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Thematic Opinion

Biomimetic nanovaccines for COVID-19

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Abstract: The outbreak of Coronavirus Disease 2019 (COVID-19) has posed a serious threat to global public health, calling for the development of safe and effective prophylactics and therapeutics against infection of its causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Currently, there is no approved vaccines or medications exist to prevent infection by SARS-CoV-2. In this emergency, more than 100 vaccines formulated using conventional approaches are being explored against SARS-CoV-2 across the world. The vaccines formulated using nanotechnology are also on the race of clinical trials. The aim of this article is to provide an insight into the ways of vaccine production by conventional and nanotechnology platforms and expand the understanding on the possibilities and limitations of these approaches for vaccine developments.

Keywords: *biomimetic; Coronavirus; COVID-19; nanotechnology; vaccine*

सारांश: कोरोनाभाइरस रोग २०१९ (COVID-१९) को प्रकोपले विश्वव्यापी जनस्वास्थ्यलाई गम्भीर खतराको रूपमा खडा गरेको छ, जसले यसको कारक तत्व (SARS-CoV-2) को संक्रमणको बिरूद्ध सुरक्षित र प्रभावकारी खोप वा औषधिको विकास गर्न अती आवश्यक भएको छ। हाल, SARS-CoV-2 द्वारा संक्रमण रोक्न कुनै अनुमोदित खोप वा औषधिहरू अवस्थित छैनन्। यस आपतकालमा, पारंपरिक दृष्टिकोण प्रयोग गरी तयार पारिएका १०० भन्दा बढी खोपहरू SARS-CoV-2 विरुद्ध विश्वभरि अन्वेषण भइरहेको छ। साथसाथै, न्यानोटेक्नोलजी प्रयोग गरेर बनाइएको खोपहरू पनि क्लिनिकल परीक्षणको दौडमा छन्। यस लेखको उद्देश्य पारंपरिक र न्यानोटेक्नोलोजी माध्यमहरू द्वारा खोप उत्पादनका तरीकाहरूको बारेमा अन्तरदृष्टि प्रदान गर्नु र खोप विकासको लागि यी दृष्टिकोणहरूको सम्भाव्यता र सीमितताहरूमा प्रकाश पार्नु हो।

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1. Introduction

On March 11, 2020, the World Health Organization (WHO) announced the COVID-19 outbreak as a pandemic. According to the WHO, there are almost 5.5 million confirmed cases of people infected with COVID-19 and 346,000 deaths around the globe as of May 24, 2020. COVID-19 is caused by a new coronavirus, named SARS-CoV-2. SARS-CoV-2 belongs to the family Coronaviridae that comprises a single-strand, positive-sense RNA genome ranging from 26-32 kilobases in length (Su et al. 2016). Structurally, SARS-CoV-2 is studded with CoV spike (S) proteins on its outer layer which play the most significant roles in viral attachment, fusion and entry to human cells through angiotensin-converting enzyme 2 (ACE2) receptors. Recently, researchers have identified the receptor-binding domain (RBD) in SARS-CoV-2 S protein and confirmed that the RBD protein has strong binding affinity to human ACE2 receptors (Tai et al. 2020). Current studies have revealed that cell entry of SARS-CoV-2 is facilitated by binding of S protein to ACE2 receptors followed by S protein priming by host cell proteases. It is now elucidated that SARS-CoV can use cysteine proteases cathepsin B and L (CatB/L) (Simmons et al. 2005) and transmembrane protease serine 2 (TMPRSS2) (Shulla et al. 2011) (Matsuyama et al. 2010) for S protein priming for their entry to the host cells.

After binding to the receptors, SARS-CoV-2 use the endocytic and/or non-endocytic route to enter the cell through fusion of the viral and cellular membranes (Figure 1). The endocytic route leads with the formation of a vesicle to internalize the virus while the non-endocytic route of entry involves the direct transport of viral RNA through the plasma membrane into the cell. Following the internalization, the virus fuses with the vesicle to release its RNA to cytosol. The genome RNA is converted to a messenger RNA (mRNA) by transcription in cytosol and the mRNA is translated into capsid proteins, spike proteins and other necessary proteins in the endoplasmic reticulum and Golgi apparatus to create the structural components of the virus. Once new copies of viral genome are made, the viral components assemble around the genome and release from the cell by budding or lysis as active viral particles.

