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Review Article

New Vaccine Technologies for Rapid Response against Emerging, Reemerging Infections and Biological Threats: Lessons from COVID-19 for Future

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Abstract
Vaccination is the most effective method to prevent dangerous infectious diseases and save lives. The expansion of human communication, the rapid spread of emerging infections worldwide, and the creation of dangerous pandemics like COVID-19 is worrying. On the other hand, with the emergence of new technologies such as genetic engineering of microorganisms, genome editing, and synthetic biology, the possibility of abusing these tools for illegal use is the next concern. In this situation, the need for rapid vaccination technologies and programs was given special importance. Recently, new vaccine platforms such as viral vector and mRNA vaccines have shown great promise that they can be used to prepare and protect human lives against dangerous infections. One of the most important factors for vaccination is the rapid development and approval of vaccines. In this review, we have given a perspective view of new vaccine technologies to rapidly develop vaccines to combat emerging infections and the biodefence against biological criminals.

Keywords: Vaccine, DNA Vaccines, mRNA Vaccines, Viral Vector, Vaccine Delivery System
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Introduction

Vaccination is an efficacious preventive intervention for saving lives from potentially dangerous infections, diseases,[1] and potential criminal biological attacks.[2] Due to more than 200 years of efforts from the first developed vaccine in 1796, thousands of vaccine candidates have been developed to prevent potential human infections (Figure 1).[3] The clear and satisfactory results of approved vaccines and successful vaccination programs are seen in most developed nations in eradicating and decreasing dangerous epidemic infections that have claimed the lives of millions of people in the past.[4]

Unfortunately, most infectious diseases that could be prevented with vaccines still threaten many lives, especially in developing and underdeveloped countries.[5] Global Alliance for Vaccines and Immunization reported that more than 1.5 million children lost their lives from preventable infections with vaccination. Vaccination programs have been adequately established in recent decades, and most infections have been successfully eradicated.[6] Generally, prophylaxis vaccines comprise one or more antigens that induce the highly evolved immune system for a correct response and memory of a specific pathogen.[7] Besides the simplicity of vaccination and vaccines, achieving an acceptable and especially long-lasting immunization for many infectious diseases has been challenging. For this reason, improved deep immunological understanding will help the development of vaccines for challenging pathogens, including *Mycobacterium tuberculosis*, HIV, and other pathogens with rapid antigenic changes or evolution.[8] In addition to immunological understanding improvements, there is a need for new technological improvements for trusted and fast development of vaccines against newly emerging infectious diseases such as COVID-19 and laboratory-engineered pathogens or potential infections for bioterrorism application.[9] In contrast to traditional vaccine platforms, including live attenuated and subunit vaccines, the third-generation platform of vaccines leads to fast designing and production of large doses for urgent vaccination applications.[10] In this review, we will focus on the new technologies and efforts for future programs for fast and urgent vaccination for natural and potential human-made infectious threats.

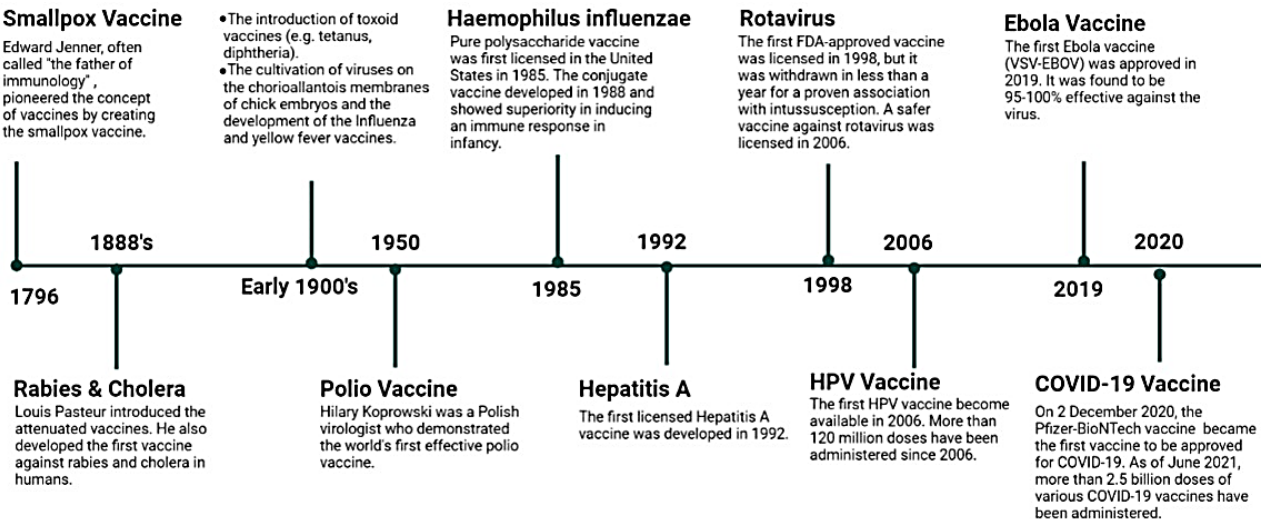

Figure 1. Vaccine Development Throughout History.[3]

