles employed to move medications might be hazardous to the brain [22].
42. For determining the interaction of hazardous effects of nanoparticles with biological systems, doses are quantified in terms of the mass because the adverse effects of any substance are hooked into the mass of the substance [39].
43. A recent study revealed that carbon nanotubes have high toxicity, implying that they cause harm via a unique mechanism, distinct from that of poisonous dust [36].
44. Induction of oxidative stress in cells and organs is assumed to be one mechanism of nanoparticle toxicity [38].
45. The toxicological study should include testing for nanoparticle interactions with proteins and various cell types [37].
46. Reducing the dimensions to the nanoscale level leads to a huge rise within the surface-to-volume ratio, which suggests that more chemical molecules are present on the surface, increasing the intrinsic toxicity 40. in comparison on a mass dosage basis, this might be one among the explanations why nanoparticles are generally more harmful than bigger particles of an equivalent insoluble material [29].
47. Nanotubes are a specific sort of fiber with a diameter of a couple of nanometres but a length of several micrometers. The carcinogenic effects of particular asbestos fibers should be considered while assessing risks [45].
48. Nanoparticles could also be ready to translocate from the lungs into the bloodstream, exposing internal organs to varying degrees of systemic exposure. Inducing an inflammatory response might cause neurological reactions that are damaging to the human body [23].

Table 2: List of Nano vaccines under clinical trial

Name of vaccines	Type of vaccines	Phases	Remarks
mRNA-1273	(LNP)-encapsulated modified mRNA	Phase I Phase II	The first vaccine completes the preclinical trials and enters the human clinal trials. The vaccine is found to be safe and effective in volunteers aging between 18-55 years. After getting positive results from the phase I and II trials now started dosing the vaccine for phase III trials. [10,35]
BNT162b2	3LNP-mRNAs	Phase III	Phase I was conducted in the US and Germany approved that the vaccine is safe and has high immunogenic properties. Now BioNTech and Pfizer are recruiting volunteers from the US, Brazil for the conduction of phase II/III trials. 41
NVX-CoV2373	Recombinant protein nanoparticle vaccine	Phase II	Phase I clinical trials was conducted in Australia. After the trials, the vaccine is termed as safe and is capable of producing antibody responses against SARS-COV-2. Now the phase II clinical trials are conducting in South Africa. [38]
INO-4800	DNA plasmid vaccine	Phase I /Phase II	In Phase 1 clinical trial is started with 40 healthy volunteers where 94% of participants showed protective immunogenic responses six weeks after two doses (1 and 2mg) of INO-4800. [32] After the early satisfactory results, Inovio is now recruiting healthy participants for the conduction of the Phase 2/3 trial in South Korea and China. [11]
Covigenix	DNA vaccine	Phase I /Phase II	In the pre-clinical studies, the vaccine shows all the potency requirements i.e. high immunogenic responses, safety, and efficacy. So, after completing the preclinical trials now the vaccine is about to enter phase I/II trials. [13]
ZyCov-D	plasmid DNA vaccine	Phase I /Phase II	Vaccine shows positive and protective immunogenic results in mice, rats, guinea pigs, and rabbits, now the company is enrolling healthy volunteers for phase I/II trials. [62]
GX-19	DNA vaccine	Phase I	After completing the preclinical trials now 120 volunteered are recruited for the initiation of phase I clinical trials. [4]
LNP-nCoVsaRNA	mRNA vaccine	Phase I	Acuitas Therapeutics (Vancouver) in collaboration with Imperial College (London) developed a self-amplifying RNA (saRNA) lipid nanoparticle encapsulated with the pre-fusion stabilized SARS-CoV-2 S protein. They are characterized for both the humoral and cellular immune response, as well as the neutralization capacity of a pseudo-typed SARS-CoV-2. The mice were immunized with two injections, one month apart, at specific doses ranging from 0.01 µg to 10 µg. After 6 weeks, robust SARS-CoV-2 S protein-specific IgG antibodies were seen in animals in a dose-dependent manner. [33]
LNPCureVac - CVnCoV	mRNA vaccine	Phase II	After the preclinical trial the vaccine has secured confirmation from the German Health Authority Paul-Ehrlich-Institute (PEI) and Belgian Federal Agency for Medicines and Health Products (FAMHP) for the conduction of phase I clinical trials. [12]
AG0302-COVID19	DNA Plasmid	Phase III	After the preclinical trials, then phase I trials started June 29 2020 with 30 healthy volunteers aged 20-65. The trial is conducted in Osaka, Japan. Then after the phase II trials started on August 31 2020 with 30 healthy volunteers aged 20-65. The trials are conducted in Osaka, Japan. After that phase III trials are initiated on November 23 2020 with 500 healthy volunteers aged above 18. The trials are going on in Japan.

