**Introduction**

In December 2019, a new coronavirus belongs to the family Beta corona virus emerged. All beta coronaviruses are unique(1, 2) . However, they have certain degrees of structural and genomic similarity, so that the SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) genome with MERS-CoV (Middle East respiratory syndrome-related coronavirus) and SARS-CoV (Severe acute respiratory syndrome coronavirus)50% and 77% (respectively) are structurally similar(3). Unlike SARS-CoV in 2002 and MERS-CoV in 2012, which showed a relatively low prevalence,SARS-CoV-2 has shown an unprecedented outbreak of infection. as a result 11 March, 2020 WHO(The World Health Organization) has declared COVID-19 infection a global pandemic(4). Design and production of effective vaccine, due to the presence of asymptomatic patients carrying COVID-19 is vital and the only factor that leads to the complete removal of restrictions (4, 5) .

**Vaccine production strategies**

To design any vaccine, including the SARS-CoV-2 vaccine, first must have complete knowledge of genomic and structural information, antigenic properties, adjuvant, production system, and vaccine delivery. This information is readily available to researchers today (6-8). With structural information of the virus along with bioinformatics studies and epitope mapping, production of recombinant vaccines faster than weakened live and inactivated vaccines are possible (9-11). It should be noted that the available information on the production of vaccines against SARS / MERS, has been very helpful in the development of the SARS-CoV-2 vaccine. The use of nanotechnology systems is also a very good tool for the production of new and efficient vaccines(12, 13).

SARS-CoV-2 virus is a envelope virus with positively polarized single-stranded RNA as genetic material and spike glycoprotein protruding from the outer surface like a crown. Beta-coronaviruses have four important structural proteins, which include:Spike protein (S), Envelope protein (E), Membrane protein (M),and nucleocapsid (N) proteins(14). protein S can be an effective target for vaccine production due to its involvement in the entry of the virus into the cell. This protein has two domains, S1 and S2, which are responsible for binding to the virus receptor (ACE2: Angiotensin-converting enzyme2) and virus fusion,respectively. The S1 domain varies among coronaviruses, while the S2 domain genomic sequence is more conserved(15).

The whole structure of protein S as well as regions derived from S1, S2, RBD( Receptor-binding domain ) are the main target epitopes for induction of neutralizing antibodies against SARS-CoV-2. Serum analysis of patients recovering from SARS-CoV-2 has been shown to have the presence of neutralizing antibodies against protein S domains(S1, RBD, S2)(16, 17). Protein S is highly glycosylated and computer surveys

 has shown that glycosylated S proteins are stable and have more organized structure than unglycosylated S proteins. Therefore, S protein glycosylation in vaccine design should be considered.One of the advantages of DNA / RNA Based vaccines is that after expression in the host body, glycosylation is inherently performed on them and for recombinant vaccines, the use of glycoengineering methods for glycosylation can be useful(18-20).

**Vaccines produced against SARS-CoV-2**

**1. Live Attenuated Vaccines**

 live attenuated vaccines ( LAVs ) are non-pathogenic replicating viruses.The purpose of designing LAVs is immunization with a single injection dose, without causing disease. These vaccines as one leading candidates in the production of the COVID-19 pandemic vaccine like as CDX-500 vaccine from Codagenix (4, 21). LAVs have disadvantages such as transmitting the virus, becoming pathogenic, and reactivating in people with weakened immune systems as well as recombination with related viruses that are circulating in the community, especially new infections that their pathogenicity is not yet known. LAVs also generally require a "cold transmission chain" as well as loss of virus efficacy and replication potential of viruses during vaccine production are very important challenges in this regard. primary studies have shown that a series of silent codon changes in this group of vaccines, has positive effects in reducing the reversibility of the virus to the wild(4, 21).

**2. Inactivated Vaccines**

Inactivated vaccines ( IVs) viruses are inactivated by heat, chemicals, and a combination of both. These vaccines are not able to reproduce and are safer than LAVs. Although IVs are more stable than LAVs But it should be noted that inactivation reduces the immunogenicity of the vaccine and ultimately requires repeated doses to increase the long-term immunity of the vaccine. In addition, they also need cold transmission chains(22). Several IVs vaccines are being developed against COVID-19 like as Sinavac vaccine (Table1)(4).

