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Emerging Frontiers in Vaccine Development: A Review of Changing Paradigm

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Abstract

The technology behind vaccine development varies significantly from one vaccine to another depending on the time when the vaccine was first developed. Over the years, the vaccine innovation time has significantly shortened with the advancement of knowledge in the fields of molecular and cell biology, and discoveries in the field of biotechnology. The first vaccines created were tested in a kind of trial-and-error approach which sometimes had deadly side effects. These vaccines used either living, weakened, or completely dead pathogens. The use of whole pathogen vaccines was seen to be time consuming and unpredictable because even though it would cause an immune response, it could vary from person to person, and always had the risk of pathogens returning to virulence causing sometimes fatal outcomes. The next major technology used to create vaccines was subunit vaccines which utilize purified antigens inactivated through various methods. This technology is quite prevalent among the vaccines that are currently in circulation, making them quite effective, and free from fatal side effects. The viral vector vaccine technology has been around for a few decades and utilizes knowledge of molecular genetics to the greatest extent. It uses intermediate vectors to deliver genetic instructions to trigger an immune response within the subject body. The introduction of nucleic acid vaccines is the newest technology and has come to a great deal of attention during the SARS-CoV-2 immunization efforts. The technology primarily utilizes the delivery of genetic information using messenger ribonucleic acid (mRNA) to create characteristic pathogen-specific proteins that in turn generate an immune response in the recipients.

Keywords

Vaccine Technology, DNA Vaccine, mRNA Vaccine, COVID-19 Vaccine, Vaccine Development

1. Introduction

A vaccine is a mixture of chemical agents that stimulates one's immune system to produce antibodies. It functions in a way that is similar to the body's exposure to the disease, without actually getting the disease. By stimulating the immune system, vaccines help in building immunity to the disease such that when the person is exposed to the disease-causing germs (bacteria or virus), his/her immune system recognizes the foreign protein (*i.e.*, antigen) and is able to fight it. Vaccines contain either a part of the germ or a dead or weakened version of the same germ, such that when a vaccine is administered into a healthy person it is no longer able to cause the disease. So, vaccines unlike drugs cannot cure someone from the disease rather they prevent the disease or protect the person from its severity [1].

At present vaccines are routinely given to the general population for chickenpox, diphtheria, flu, hepatitis A and B, *Haemophilus Influenzae* type B (HiB), human papillomavirus (HPV), measles, meningococcal, mumps, polio, pneumococcal, rotavirus, rubella, tetanus, whooping cough, and most recently for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or commonly referred as COVID-19). Vaccines are available for travelers to protect them from adenovirus, anthrax, cholera, Japanese encephalitis (JE), rabies, smallpox, tuberculosis, typhoid fever, and yellow fever [2].

Vaccine ingredients typically include antigens, stabilizers, adjuvants, antibiotics, and preservatives—all of which play key roles either in manufacturing the vaccine, helping to boost the body's response, or ensuring that the final product is safe and effective [3]. The use of ingredients varies depending on the type of vaccine, manufacturing process, and its desired effectiveness. Similar to any product produced through a bulk scale manufacturing process, a vaccine may also contain non-intended residual by-products. Awareness of actual components of a vaccine is highly desired in avoiding adverse events following immunization [4].

Immunization by means of inoculation has been in practice for several hundred years. It is known that Buddhist monks used to drink snake venom to develop immunity to snakebites and at least since the 17th century in China, people used to practice variolation which is a form of inoculation with cowpox to develop immunity against the smallpox virus. In 1796, Edward Jenner, the founder of vaccinology in the West, was the first person to test a method of inoculation against smallpox using the scientific approach. The arm-to-arm inoculation approach that Edward Jenner developed using vaccinia virus (cowpox) led to the development of the smallpox vaccine in 1798 [5].

Louis Pasteur, best known for his discoveries on bacterial fermentation, developed inactivated rabies vaccine in 1885 [6], a live attenuated cholera vaccine in 1897, and inactivated anthrax vaccine in 1904 [5] for use in humans. Although no approved vaccine is available against plague caused by Yersinia pestis, a Gram-negative bacterium, some countries have used vaccines made from live

bacteria for immunization since the 1920s [7]. In 1921 after almost 20 years of research Albert Calmette and Camille Guérin introduced the Bacillis-Calmette-Guerin (BCG) vaccination for human trials to fight against tuberculosis, caused by intracellular pathogen M. tuberculosis [8]. In 1923, Alexander Glenny developed tetanus vaccine using inactivated tetanus toxin with formaldehyde to protect against Clostridium tetani, a bacterium that causes disease of the nervous system [9]. Similar method was used in developing the diphtheria vaccine in 1926 to protect against Corynebacterium diphtheriae, an aerobic, gram-positive bacteria [10].

Vaccine to protect against respiratory disease whooping cough or pertussis caused by gram-negative Bordetella pertussis bacteria [11] was developed in 1939 by Pearl Kendrick, Grace Eldering and Loney Gordon. The highly effective pertussis vaccine used whole cell inactivated bacteria, which in late 1940s was combined with diphtheria and tetanus toxoids [12]. The combined vaccine was called DTP. Later, in 2005, an improved formulation containing a tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis was developed and marketed as Tdap vaccine for use in adults and adolescents [13].

Although the development of bacterial vaccines proliferated from late 1800s to mid-1900s, the development of viral vaccines did not quite take off until discovery of laboratory-based cell culture techniques around the mid-1900s. The "roller tube" apparatus created by researcher George Otto Gey in the 1930s revolutionized the cell culture approach by simulating the living body condition in test tubes whereby tissue cells were alternately exposed to periods of nutrient supply and waste removal.