Basic Concept of Immunization and Vaccine Designing

Generally, immunization (from the word immune, meaning protected) is the immunity that is the result of exposure of the immune system to an antigen (active immunization) or exogenous humoral immunity (passive immunization). Immunization could be achieved by natural infections or by utilizing vaccines to prevent infectious diseases (prophylactic vaccines) or treatment (therapeutic vaccines) of some diseases, such as malignancies.[11] The complete adaptive immunity for a primary infection results from the induction of humoral immunity (antibody production, especially for pathogen's surface antigens) by B lymphocyte cells and cell-mediated immunity (CMI) by T lymphocyte cells. In this regard, concurrent humoral and CMI immunity will provide high immunity for the same infection in possible future exposures.[12] The vaccination goal is the same as natural exposure to a pathogen; induction of humoral immunity and CMI. Different platforms of vaccines (Figure 2) have been developed and tested successfully for immunization; live attenuated vaccines (oral polio, varicella, and measles vaccines),[13] inactivated vaccines (polio, pertussis, rabies, and typhoid vaccines),[14] toxoids (diphtheria and tetanus toxoids),[15] subunit vaccines (*Haemophilus influenza* type b, *Salmonella typhi*, and hepatitis B),[16] and finally next generations of vaccines including viral vector and mRNA vaccines.

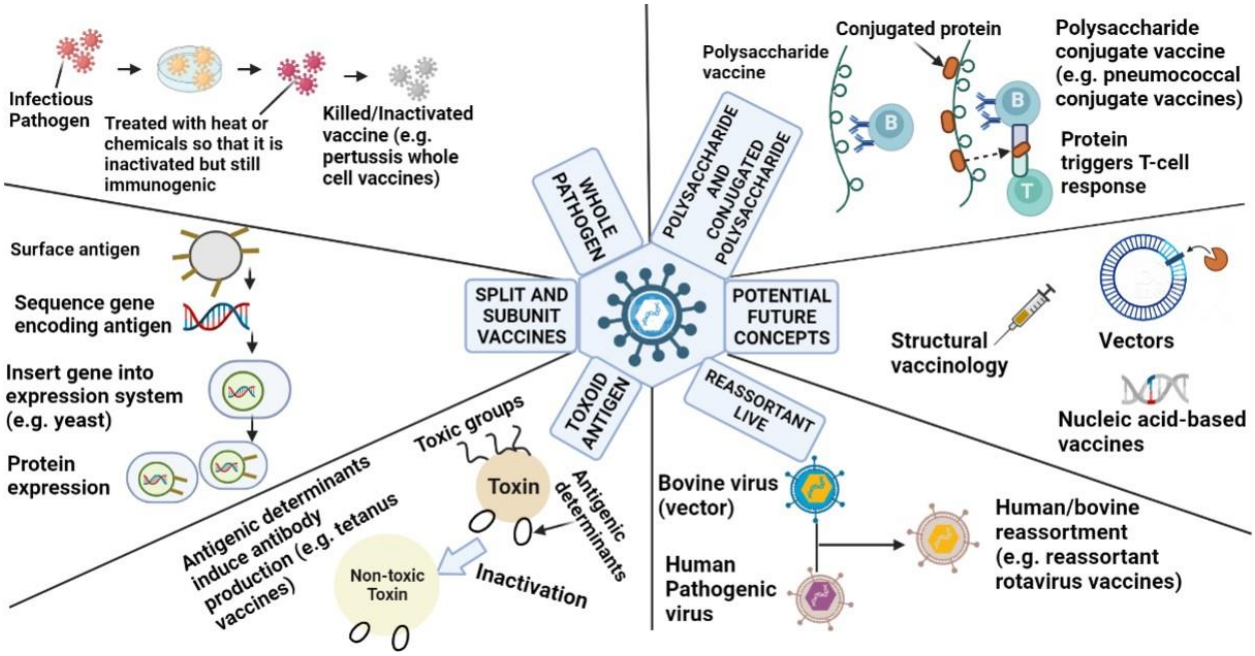

Figure 2. Different Vaccine Platforms.