**3-Viral Vector Vaccines**

A number of engineered mammalian viruses have been used to produce various vaccines. Several adenovector vaccine candidates are being developed for COVID-19. Leading adenovirus vectors in vaccine development SARS-CoV-2, Adenovirus type5 (Ad-nCoV) and vector Chimpanzee adenovirus (ChAdOx1 ) respectively by CanSino Biological and Oxford University have been used (Table1)(4). One of the challenges of using vectors Adenovirus has been reported to have high levels of pre-existing immunity against Ad5, due to Ad5 vector problems, ChAdOx1 vector as an alternative that does not already have immunity in humans,it has been used as a vaccine production infrastructure (23, 24). The Oxford-AstraZeneca vaccine is another example of an adenovctor-based vaccine. The effectiveness of this vaccine varies between 62 and 90%. The storage temperature of this vaccine is between 2 and 8° C and can be stored for up to 6 months, One of the main advantages of this vaccine is its cheapness to other approved vaccines for injection (25) .

**4-Next generation vaccines based on advances in nanotechnology**

Viruses are nanoscale objects; So they can be considered as natural nanomaterials. According to this definition LAVs, IVs, and viral vectors are considered as nanomaterials. Nanoparticles, what natural and synthetic, they mimic the structural features of viruses.This action makes it possible to develop next generation vaccine production technologies in biotechnology and nanochemistry(4).

**5-Nucleic acid-based vaccine design**

**5.1.DNA- based vaccine**

Using genetic codes to directly produce viral proteins is a promising alternative to traditional vaccine design and production. Both DNA and mRNA have been used in the production of vaccines against COVID-19 (4). The practical advantages of these vaccines are the production of high antibodies , specific immune responses of TCD4 + cells ,and stimulation of cytotoxic T CD8+ responses which play a pivotal role in eradicating the virus(26-28). Leading companies in the production of DNA vaccines against COVID-19 are Inovio Pharmaceuticals and Entos Pharmaceuticals(Table1).

**5.2. RNA-based vaccines**

mRNA-based vaccines are produced in vitro through a transcriptional mechanism. In this case, the need for the cell as well as the barriers that will occur during transcription in the cell are eliminated. vaccines of BioNTech-Pfizer and Moderna companies that are available in the market have used this technology to make them(Table1)(4, 29). Both vaccines are 94 to 95% effective. The Pfizer vaccine produces 52% immunity in the first injection and reaches 95% after the second injection. It should be noted that the Pfizer vaccine should be stored at -70 ° C, which requires special freezers. Also, the shelf life of this vaccine is 5 days. while the Moderna vaccine can be stored in a normal freezer for 1 month at a temperature of -20 ° C. Therefore, due to the same effectiveness, the demand for Moderna vaccine will be higher(30, 31).

**Advantages of RNA-based vaccines over DNA-based vaccines**

Although DNA vaccines are more stable than mRNA vaccines, mRNAs do not enter the genome, so they do not cause mutations. In addition to the half-life, the stability and immunogenicity of mRNAs can be controlled and adjusted through various changes for it(4, 32).

**6.Subunit vaccines**

Subunit vaccines contain components of the SARS-CoV-2 structural compound that, together with adjuvants, can induce a protective immune response in the host.subunit vaccines of the SARS-CoV-2 have been designed and manufactured using the complete structure of S protein or S1 / S2 subunits with Adjuvant. Novavax is a leader in the development of this class of vaccines (Table1). In addition, subunit vaccines can be made from nanoprotein particles or virus-like particles (VLP). VLP vaccines were produced using recombinant expression systems and, using genetic engineering methods, enabled the binding of ligands and immunomodulatory systems in these vaccines. Both self-aggregating protein nanoparticles and VLPs are stable compounds of similar size and can be produced on a large scale by fermentation or molecular methods(4, 33).