CONCLUSION

The recent outbreak of the COVID-19 virus causes huge destruction to all or any of the continents around the world claiming thousands of lives by the disease. So, there's an urgent got to develop an efficient therapeutic strategy to save lots of many lives. Although researchers and scientists from universities and corporations from everywhere the planet are put considerable efforts to seek out a cure for COVID-19 no specific therapy has been identified. While several vaccines made by conventional technologies are still under preclinical and clinical evaluation now, new vaccines using nanotechnology also are on the race of coronavirus vaccine trials. The scope of nanotechnology-based vaccines is going to be determined by the outcomes of ongoing clinical trials.

Nanotechnology-based mRNA, DNA, and recombinant protein vaccine offer large advantages like they will be delivered into the targeted host cells to create immunity against the coronavirus wouldn't are feasible without nanoparticles as a delivery vehicle. Nanoparticles also

provide the solutions to deal with the unmet delivery challenges related to the utilization of naked DNA plasmids or mRNA for vaccine development. Such solutions provide the power to deliver DNA plasmids or mRNA vaccines in areas previously unreachable. additionally, nanotechnology-based vaccines are easy to style, synthesize, or proportion in larger volumes compared to the traditional vaccine approaches (e.g., inactivated and live-attenuated strains). Nanotechnology thus has great potential to be a crucial tool for tackling the COVID-19 outbreak and will be a crucial technology to prevent future disease outbreaks.