Using this approach researchers John Enders, Thomas Weller, and Frederick Robbins successfully grew poliovirus in static flasks without using nervous tissue, which earned them a Nobel Prize in Physiology or Medicine in 1954 [14]. Later, vaccine development shifted from the use of tissue culture to the use of single cell lines (*i.e.*, cell strains). This shift saw one of its first successes in development of polio vaccine using a monkey kidney cell strain. Using these methods Jonas Salk developed the inactivated poliovirus vaccine (IPV) or Salk vaccine in early 1950s which began its mass scale testing on children in 1954 [15]. Around the same time Albert Sabin developed the live attenuated polio virus vaccine, which after successful field testing in the Soviet Union in 1957, was approved by the U.S. Public Health Service in 1960 [16]. It was Sabin's oral polio vaccine which ultimately led to worldwide eradication of polio [17].

In the coming years the world saw development of measles (1963), mumps (1967), and rubella (1969) vaccines, which were subsequently combined into a single MMR vaccine in 1971. The trend of new vaccine development continued as our understanding of molecular and cell biology, genomics, disease physiology, and technologies in developing vaccines improved, which led to development of vaccines for hepatitis B (1981), HiB (1985), chickenpox (1995), rotavirus (1998), hepatitis A (2000), pneumococcal disease (1983 and 2000), HPV (2006),

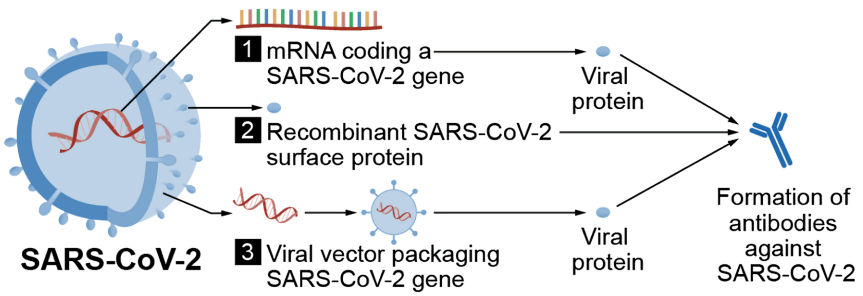
meningococcal serogroup B (2014), and most recently COVID-19 in late 2020. Figure 1 presents a schematic representation of three different types of COVID-19 vaccines that are currently being used for mass scale immunization efforts.

There are five commonly used ways vaccines are currently administered to subjects: intramuscular, subcutaneous, intradermal, oral, and intranasal. The last two approaches do not involve the use of needles. The effectiveness of a vaccine depends on its delivery to a target part of the body and relies on the body's own transport mechanism. Therefore, the route of vaccine administration varies depending upon the desired part of the body where its action is targeted to achieve optimum results [18]. Sometimes the choice of vaccine administration routes is also influenced by the components of a vaccine, and condition of the recipient.

As discussed above, vaccine development has come a long way since the inception of the concept. The technology behind vaccine development varies significantly from one vaccine to another depending upon the time when the vaccine was first developed. The vaccine innovation time has significantly shortened with the advancement of knowledge in the fields of molecular and cell biology, and discoveries in the field of biotechnology. Awareness in public health has resulted in ease of live in-person studies that are needed before fully authorizing a vaccine for general circulation. Technological advancements including use of machine learning and artificial intelligence [19] have further supported mass scale production of the vaccines within a much shorter timeframe than the past. The primary purpose of this study is to evaluate the technology behind select key vaccines and to systematically document its evolution, especially over the last century.

2. Methodology

In preparing this paper authors conducted an extensive review of available literature on different vaccines that are currently in circulation, different components used in these vaccines, and the underlying guiding principles or technologies used in developing the vaccines. Multiple databases including PubMed, PubMed Central, EMBASE, and Google Scholar were utilized this process. A total of 86 references were reviewed which documented a chronological evaluation of vaccine development which progressed from minimalistic use of technologies



Source: GAO analysis of The Pharmaceutical Journal infographic. | GAO-20-583SP

Figure 1. Three different types of COVID-19 vaccines that are in circulation.

from earlier days to a more robust approach using molecular genomics aided by machine learning in the recent years. Vaccines were segregated based on underlying principles used in developing them and are summarized below.

3. Discussion on Vaccine Technologies

As we review the underlying principles used in development of the vaccines, one will note it can be broadly classified into four major categories: 1) whole pathogen vaccines, 2) subunit vaccines, 3) viral vector vaccines, and 4) nucleic acid vaccines [19] [20]. Each category of vaccine is further subdivided based on technologies behind the vaccine manufacturing resulting in a total of 12 different approaches. The following sections provide a brief overview of each of these approaches, their advantages, and disadvantages.

3.1. Whole Pathogen Vaccines

In this vaccination approach either live or dead whole cell of the pathogen is used for vaccinating the target population. Although active pathogen cells were used for vaccination in the recent past, presently only attenuated or dead pathogen cells are generally used for vaccination. The following sections describe in detail this type of vaccination approach.

3.1.1. Variolation

Historically also referred to as "inoculation", is one of the oldest vaccination approaches, that deliberately exposes the target population to a live disease-causing agent. It was first used to protect people from smallpox infection, a contagious, disfiguring disease [21] caused by variola virus and often left severe scars on survivors [22]. One of the first modern uses of the variolation technique was in 1796, English doctor Edward Jenner had noticed that the milkmaids who contracted cowpox previously, had not contracted smallpox. Dr. Edwards knew that about variolation but assumed that the previous exposure to cowpox had protected them from smallpox. In order to confirm this hypothesis, Dr. Jenner took a sample of cowpox from a milkmaid and inoculated it into the arm of another worker, who had not previously been exposed to either disease. The person who received the sample of cowpox did not contract smallpox which validated Dr. Jenner's theory.