**7. Peptide vaccines**

The most important thing to design a vaccine is its safety. Many vaccines are designed in such a way that the whole structure of the protein is used to make the vaccine. For example, the use of full-length S protein contains different epitopes in its structure, which leads to the production of a wide range of cellular and humoral responses in the vaccine recipient. , however, Recent studies on protein candidates for the SARS and MERS vaccines have indicated risks of ADE(antibody-dependent enhancement) infection(5, 34, 35). The presence of neutralizing antibodies can contribute to increased infectivity and lead to allergic inflammation and life-threatening. However, no evidence is available yet, but the immunological data of patients with SARS-CoV-2 have evidence of ADE in this group of patients, and a high IgG titer against the virus may have worse consequences(36, 37). However, targeting the S protein, the SARS-CoV-2 virus, with a variety of different epitopes that stimulate the immune system, can be very helpful. Preliminary studies indicate the presence of various epitopes in the S protein of the virus that can stimulate B and T cells(9, 38). Peptide vaccines are the simplest form of vaccine that are easily designed and approved and produced very quickly(4). Peptide vaccines can be a combination of peptides with an adjuvant or a nanoparticle carrier, and a nucleic acid vaccine encoding peptides(39). For example, OncoGen and the University of Cambridge / DIOSynVax in making vaccines from Peptide sequences have used S protein,In order to increase the stimulation of B and T cells, some domains of immune system stimulation have been predicted in them. However, extensive research is ongoing in this area(4).

Table 1. Candidate vaccines against COVID-19 infection

|  |  |  |
| --- | --- | --- |
| Vaccine production strategies | Type of vaccine | Vaccine manufacturer |
| Complete viral particle inactivated with formalin with alum adjuvant | Inactivated vaccine | Sinovac |
| SARS-CoV-2 inactivated vaccine | Inactivated vaccine | Beijing Institute of Biological Products, Sinopharm |
| SARS-CoV-2 inactivated vaccine | Inactivated vaccine | Wuhan Institute of Biological Products, Sinopharm |
| SARS-CoV-2 inactivated vaccine | Inactivated vaccine | Institute of Medical Biology, Chinese Academy of Medical Sciences |
| S protein fusion with adjuvant and M-matrix | Subunit vaccine | Novavax |
| Recombinant intramuscular vaccine in adenovirus type 5 vector (Ad5-nCoV) | Non-replicating viral vector vaccine | CanSino Biological Incorporation, Beijing Institute of Biotechnology, Canadian Center for Vaccinology |
| Chimpanzee Adenovirus Vector Vaccine (ChAdOx1) | Non-replicating viral vector vaccine | University of Oxford, AstraZeneca |
| Approach 1: Dendritic cells expressing the SARS-CoV-2 mini-geneApproach 2: Synthetic presenting cells expressing the SARS-CoV-2 mini-gene | Non-replicating viral vector vaccine |  Shenzhen Geno-Immune Medical Institute |
| DNA vaccine is optimized and administered by electroporation | DNA vaccine | Inovio Pharmaceuticals |
| Oral DNA vaccine (bacTRL-Spike) encoding S protein of SARS-CoV-2 | DNA vaccine | Symvivo |
| mRNA vaccine with lipid nanoparticles | RNA vaccine | BioNTech, Pfizer, Fosun Pharma |
| mRNA vaccine of the S2 region of the S protein of the virus encapsulated with nanolipids | RNA vaccine | Moderna |

Another class of vaccines are phage-based vaccines, which can also be classified as peptide vaccines. Phage-based vaccines have important advantages, such as: They have not had any specific side effects in the body. In fact, bacteriophages are not an infectious agent for humans. It is very unlikely that bacteriophages can bind to cellular receptors and activate cellular signaling pathways. On the other hand, phages can activate both arms of the immune system, namely cellular and humoral immunity, which is one of the goals of designing effective vaccines. Furthermore, phages themselves can act as effective adjuvants and induce stronger immune responses in the immune system, and this feature eliminates the need for adjuvants required in recombinant peptide vaccines.Also, although vaccination routes are usually intradermal, subcutaneous, or intramuscular, phage vaccines can also be given orally because bacteriophages are stable in the gastrointestinal tract. However, since phage may infect the gut microbiome, non-lytic phages should be used during oral administration. (40-43). Despite the benefits mentioned, unfortunately to date there has been no worldwide report on the production of a phage vaccine against COVID-19. Despite the benefits mentioned, unfortunately, to date, there has been no worldwide report on the approval of a phage vaccine against COVID-19. Therefore, steps have been taken in this regard, which are mentioned in this article, hoping that it will be fruitful.

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