The released viruses are recognized as foreign particles by the immune system triggering antigen presenting cells (dendritic cells and macrophages) to engulf the particles, chop them into smaller pieces (antigens) and display the antigen portion to activate T cells. Dendritic cells may present the antigens through major histocompatibility complex (MHC) class I and class II molecules to CD8+ and CD4+ T-cells respectively to produce cytotoxic T lymphocytes (CTL) and mature macrophages that can attack the infected cells. Activation of CD4+ Th2 cells induces B cells to secrete antibodies for neutralization of the viruses. B cells, upon encounter with viral antigens in mucosal route, also activates into plasma cells to produce neutralizing antibodies. The generated immune responses if produce long lived memory B and T cells will provide immunity against potential infection by the same virus in the future.

A myriad of vaccines against coronavirus can be made by traditional and nanotechnology approaches. Although advances in nanoscience and nanotechnology have made significant impact on the developments of therapeutics and diagnostics for better healthcare of humans, no drugs or vaccines are currently available to protect from COVID-19. Due to sudden outbreak of the new disease, there are no literatures to demonstrate the successful applications of nanotechnology against COVID-19. In this circumstance, the following insights will help to understand the methods of vaccine formulation and aim to develop an effective vaccine against SARS-CoV-2 using nanotechnology approach. Although the applications of nanotechnology cover a vast array of nanomaterials and nanomedicines, I have focused this article primarily on the biomimetic vaccines developed using nanotechnology approaches. Due to space limitation, I have briefly described conventional vaccines to give a clear understanding of the significance of biomimetic vaccines and their current clinical trials here.

2. 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 nonreplicating agents, but in either case the structure of cell membranes or proteins of the pathogens remain intact to be able to interact with the immune system. Some of the conventional vaccines and their advantages are summarized below.

2.1. Viral vaccines

Viral vaccines are derived from disease-causing viruses and produced either as attenuated (live) or inactivated (dead) viruses. Attenuated vaccines contain the live form of the viruses that have been weakened to prevent serious diseases after vaccination. Because attenuated vaccines replicate in vaccinated individuals and mimic the natural infection, they induce effective immune responses. However, these vaccines are not recommended to immune-compromised individuals. Many

conventional vaccines including measles, mumps, and rubella vaccine (MMR), smallpox and chickenpox vaccines are made in this way. On the other hand, inactivated vaccines contain killed viruses and hence they do not replicate in vaccinated individuals. Although inactivated vaccines can induce protective immune responses, multiple doses may need to be administered to maintain the long-lasting immunity. Polio and Hepatitis vaccines are common examples of inactivated vaccine.

As described above, attenuated or inactivated coronaviruses can be produced as vaccines against COVID-19 and in fact, many of them are in clinical trials now. Recently, phase I / II clinical trials are being conducted in healthy adults aged 18~59 years to evaluate the immunogenicity and safety of inactivated SARS-CoV-2 vaccine (NCT04352608, NCT04412538).

2.2. Viral vector vaccines

Viral vector vaccines are safer substitutes for the viral vaccines as they can be modified by genetic engineering. Viral vector vaccines also use live viruses, such as measles virus and adenovirus, to deliver DNA into human cells, express immunogenic antigens, and elicit immune responses. These viruses are weakened to prevent respective diseases. Ebola and Dengue vaccine are examples of viral vector-based vaccines. There are several other advantages of using these viral vectors.