Patients who received the variolation treatment had only 1-2% mortality rate compared to the 30% mortality rate without inoculation. In this approach subjects, without any prior history of smallpox, were exposed to materials from smallpox sores. Primarily subjects were exposed through dermal inoculation or inhalation, and afterwards they were observed for fever and rash, common symptoms of smallpox. Mortality rate among subjects exposed to smallpox through variolation was significantly lower than those exposed to the disease naturally [22].

The variolation technique came with certain risks. These risks included the possibility to contract a milder form of the virus. Also, there was a possibility for

an epidemic to spread from a patient. The possibility of death was also a risk factor for patients [23].

3.1.2. Live Attenuated (Weakened)

The first reporting of the isolated cholera bacillus was in 1884 by Robert Koch. After the discovery of cholera, within a year a vaccine was being given to people which was a cultured unattenuated Vibrio cholerae. This vaccine led to development of other inactivated whole cell vaccines, most of which provided short term protection. This was the opposite of the cholera vaccine, as it proved to have an efficacy rate of about 80% [24]. Since newer vaccines could only provide protection for a shorter time period and had local reactions, this led to higher rates of fever and malaise among vaccine recipients.

Live attenuated vaccines use a small, weakened piece of the virus and injects it with a person. This is meant to essentially expose one's immune system to the virus. The interaction between the virus and immune system creates a long-lasting protection against a particular virus. In most cases one or two doses of an attenuated vaccine can give one a lifetime protection against that particular virus [19].

The main advantages of using an attenuated vaccine were that it would force the immune system to respond to the controlled infection [25]. This meant that essentially the vaccine would cause the immune system to initiate a response. Some additional pros of using the live attenuated vaccines are that the immune response is fast and effective. The live microorganism found within the vaccine provides the body with enough time for the cell to have a memory produced of the virus. Also, the attenuated vaccine is able to properly replicate within the host cells. This allows the pathogen to create its own defense within one's body.

The attenuated vaccine required more development because along with the potential advantages, came significant risks. Some cons to live attenuated vaccines are that they can revert to the original form and have the possibility to cause the disease. Also, the disease can be potentially even more harmful for those with a compromised immune system. So, in certain cases, it is recommended that people not receive a live attenuated vaccine. If one is pregnant, a live attenuated vaccine may pose risks for the fetus as the virus may cross the placenta and infect the fetus. The Canadian Task Force on Preventive Health Care recommends that all women of childbearing age should be evaluated for the possibility of pregnancy before immunization with such vaccines. It is also generally recommended that non-pregnant women immunized with a live or live-attenuated vaccine should be counselled to delay pregnancy for at least four weeks [26]. For example, pregnant women receiving the live-rubella vaccine made the fetus susceptible to congenital rubella syndrome which may lead to deafness, cardiac defects, and bone damage in the child. Also, if one is finishing cancer treatment it is recommended that they do not receive a live attenuated vaccine for at least six months due to the possibility that the immune system may be too weak and not able to control small infection [27]. Patients who re-

ceive live attenuated vaccines are also susceptible to potential errors in immunization as the vaccine may come in a powder form [28]. To properly administer the vaccine, it should be diluted. Errors in diluting the powder can lead to programmatic errors.

3.1.3. Inactivated (Killed) Vaccine

Inactivated or killed vaccines contain microorganisms which have been chemically or physically killed. As the microorganisms are killed or inactivated, they cannot cause any disease but instead only promote an immune response. Since the pathogen is already dead, this type of vaccine may not always promote a response by the body's immune system, which leads to such vaccines sometimes needing several doses to trigger a significant immune response [29]. Inactivated vaccines do not pose any significant risks to people as they do not contain any live microorganism. Hence, such vaccines cannot infect the fetus or cause a severe immune reaction making them safe to be given to both pregnant women and cancer patients.

In the 1880s Dr. Robert Koch and his colleagues noted naturally acquired immunity among cholera patients from subsequent infections during the same epidemic [30]. This led to development of the first cholera vaccine by Dr. Jaime Ferran in 1885 which used live bacteria. Subsequently, in 1893 Sawtschenko and Sabolotny developed the first oral cholera vaccine (OCV) using a killed cholera bacteria "broth" [31]. OCVs were later proven to be much more effective in terms of efficacy and duration of protection than those administered via injection as oral vaccines stimulated intestinal immune cells resulting in superior antibody responses against enteric infections [32].

There are currently four inactivated whole cell vaccines available in the market, which are:

- Dukoral: A killed whole cell oral vaccine that was licensed in 1991. This vaccine is administered with a sodium bicarbonate buffer to protect it from degradation by gastric acid.
- Shanchol: This vaccine was first licensed in India in February 2009, which was later licensed for use by WHO in November 2011. This bivalent killed oral cholera vaccine is administered as a two-dose regimen without the need for an oral buffer.
- mORC-Vax: Contain inactivated whole cells of O1 classical and El Tor biotypes plus O139.
- Oravacs: Inactivated whole cell (monovalent and bivalent) vaccine which comes in an enteric coated capsule. The bivalent vaccine was proven to be safe and effective in both children and adults [33]. Its efficacy is sustained for 3 to 5 years after vaccination [34] and it is currently only licensed in China and Philippines.

As of 2021, there were only 245,393 cases of cholera which had been reported to the World Health Organization (WHO). From those cases approximately 3034 deaths were also reported. This is a significant decline in the number of

cases of cholera, however, the overall death rate still remains high. Within the past decade, the number of cholera cases has increased, leading to concern. The mass production of the OCVs were introduced into regions where it is still present in a large degree [35]. Only the inactivated OCV is available as the live OCV has been discontinued. Other than Vietnam, where cholera is still prevalent, countries do not use cholera vaccine as part of their vaccination regimen with the exception of its use among visitors traveling to cholera-prone areas.