REFERENCES

1. Ferreira AJ, Cemlyn-Jones J, Robalo Cordeiro C. Nanoparticles, nanotechnology and pulmonary nanotoxicology. Rev Port Pneumol [Internet]. 2013; 19(1): 28–37. Available from: http://dx.doi.org/10.1016/j.rppneu.2012.09.003
2. Gill P. Nanocarriers, nanovaccines, and nanobacteria as nanobiotechnological concerns in modern vaccines. Sci Iran [Internet]. 2013; 20(3): 1003–13. Available from: http://dx.doi.org/10.1016/j.scient.2013.05.012
2. Emery SL, Erdman DD, Bowen MD, Newton BR, Winchell JM, Meyer RF, et al. Real-Time Reverse Transcription-Polymerase Chain Reaction Assay for SARS-associated Coronavirus. Emerg Infect Dis. 2004; 10(2): 311–6.
3. Liu D, Liu J, Ma B, Deng B, Leng X, Kong D, et al. A simple self-adjuvanting biomimetic nanovaccine self-assembled with the conjugate of phospholipids and nucleotides can induce a strong cancer immunotherapeutic effect. Biomater Sci. 2021; 9(1): 84–92.
4. Gheibi Hayat SM, Darroudi M. Nanovaccine: A novel approach in immunization. J Cell Physiol. 2019; 234(8): 12530–6.
5. Arnold D. Pandemic India: Coronavirus and the uses of history. J Asian Stud. 2020; 79(3): 569–77.
6. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol [Internet]. 2021; 19(3): 171–83. Available from: http://dx.doi.org/10.1038/s41579-020-00461-z
7. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021; 19(3): 155–70.
8. Bergmann CC, Silverman RH. COVID-19: Coronavirus replication, pathogenesis, and therapeutic strategies. Cleve Clin J Med. 2020; 87(5): 321–7.
9. Sinha N, Balayla G. Hydroxychloroquine and COVID-19. Postgrad Med J. 2020; 96(1139): 550–5.
10. Maier HJ, Bickerton E, Britton P. Coronaviruses: Methods and protocols. Coronaviruses Methods Protoc. 2015; 1282(1): 1–282.
11. Karia R, Gupta I, Khandait H, Yadav A, Yadav A. COVID-19 and its Modes of Transmission. SN Compr Clin Med. 2020; 2(10): 1798–801.
12. Chauhan G, Madou MJ, Kalra S, Chopra V, Ghosh D, Martinez-Chapa SO. Nanotechnology for COVID-19: Therapeutics and Vaccine Research. ACS Nano. 2020; 14(7): 7760–82.
13. Zhu G, Zhang F, Ni Q, Niu G, Chen X. Efficient Nanovaccine Delivery in Cancer Immunotherapy. ACS Nano. 2017; 11(3): 2387–92.
14. Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID- 19 . The COVID-19 resource centre is hosted on Elsevier Connect , the company ' s public news and information website . Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories , such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source . These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active . 2020; (January).
15. Sekhon B, Saluja V. Biosimilars: an overview. Biosimilars. 2011; Volume 1: 1–11.
16. Zhu G, Liu Y, Yang X, Kim YH, Zhang H, Jia R, et al. DNA-inorganic hybrid nanovaccine for cancer immunotherapy. Nanoscale. 2016; 8(12): 6684–92.
17. Maina TW, Grego EA, Boggiatto PM, Sacco RE, Narasimhan B, McGill JL. Applications of Nanovaccines for Disease Prevention in Cattle. Front Bioeng Biotechnol. 2020; 8(December): 1–20.
18. Patel M, Shahjin F, Cohen JD, Hasan M, Machhi J, Chugh H, et al. The Immunopathobiology of SARS-CoV-2 Infection. FEMS Microbiology Reviews. Oxford University Press; 2021. 1–31 p.
19. Borm PJA. Future interactions in Particle Toxicology: The role of PFT. Part Fibre Toxicol. 2008; 5: 1–2.
20. Yadav HKS, Dibi M, Mohammad A, Srouji AE. Nanovaccines formulation and applications-a review. J Drug Deliv Sci Technol [Internet]. 2018; 44(September 2017): 380–7. Available from: https://doi.org/10.1016/j.jddst.2018.01.015
21. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends Immunol. 2020; 41(12): 1100–15.
22. Lin P, Wang M, Wei Y, Kim T, Wei X. Coronavirus in human diseases: Mechanisms and advances in clinical treatment. MedComm. 2020; 1(3): 270–301.
23. Liu L, Liu Z, Chen H, Liu H, Gao Q, Cong F, et al. Subunit Nanovaccine with Potent Cellular and Mucosal Immunity for COVID-19. ACS Appl Bio Mater. 2020; 3(9): 5633–8.
24. Nandedkar TD. Nanovaccines: Recent developments in vaccination. J Biosci. 2009; 34(6): 995–1003.
25. Sivasankarapillai VS, Pillai AM, Rahdar A, Sobha AP, Das SS,

Mitropoulos AC, et al. On facing the SARS-cov-2 (COVID-19) with combination of nanomaterials and medicine: Possible strategies and first challenges. Nanomaterials. 2020; 10(5): 1–23.

26. Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP, et al. Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N Engl J Med. 2020; 383(16): 1544–55.

27. Rashidzadeh H, Danafar H, Rahimi H, Mozafari F, Salehiabar M, Rahmati MA, et al. Nanotechnology against the novel coronavirus (severe acute respiratory syndrome coronavirus 2): Diagnosis, treatment, therapy and future perspectives. Nanomedicine. 2021;16(6):497–516.