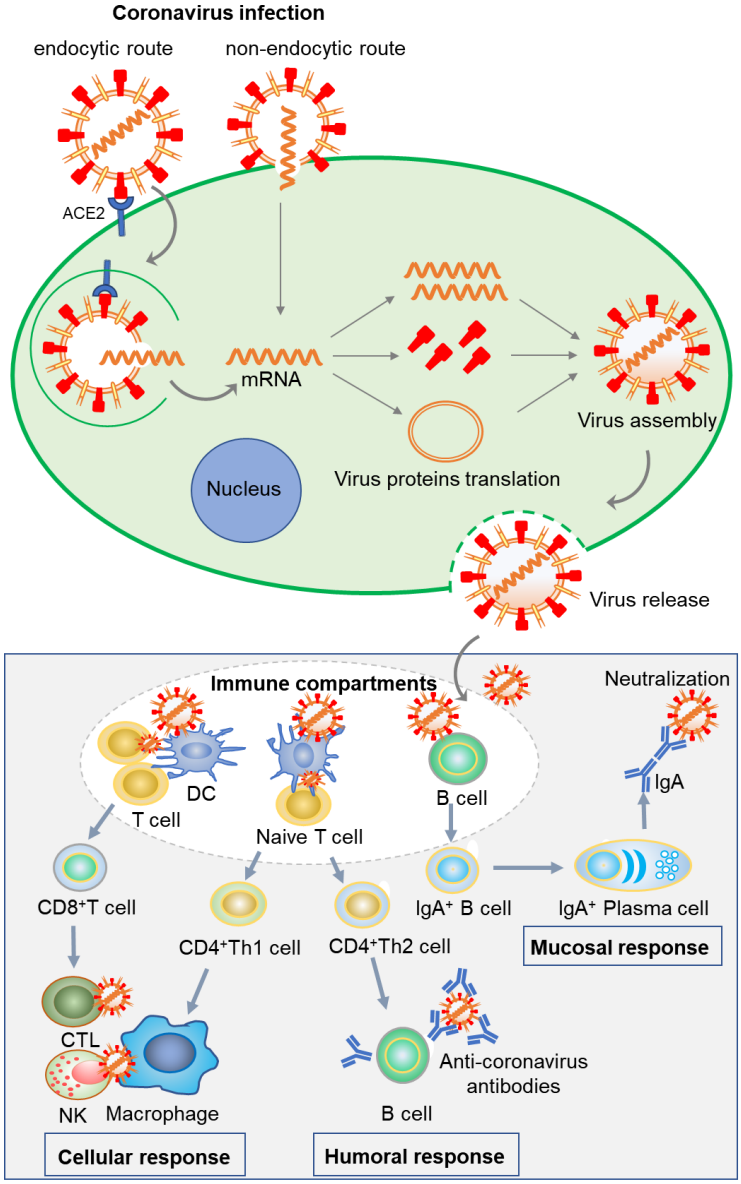

Figure 1. Illustration of replication of SARS-CoV-2 after entering a cell and the immune responses thereafter.

They can be genetically modified to allow or prohibit them to replicate within the cells. It is also feasible to combine multiple DNAs encoding immunogenic antigens into a single viral vector to simultaneously protect against a broad range of infectious diseases. However, the practice of viral

vector vaccines raises safety concerns for use in humans due to the risk of integration into the host genome leading to other diseases (Rauch et al. 2018). Another problem is the neutralization of viral vector vaccines by pre-existing immunity in individuals previously exposed to the vector (Ura, Okuda, and Shimada 2014).

An investigational vaccine called ChAdOx1 MERS, which uses a non-replicating chimpanzee adenovirus to deliver a Middle East respiratory syndrome coronavirus (MERS-CoV) S protein, prevented MERS-CoV disease upon lethal challenge in mice