In order to produce an aliquot that contains enough virus cells that can trigger an adequate immune response in the recipients, two types of growth mediums are used: eggs or animal cells. Based on the culture or growth medium used such vaccines can be classified into two sub-categories: 1) egg-based vaccine and 2) cell-based vaccine.

1) Egg-Based Vaccine: One of the most common vaccines, the flu vaccine, is made using egg-based manufacturing which allows producing large amounts of virus and high virus titer [36]. The process has been occurring for more than 70 years to produce both inactivated and live attenuated vaccines. In this process first the candidate vaccine viruses (CVVs) are grown in eggs following Food and Drug Administration's requirements, which are then injected into fertilized hen's eggs, and incubated for several days. This allows the viruses to replicate. Subsequently, allantoic fluid containing the virus is harvested from the egg, any debris present are cleared by centrifugation, aliquoted, and transferred to a ultra-low temperature for storage [37].

For inactivated vaccines, the viruses are then inactivated (*i.e.*, killed) followed by purification of virus antigen (e.g., influenza vaccine flu shots). For live attenuated vaccines, the live viruses are weakened as part of the manufacturing process (e.g., nasal spray flu vaccine containing live attenuated influenza virus).

2) Cell-Based Vaccine: In contrast to the previously described egg-based vaccines, cell-based vaccines grow viruses in cultured mammalian cells [38]. One common example of such a vaccine is cell-based flu vaccine that uses animal host cells (Madin-Darby Canine Kidney, or MDCK cells) for growing the virus. In such vaccine development first the target virus is grown in cells to produce candidate vaccine viruses (CVVs) by CDC or one of its laboratory partners, which are subsequently transferred to the vaccine manufacturer. The cultured mammalian cells are then inoculated with CVVs which are then allowed to replicate. After a few days of replication, fluid from cells are collected that contain the CVVs, which then undergoes few cycles of purification and testing prior to its release as a vaccine dose to the market. Flucelvax Quadrivalent is such a cell-based flu vaccine that is currently licensed in the United States for use in subjects 4 years and older.

Some of the key advantages of the cell-based vaccines over the egg-based ones are: a) CVVs used in cell-based vaccines tend to provide superior protection over their egg-based counterparts; b) egg-based vaccines may contain certain proteins which could be harmful to those allergic to egg-based preparation; c) CVVs used in developing cell-based flu vaccines are more closely related to the

"wild" types typically in circulation during the flu season, which in egg-based productions may be impacted due to the egg-adapted changes causing critical differences in terms of subject body's immune response; d) cell-based vaccines have a shorter startup time due to effective utilization of cell culture and cell banking; and e) cell-based productions are not dependent on the egg supply which may be impacted due to multitude of reasons including disease affecting the livestock, poor quality of livestock feed, high market demand, supply chain issues, to name a few [36].

3.2. Subunit Vaccines (Purified Antigen)

Subunit vaccines, also known as acellular vaccines, are similar to inactivated whole-cell vaccines, however, instead of the whole cell it contains a specific antigenic part of the pathogen (26), fragments of protein and/or polysaccharide, that generates a strong immune response in the recipient [39]. During future infections by the specific pathogen the immune system memorizes the targeted part of the pathogen from vaccination and replicates the immune response. For example, acellular pertussis (aP) vaccine is a protein-based subunit vaccine that contains the inactivated pertussis toxin from the Bordetella pertussis bacteria. Another example of a subunit vaccine is Hepatitis B vaccine that contains the hepatitis B surface antigen (HBsAg) protein produced by the hepatitis B virus [40]. Since subunit vaccines do not use any live or attenuated pathogens there is no change of pathogens to return to virulence making them safe for use even in pregnant or immunocompromised individuals or those suffering from chronic illness [41].

However, such vaccines have some key disadvantages that must also be considered. Typically, the immunity imparted by subunit vaccines are not long-lasting, requiring periodic booster doses. A subunit vaccine developed with only one structural protein from a pathogen may not be quite effective as typically pathogens contain a number of structural proteins that identifies them from others [42]. In case of a protein-based subunit vaccine, if the isolated protein is denatured as it is introduced in the recipient body, the antibodies it will generate may not effectively bind with the pathogen's protein encountered during infection resulting in low vaccine efficacy. Similarly, in the case of polysaccharide-based subunit vaccines that target the polysaccharide encapsulation of certain pathogens (mainly bacteria), due to the small size of these molecules the immune system may miss them during infection phase resulting in decreased vaccine efficacy.

To overcome these shortcomings and improve vaccine effectiveness, subunit vaccines often use carriers in addition to purified antigen(s) making them conjugate vaccines. The antigenic efficacy is further enhanced by use of improved carriers, use of ligands and adjuvants which increases cellular and humoral immune responses [43]. Also, use of a combination of surface proteins (*i.e.*, antigens) specific to a pathogen improves its efficacy [44].

3.2.1. Recombinant Protein Vaccines

The manufacturing of recombinant protein vaccines replies upon the recombinant DNA technology which allows DNA from more than one sources to be combined into one [19]. In recombinant protein vaccine a part of pathogen's DNA encoding a specific antigen (typically a surface protein from the pathogen) is inserted into bacteria or yeast cells [20] [45] which in turn produces the antigen as its own. This antigen upon purification is used as the active ingredient for the vaccine which triggers humoral immune response in the recipients [46]. Due to the absence of any cellular materials or antigens produced directly by the pathogen, such vaccines are safer than their traditional counterparts. They do not trigger autoimmunity and are quite simple and affordable to produce making them more readily available in developing countries [46]. However, recombinant protein vaccines routinely need adjuvants to increase its long-term immune efficacy in the recipients [19].