28. Bachmann MF, Jennings GT. Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol. 2010; 10(11): 787–96.

29. Paulis LE, Mandal S, Kreutz M, Figdor CG. Dendritic cell-based nanovaccines for cancer immunotherapy. Curr Opin Immunol [Internet]. 2013; 25(3): 389–95. Available from: http://dx.doi.org/10.1016/j.coi.2013.03.001

30. Teleanu DM, Chircov C, Grumezescu AM, Teleanu RI. Neuronanomedicine: An up-to-date overview. Pharmaceutics. 2019; 11(3): 1–23.

31. Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, Verma V. COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. PLoS Pathog [Internet]. 2020; 16(8): e1008762. Available from: http://dx.doi.org/10.1371/journal.ppat.1008762

32. Bonner JC. Nanoparticles as a potential cause of pleural and interstitial lung disease. Proc Am Thorac Soc. 2010; 7(2): 138–41.

33. Young B, Tan TT, Leo YS. The place for remdesivir in COVID-19 treatment. Lancet Infect Dis [Internet]. 2021; 21(1): 20–1. Available from: http://dx.doi.org/10.1016/S1473-3099(20)30911-7

34. Li Y Der, Chi WY, Su JH, Ferrall L, Hung CF, Wu TC. Coronavirus vaccine development: from SARS and MERS to COVID-19. J Biomed Sci. 2020; 27(1): 1–23.

35. Campos EVR, Pereira AES, De Oliveira JL, Carvalho LB, Guilger-Casagrande M, De Lima R, et al. How can nanotechnology help to combat COVID-19? Opportunities and urgent need. J Nanobiotechnology [Internet]. 2020; 18(1): 1–23. Available from: https://doi.org/10.1186/s12951-020-00685-4

36. Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini E, et al. Immune-mediated approaches against COVID-19. Nat Nanotechnol [Internet]. 2020; 15(8): 630–45. Available from: http://dx.doi.org/10.1038/s41565-020-0732-3

37. Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, et al. COVID-19 vaccine development and a potential nanomaterial path forward. Nat Nanotechnol [Internet]. 2020; 15(8): 646–55. Available from: http://dx.doi.org/10.1038/s41565-020-0737-y

38. Singh B. Biomimetic nanovaccines for COVID-19. Appl Sci Technol Ann. 2020; 1(1): 176–82.

39. Skwarczynski M, Toth I. Peptide-Based Subunit Nanovaccines. Curr Drug Deliv. 2012; 8(3): 282–9.

40. Strizova Z, Smetanova J, Bartunkova J, Milota T. Principles and Challenges in anti-COVID-19 Vaccine Development. Int Arch Allergy Immunol. 2021; 182(4): 339–49.

41. Talebian S, Conde J. Why Go NANO on COVID-19 Pandemic? Matter. 2020; 3(3): 598–601.

42. Boopathi S, Poma AB, Kolandaivel P. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J Biomol Struct Dyn [Internet]. 2020; 0(0): 1–10. Available from: https://doi.org/10.1080/07391102.2020.1758788

43. Tian R, Ke C, Rao L, Lau J, Chen X. Multimodal stratified imaging of nanovaccines in lymph nodes for improving cancer immunotherapy. Adv Drug Deliv Rev [Internet]. 2020; 161–162: 145–60. Available from: https://doi.org/10.1016/j.addr.2020.08.009

44. Vahedifard F, Chakravarthy K. Nanomedicine for COVID-19: the role of nanotechnology in the treatment and diagnosis of COVID-19. Emergent Mater. 2021; 4(1): 75–99.

45. Vijayan V, Mohapatra A, Uthaman S, Park IK. Recent advances in nanovaccines using biomimetic immunomodulatory materials. Pharmaceutics. 2019; 11(10): 1–26.

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