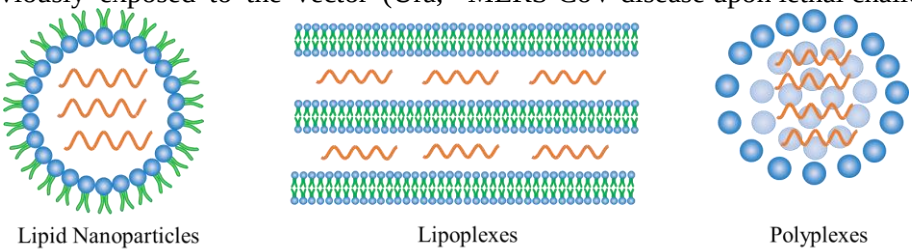

Figure 2. Illustration of nanotechnology-based particles formation

(Alharbi et al. 2017). Consistently, a single intramuscular vaccination with ChAdOx1 MERS protected rhesus macaques from six different MERS-CoV strains (van Doremalen, Haddock, et al. 2020). As MERS-CoV is a relative of SARS-CoV-2, they recently created ChAdOx1 nCoV-19 vaccine candidate to deliver SARS-CoV-2 S protein. A single vaccination with ChAdOx1 nCoV-19 elicited a robust humoral and cellular immune response in mice as well as rhesus macaques protecting the vaccinated animals challenged with SARS-CoV-2 (van Doremalen, Lambe, et al. 2020). Currently, ChAdOx1 nCoV-19 is under investigation in a phase I clinical trial (NCT04324606). The clinical trial was started on April 23, 2020 in UK with 1090 volunteers as of May 24, 2020. Thus, this study is a crucial step towards the development of SARS-CoV-2 vaccine.

Recently, the first-in-human clinical trial of Ad5 vectored COVID-19 vaccine (NCT04313127) was reported in the Lancet journal (Zhu et al. 2020). The vaccine was tolerable and immunogenic in healthy adults with mild or moderate adverse effects at low or middle doses of vaccination. At high doses, although there were severe fever, fatigue, and muscle or joint pain, the adverse effects were temporary. The effects were consistent with Ad5 vectored Ebola vaccine in healthy adults in phase I trial (Zhu et al. 2015). Based on the safety profile of phase I study, Ad5 vectored COVID-19 vaccine showed great potential for the control of the COVID-19 outbreak. Further investigation with low and middle doses of vaccine will evaluate the safety and immunogenicity in Phase II clinical trial (NCT04341389).

A novel nanoparticle-based technology is being explored for vaccine delivery. The nanoscale artificial antigen presenting cell (aAPC) can mimic an antigen presenting cell and present viral proteins to T cells to activate the immune system (Perica et al. 2014). Based on the system, a new Covid-19 aAPC vaccine is developed by using lentiviral vector system to express SARS-CoV-2 antigens to the aAPC and subsequently inactivated so that they cannot proliferate when administered in humans. The Covid-19 aAPC vaccine is proposed to develop as a universal vaccine which is under a trial now (NCT04299724). In another investigation, a lentiviral engineered to express COVID-19 antigens (LV) is transfected in dendritic cells (DC) and the resulting LV-DC is used to activate cytotoxic T lymphocytes (CTL). The activated vaccine is under Phase I clinical trial of lentiviral minigene vaccine of COVID-19 (NCT04276896).

3. Biomimetic nanovaccines

Biomimetic vaccines contain biomimetic vehicles that are loaded with target antigens. Various types of biomimetic nanovaccines such as lipid nanoparticles, protein nanoparticles, and virus-like particles can be produced with the aid of biotechnology, nanotechnology, or both. Some of the nanovaccines and their advantages are summarized below.

3.1. Virus-like particles

Virus mimicking particles have garnered great attention for vaccine development because they can trigger a strong immune response. Because the particles resemble with 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. Although many virus-like particles-based vaccines against viral infections have been undergoing clinical trials (Chroboczek, Szurgot, and Szolajska 2014), only a few of them have been licensed and commercialized. 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, coronaviruslike particles with targeted structural proteins could be a potential vaccine candidate for COVID-19 control. In fact, the assembly of viral membrane proteins into particles have 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 (Vennema et al. 1996). The released particles were spherical and identical to coronavirions 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 (de Haan et al. 1998). Similarly, coronavirus like particles that express one or more structural proteins of SARS CoV were produced using a heterologous expression system (Mortola and Roy 2004). In this study, a recombinant virus was used to express high level of S, E and M proteins simultaneously which self-assemble to produce and release the coronaviruslike particles.

3.2. Nucleic acids vaccines

Currently, vaccines using nucleic acids (DNA or RNA), based on 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 process 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 (Figure 2). 1. 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. 2. Lipoplexes: The formulation is a complex of embedded mRNA in 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 immune system. 3. 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.