One of the first vaccines made using this approach was hepatitis B vaccine. DNA fragments encoding hepatitis B surface protein (HBsAg) was inserted into yeast cell DNA which in turn produced the necessary antigen that was harvested for vaccine manufacturing [36] [47]. Similarly, in 2013 U.S. Food and Drug Administration (FDA) approved a recombinant influenza vaccine which utilizes hemagglutinin (HA), a surface protein from influenza virus. HA producing gene from influenza virus is recombined with DNA of baculovirus which in turn produces HA antigen in the host cells [48]. Purified HA protein upon introduction as a vaccine inside the recipient body produces the necessary antibody response that builds immunity against future influenza infections.

3.2.2. Use of Toxoid

The bacteria which cause tetanus produce tetanospasmin, a toxin. Those who develop an immune response against this toxin are also protected against the disease [36]. Based on this principle, vaccines have been developed using toxins which are inactivated by treating them with chemicals or physical methods (such as heat). The inactivated toxin used in the vaccine is called a toxoid, which upon introduction in the subject body produced anti-toxoid antibodies. These antibodies during future infections bind with the toxin produced by the target (vaccine) pathogen, thereby neutralizing it [49] such that the host does not develop harmful effects from the infection. In the case of tetanus vaccine, the potent tetanus neurotoxin (TeNT) is treated with formaldehyde and lysine to produce non-toxic but immunogenic tetanus toxoid (TTd) [50]. For diphtheria vaccine, diphtheria toxin (DT) is incubated with formalin which converts DT to toxoid [10].

Some pros of toxoid-based vaccines are that they cannot cause the disease they are protecting against. They produce only local systemic reactions upon introduction into the host. Hence if one develops a reaction, it typically is not severe and only develops close to the injection site. Toxoid based vaccines produce long lasting immunity to the subjects which get easily distributed throughout the

bloodstream and extravascular spaces [51], and are known to infer transplacental immunity. The only con to this type of vaccine is that in some cases, several doses may be needed to fully activate the immune response.

3.2.3. Use of Virus Like Particles

The virus-like particles (VLPs) are nanoscale structures which mimic the form and size of a virus [52] and contain viral proteins, but lack viral genetic materials, thereby making them unable to cause infections. The particles mostly resemble pathogens-associated structural patterns (PASP) that can be easily recognized by all cells in the immune system. The VLPs are typically icosahedral or rod-shaped, 20 - 200 nm in size and are produced through self-assembly of the recombinant viral structural proteins [53], which are organized into single-layered, two-layered, or multi-layered fashion [54]. Depending on the presence or absence of lipid envelopes around them VLPs are classified into enveloped and non-enveloped [53]. Virus capsid, envelope, and proteins can be part of VLP structures. VLPs can be produced in a variety of organisms, including plants, mammals, yeast, and bacteria [55] All VLPs also have the ability to be used as carriers for various nanomaterials and imaging substances.

Although most VLPs are primarily produced using a single virus, chimeric VLPs are created by assembling the structural proteins found in various viruses. The shape and size of the VLPs are like those of the target viruses for which the vaccines are being developed. The immune system in the recipient's body is activated upon introduction of the VLP which in turn produces appropriate antibodies and cell-mediated immune responses [53]. Later on, when an infection occurs due to the target virus, the recipient's immune system recognizes it as a known antigen due to the similarities between the viral structure and the VLP. This in turn invokes the immune response which due to its prior memory can effectively neutralize the infection.

Since VLPs do not carry viral genetic materials, they cannot reproduce within the recipient cells, making them unable to cause the disease. This makes VLPs safe for elderly patients or those who are immunocompromised to develop immunity to the disease-causing virus without having an active infection. One of the commonly used VLP vaccines that is currently in circulation is the human papillomavirus (HPV) vaccine Gardasil-9. It is made of the purified VLPs containing the major capsid (L1) protein of HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 [54]. VLPs also have been developed using structural proteins from human immunodeficiency virus (HIV), adeno-associated virus, Hepatitis B virus (HBV), Hepatitis C virus (HCV) and bacteriophages [55] [56] [57].

3.3. Viral Vector Vaccines

In some vaccine development approaches, a modified form of a virus is used to deliver critical genetic instructions to the cell which in turn generate the expected immune response in the body. Such carrier virus is different from the virus that is being targeted to generate the protection for and is called a vector vi-

rus [58]. Examples of such vector viruses include adenoviruses, poxviruses, adeno-associated virus, alphavirus, herpesvirus, measles virus, vesicular stomatitis virus to name a few [59]. The disease-causing genes from these viruses are removed making them harmless to cause any infection on their own.

Certain genetic instructions that encode characteristic antigen for the target pathogen (for which the vaccine is being developed) are inserted within the viral vector. Once the vector is injected into the host body, it starts infecting the host cells and inserts its genetic material into the host cells nuclei. This also includes the antigen producing gene from the target pathogen. It leads to the host cell manufacturing the antigen (from the pathogen), which in turn generates an immune response by the host's immune cells as they detect the foreign protein [60]. Since the full genetic makeup of the target pathogen (against which the vaccine protection is being developed) is not included within the vector, it does not develop a full-fledged infection from the target pathogen. **Figure 2** presents a schematic representation of the process.

One of the key disadvantages of using viral vectors (replicating or non-replicating) for vaccination is the "anti-vector immunity", a host body's immune response to the vector itself if the host had previously seen an infection from the

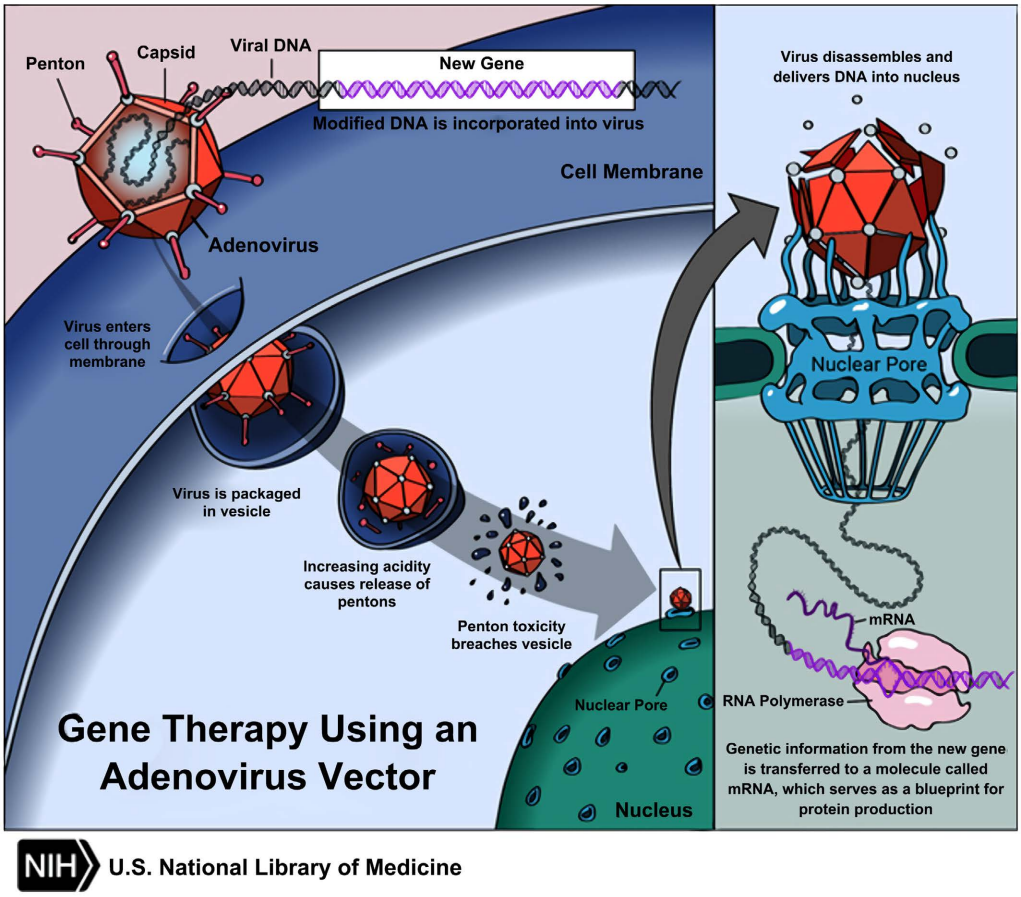


Figure 2. Schematic representing use of viral vectors in carrying certain genetic information to recipient cells.

vector. Having a premeditated immune response to the vector significantly reduces the effectiveness of the body's response to the vaccine antigen, thereby reducing the intended vaccine efficacy. The same is true if a repeat or booster dose is needed, which many a times require use of a different vector to overcome the "anti-vector immunity" from the initial dose [60].

There are two types of such viral vectors used in vaccine development: replicating and non-replicating.

3.3.1. Replicating Viral Vector Vaccines

As the name suggests, replicating viral vectors after being introduced inside the host body can replicate within the host cells, which in turn produce vaccine antigen. Since the vector virus itself is harmless and only produces the vaccine antigen that the body can safely handle by generating an immune response, the host body does not develop a full-blown viral disease from the target vaccine virus. The primary advantage of utilizing a replicating viral vector is that it generates a complete immune response in the host body that not only stimulates the body's natural or innate immunity, but also produces humoral, cellular, and mucosal immune responses [59].

Modified adenoviruses, hereinafter referred to as Ad vectors, which in wild type (unmodified form) develops common cold in humans, are one of the most commonly used replicating viral vectors. Pox viruses, measles virus, and vesicular stomatitis virus are also used as replicating viral vectors. At present recombinant vesicular stomatitis virus (rVSV)-Zaire Ebola virus vaccine and the live attenuated tetravalent dengue vaccine are the only FDA approved vaccines that utilize replicating viral vectors [61].

3.3.2. Non-Replicating Viral Vector Vaccines

The viral vectors when genetically modified such that they can no longer replicate (*i.e.*, replication-defective) within the host body are called non-replicating viral vectors. These types of vectors upon introduction into the host cell's nucleus can only produce the vaccine antigen which in turn produces the immune response by the host body [62]. Some of the commonly used non-replicating viral vectors include adenoviruses, adeno-associated virus, alphavirus, herpesvirus, and poxviruses [57].

The Johnson & Johnson/Janssen, Oxford-AstraZeneca and Gam-COVID-Vac (Sputnik V) COVID-19 vaccines that are currently in use in various parts of the world utilize non-replicating viral vectors. Although all three vaccines named here include genetic coding for a stabilized variant of the SARS-CoV-2 spike protein, however, they differ in their non-replicating viral vectors: the Janssen COVID-19 vaccine uses a human adenovirus, Ad26, while the Oxford-Astra Zeneca COVID-19 vaccine uses a chimpanzee adenovirus (ChAdOx1, which is based on ChAdY25), and the Gam-COVID-Vac vaccine uses two human adenovirus vectors, Ad26 and Ad5 [59]. Due to the non-replicating nature of the viral vectors, these types of vaccines are quite safe from developing a vaccine-induced