Often, nucleic acids-based vaccines use LNPs to avoid degradation of nucleic acids when administered in the body. Also, many scientists are reporting promising results with nucleic acid vaccines in literatures (Kose et al. 2019) (Feldman et al. 2019) (Jagger et al. 2019). For instance, lipid nanoparticles were used to deliver mRNA-based vaccines expressing respiratory syncytial virus (RSV) F proteins. The mRNA RSV vaccine elicited robust CD4+ and CD8+ T-cell responses in rodent models of RSV infection (Espeseth et al. 2020). This pre-clinical study suggested that the mRNA vaccines mimic the natural mode of infection by viruses, highlighting a potential advantage of the nanotechnology for vaccines requiring both humoral and cellular immune responses.

By leveraging the technology, Moderna Inc. (https://www.modernatx.com/) started its Phase I study of mRNA-1273 vaccine, the first nanovaccine to enter human trials in the US, for COVID-19 on March 16, 2020 (NCT04283461). The mRNA-1273 is a novel lipid nanoparticle-enveloped mRNA-based vaccine that encodes for a full-length spike protein of SARS-CoV-2. Two months later, Moderna announced a positive interim Phase I data revealing that the mRNA-1273 vaccine was safe, well tolerated and elicited higher neutralizing antibody titer levels in all eight initial participants consistent to neutralizing antibody responses to SARS-CoV-2 in COVID-19 recovered patients. Because the neutralizing antibody titer levels in the participants were also comparable to neutralizing titers that were protective in the mouse challenge with SARS-CoV-2, Moderna anticipates successful outcomes for their Phase II study with the aim of selecting a specific dose of vaccination.

On May 5, 2020, Pfizer Inc. (https://www.pfizer.com/) and BioNTech SE (https://biontech.de/) announced their Phase I/II

clinical trial of SARS-CoV-2 RNA vaccine candidates to prevent COVID-19 (NCT04368728). The mRNA vaccines are formulated in LNPs to protect RNAs from degradation by extracellular RNAses at the site of delivery. The dose-finding study is aimed to evaluate the safety and potential efficacy of the four mRNAs encoding different target antigens.

3.3. Protein vaccines

Protein subunit vaccines are also under investigation for COVID-19. Novavax's NVX-CoV2373, a recombinant spike protein nanoparticle vaccine has been listed for Phase I clinical trial (NCT04368988). Novavax's Phase I trial is investigating with or without MATRIX-M adjuvant because NVX-CoV2373 is a recombinant version of SARS-CoV-2's spike protein, where native spike protein is a trimer. The adjuvant will assist to reduce the number of antigens needed for vaccination. Clover biopharmaceuticals will be performing first-in-human study to evaluate SCB 2019, a recombinant SARS-CoV-2 trimeric S protein subunit vaccine for COVID-19 soon (NCT04405908).

4. Conclusions

The current outbreak of COVID-19 is one of the huge eruptions spreading all the continents around the globe claiming thousands of lives by the disease. So, there is an urgent need to develop an effective therapeutic strategy to save the millions of lives. Although researchers from both universities and companies have been put considerable efforts to find a cure for COVID-19, no specific therapy has yet been identified. While several vaccines made by conventional technologies are under preclinical and clinical evaluation now, new vaccines using nanotechnology are also on the race of coronavirus vaccine trials. The scope of nanotechnology-based vaccines will be determined by the outcomes of ongoing clinical trials.

In fact, nanotechnology-based mRNA vaccine offers large advantages due to speed of development and scalable production in response to infectious disease pandemic. The mRNA vaccine mimics many features of natural viral infections potentially stimulating the same pattern of immune responses to defend the real viral infections. Unlike traditional technologies, a single vaccine formulated by nanotechnology can include multiple mRNAs to express multiple antigens to defend multiple viruses. Viruses themselves are smart 'tricky" nanoparticles which are capable of invading into human cells to replicate their genetic contents and evading from the cells as new infectious viral nanoparticles. To counteract the replication of viruses, gene silencing technique such as RNA interference can be utilized to turn off the critical genes necessary for the survival of the viruses. For this, we can engineer the same infectious viruses to non-infectious and leverage them to deliver the silencing genes inside the cells through the same itinerary of the viruses. Hence, effective vaccination strategies using biomimetic nanotechnology could help to address many challenging conditions of coronaviruses outbreak.

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Ethical approval: Not applicable

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