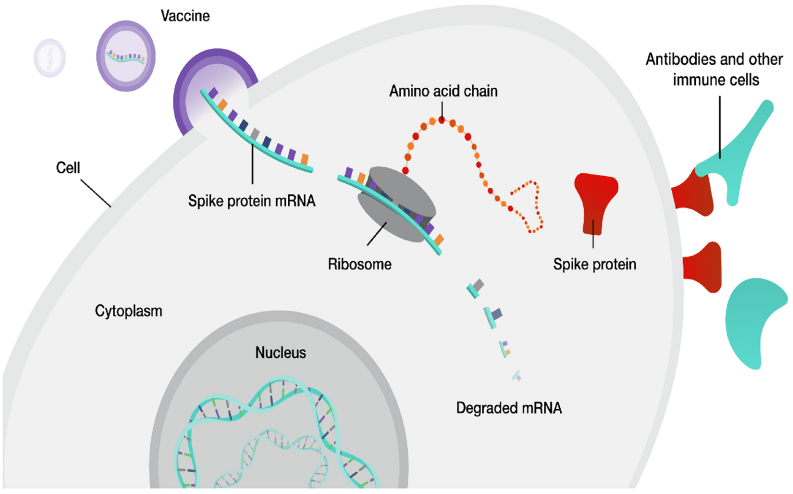
disease. At the same time, due to the non-replicating nature, the host does not always generate adequate immune responses to the vaccine pathogen needing more than one dose to generate the desired immunity.

3.4. Nucleic Acid Vaccines

The recent frontier of vaccine research and development is centered around nucleic acid vaccines, which only delivers genetic information to the recipient cells that contain instructions on producing some specific antigens by which the recipient's immune system can identify the specific pathogen during future encounters. This approach does not introduce any parts of the pathogen's cell, or any antigen(s) produced by the pathogen. It only delivers genetic instructions to the recipient cell such that they can produce a distinctive feature of the pathogen without any virulence. Currently there are two types of nucleic acid vaccines that are being studied and developed: mRNA vaccines, and DNA vaccines.

3.4.1. mRNA Vaccines

In ribo-nucleotide acid (RNA) vaccines, a messenger RNA (mRNA) sequence is introduced into the recipient's body which contains the instruction to produce a piece of protein that is characteristic of the pathogen [61]. Upon deciphering the instructions (*i.e.*, blueprint) contained in the mRNA, the recipient's cell produces the pathogen-specific protein which in turn is recognized by the recipient's immune system as foreign particle (*i.e.*, antigen) resulting in production of antibodies [62] that can neutralize the antigen. **Figure 3** presents a schematic representation of the process. The immune response recorded upon introduction of the mRNA sequence can be replicated later on when the infection occurs due to exposure of the recipient to the pathogen. Based on immune memory, the recipient is able to fight off the infection more successfully than in the case of no



Source:
https://www.genome.gov/about-genomics/fact-sheets/Understanding-COVID-19-mRNA-Vaccines

Figure 3. Schematic showing how mRNA vaccines work within a recipient cell.

prior exposure to the pathogen-specific antigen. Once recipient cells complete the protein production, they break down the mRNA so that the pathogen-specific protein production does not continue infinitely. Also, since the introduced mRNA never enters the recipient cell's nucleus, they do not alter the recipient's genetic makeup [62].

The three main types of RNA vaccines are non-replicating mRNA, *in vivo* self-replicating, and *in vitro* dendritic cell non-replicating. The simplest RNA vaccine is non-replicating mRNA because the mRNA strand is contained and delivered in the body, from there the body's cells make the antigen specific to the mRNA strand. The second type of RNA vaccine is in vivo self-replicating mRNA which is packaged with additional RNA strands which are used to make sure that it will be copied once when the vaccine enters the body's cells. This also assures the body that more antigen is produced from a smaller original amount of vaccine when inserted into the body. This in turn means a larger and more prominent immune response is expected when a natural infection occurs. The final type of RNA vaccine is in vitro dendritic cell non-replicating mRNA vaccine. The dendritic cells are immune cells which can present antigens on the cell surface. The antigens present on the cell surface are used to help stimulate an immune response. Once cells are removed from the patient's blood and transfected with the RNA vaccine, the blood is transferred back into the patient. This will lead to an immune reaction being recorded [63].

The mRNA uses lipid nanoparticles to enter the body. The fatty nanoparticle which surrounds the mRNA is produced by a combination of four different lipid molecules [64]. Out of the four different lipid molecules, one of them is "ionizable", which means that it has a positive charge making it able to attach to negatively charged mRNA. However, the positively charged molecules will lose their charge in the alkaline conditions present in the bloodstream allowing the molecules to be transported into the cells [65].

Some of the benefits of mRNA vaccines over other vaccines are that they are considerably safer. Since mRNA vaccines do not use any part of a pathogen or inactivated pathogen, they have no chance of becoming virulent later on. Also, RNA does not integrate itself onto the host [59]. In other words, the mRNA strand inserted in the body will not be able to replicate or attach onto the recipient's genome. Instead, the strand of RNA is degraded once the antigen is produced. RNA vaccines also have incredibly few side effects with an efficacy rate of close to 90% or higher [66]. Also, RNA vaccines can be produced at a much faster rate and relatively inexpensively [67] in a laboratory as the manufacturing process once standardized can produce the vaccine in mass scale.

Some key challenges which have prevented RNA vaccines to become readily available is the unintended effects have not yet been fully studied. In some rare cases, an unintended immune reaction can occur (need reference) and because of this, such conditions are difficult to predict or avoid as part of large-scale vaccine deployment. Also, the delivery of the vaccine has been a challenge. In order to effectively deliver the vaccine to the cells, the RNA must be free from the lipid

nanoparticles to be broken down quickly and for a quicker delivery, a larger molecule is used to stabilize the RNA then package it into liposomes. Lastly, unlike other vaccines, RNA vaccines need to be stored in special ultra-low temperature freezers [68] [69] maintaining a controlled environment.

One of the newest vaccines which uses RNA technology is the SARS-CoV-2 (COVID-19) vaccine. Pfizer-BionTech and Moderna have produced COVID-19 vaccines using non-replicating mRNA [70] [71]. These vaccines use the same mRNA modification and have similar efficacy rates (90% - 95%). The mRNA in both of these vaccines produces the "spike" protein. The mRNA has been modified by replacing the uridine (U) nucleotide with pseudorine [72]. This modification is done to prevent the immune system from reacting with the introduced mRNA. Once the mRNA sequence is stabilized, the spike protein will use its shape to fuse with the human cells.

3.4.2. DNA Vaccines

One of the recent and revolutionary approaches to vaccination is DNA vaccine which utilizes delivery of a plasmid carrying a DNA sequence that upon introduction into the host body produces target pathogen-specific antigen(s) [73]. This in turn produces an immune response in the host body developing protection against the pathogen without needing introduction of an attenuated or dead pathogen or antigen produced by the pathogen. Typically, the plasmid DNA is precipitated on inert particles and injected into the host cells by helium blast utilizing gene-gun delivery. This in vivo production of target antigen stimulate both B- and T-cell responses engaging MHC-I and MHC-II pathways, that is by far superior than generating antibody responses by introducing recombinant proteins [74].

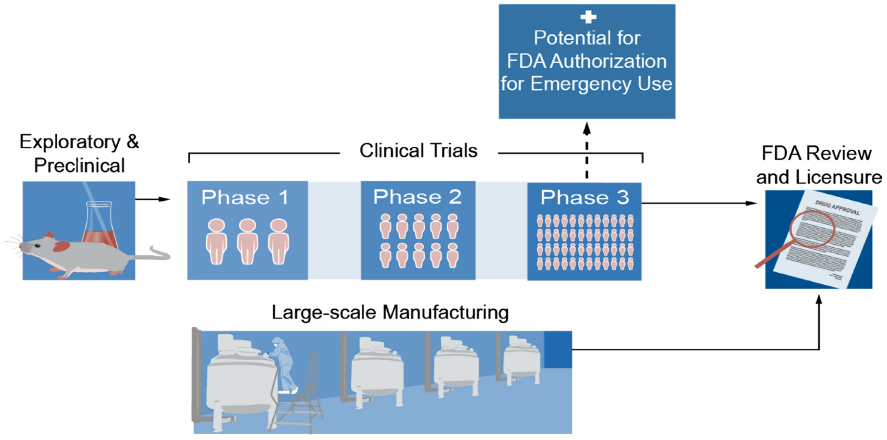
One of the key advantages of DNA-based vaccines over others is that it can induce in the host body both the humoral and cell mediated immune responses [75]. Also, plasmid DNA being relatively stable in comparison to other genetic materials (such as mRNA) makes the purification process simpler and less time consuming. Such vaccines were noted for their in vivo stability as well, as plasmids in non-integrated form were detected in muscles up to six months after their injection in the host body [76]. Since such vaccines do not use any live attenuated or inactivated pathogens, it eliminates any potential of developing in vivo pathogenic infections within the host body from injected cells returning to virulence. This is especially critical from the vaccine safety standpoint for immunocompromised recipients.

Although DNA-based vaccines offer great promise in combating diseases, in clinical studies thus far they were found to be far less potent when given to humans compared to study animals, such as mice [77] [78]. In addition, there is DNA degeneration if the plasmid DNA is unformulated [79], resulting in low amount of plasmid DNA available that can ultimately pass the nuclear membrane barrier to be transcribed by the host cell [80]. To optimize vaccine potency, the transfection plasmids can be redesigned such that they produce large

amounts of protein in the host cell. In addition, many adjuvants and other immunostimulants which are used in the preparation, alongside various formulation and delivery strategies, such as prime-boost combinations, can also be utilized to optimize vaccine efficacy. Lastly, plasmid DNA can be optimized by using different promoters such as CpG (cytosine connected to guanine using a phosphodiester bond) motifs, and codon optimization techniques [76].

4. Conclusion

This article has summarized the technological aspect of the development of vaccines. Long gone are the painstaking and time-consuming approaches of trial and error in developing a vaccine and putting it through several levels of animal clinical studies before even embarking on clinical studies involving human subjects. Figure 4 shows the typical process that any vaccine goes through before it is authorized to be used for mass scale vaccination efforts. Such rigorous efforts used to take years, if not decades before a new vaccine would get approval for introduction in the market for common usage. While growing pathogens in controlled laboratory environments, attenuating or inactivating them many challenges were faced proving to be too difficult to materialize from concept to reality. As stated earlier in the article, advances in cell culture technology, better understanding of cell biology, and molecular genetics along with advancements in computing power coupled with artificial intelligence and predictive analysis have helped filter vaccine development approaches and selection of vaccine candidates within a much shorter time frame. A rousing testament of such an effort could be seen from the wide-scale vaccine development and emergency use deployment against the SARS-CoV-2 virus. Since its world-wide virulence affecting global population during late 2019/early 2020, deployment of a number of vaccines in many of the developed nations across the world took less than a year. The fact that most of the mRNA-based vaccines authorized in the United States



Source: GAO analysis of Food and Drug Administration (FDA), Pharmaceutical Research and

Figure 4. Schematic representing key steps that vaccine undergoes prior to authorization for mass scale deployment.

had greater than 90% efficacy illustrates their effectiveness despite the highly consolidated research and development time spent. As our knowledge and understanding of pathogenesis improve, one can only hope that there will be vaccines available for those pathogenic diseases where currently there are none. We also hope that as the cost of research and development of a vaccine goes down considerably, vaccines will be available at an affordable cost to all nations irrespective of their socio-economic condition. This will bring greater access and equality across the globe.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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