Generally, all vaccines work through these three steps: Firstly, antigens and used adjuvants will be uptake by immune cells or other cells depending on the route of administration. Secondly, specified antigen-presenting cells (APCs) will be matured, and the vaccine antigens will be processed. Finally, specific B and T lymphocyte cells will be produced, and a proper immune response will be established. APCs, including dendritic cells, macrophages, and B cells, can take up the vaccine's antigens and process the antigens into peptides suitable for expressing major histocompatibility molecules (MHCs) I or II.[17] Naïve T cells interact with APCs MHC, recognize the vaccine antigen epitopes and are primed into either cytotoxic or helper T cells. In addition, B cells also will be activated through interaction with soluble or surface antigens by their receptors (BCR) and differentiated into plasma or memory B cells for the production of antigen-specific antibodies.[4] The first step of vaccine development is designing of vaccine regarding the chosen platform (e.g., subunit, viral vector, mRNA, etc.). New designing methods and tools have made significant advances in the rational designing of vaccines due to an accumulated understanding of the biological and immunological mechanisms of immunity and technological understandings in the last few decades.[18]

The mode of action and immunological interaction between the immune system and the vaccine could be used initially to understand their efficacy, safety, and expected benefit for the vaccinated individual. In addition, different bioinformatics tools,[19] and immunological databases, will be beneficial and, most times, essential for designing vaccines.[20] Choosing a particular vaccine platform can depend on several factors, such as the level of expected protection, the mode of action, the characteristics of the pathogen, or the disease elimination strategy and time.[21,22] Traditional and more established methods, including subunit vaccines, need more time to develop. However, most traditional platforms have been tested in different large populations for many years, and their advantages and disadvantages are well known.[23] Also, designing tools for these platforms are well-developed and accessible. In contrast, regarding the limitations of traditional platforms (discussed later), if time is a critical factor, for example, when an infection is spreading rapidly and is becoming an epidemic and pandemic situation, there is an urgent need for rapid vaccine development.[24] In addition, when a biological attack or bioterrorism has occurred with infections, R & D teams should turn to methods with decreased designing time, such as third-generation DNA and mRNA vaccines.[25,26] These vaccines have many advantages in design and production over traditional vaccines, which were proven in the 2020 pandemic COVID-19.[27,28] Although there are many challenges and unknowns in using new vaccine platforms, in emergency conditions such as the recent pandemic and unexpected biological attacks, the priority is the rapid design and production of a vaccine and vaccination to savelives.[29]

Vaccine Platforms

There are different platforms of vaccine candidates regarding their source of production, components, and level of immunogenicity. Most of the vaccine platforms' candidates are not approved yet. Here we will briefly discuss important approved platforms.

Live Attenuated

The first platform of vaccine produced by humans was the live-attenuated smallpox vaccine. This platform successfully eradicated smallpox and poliomyelitis. Live attenuated vaccines are associated with safety concerns and relatively low efficacy. This platform of vaccines is the infection-related pathogen that is not pathogenic for humans or less severe strains or is obtained after repeated culturing in cells from a species not permissive to the virus.[30]

Inactivated

The second class of vaccines is inactivated vaccines by applying heat, radiation, or chemicals. Inactivating destroys the ability of pathogens to replicate in human cells. However, most pathogen antigens should remain intact through the inactivation process for proper immune response induction.[31]

Toxoids

Toxoids are another platform of vaccines. Toxoids are produced from the toxins of bacteria (tetanus and diphtheria). Toxoids are not highly immunogenic, and they require an adjuvant as well as multiple-dose administration.[32]

Conjugate Vaccine

The conjugation of an antigenic protein produces 1.2. conjugated vaccines to carbohydrates to increase their immunogenicity and stability.[33]

Subunit Vaccine

Subunit vaccines comprise protein antigens, usually produced through recombinant DNA technologies in prokaryotes expression systems such as *E. coli*. Subunit vaccines show an excellent safety profile and can be modified to change properties facilitating the development of stable formulations. They often require the formulation and adjuvants to increase their efficacy.[16]

Nucleic Acid Vaccine

Finally, the latest technology for vaccine development is based on nucleic acid–with strong immunogenicity, fast production technology, and safety profile compared with live-attenuated vaccines ensuring that the immune response is directed against the transgene product, partially against the viral coating.[34] Recently in the 2020 pandemic of COVID-19,

the FDA approved mRNA vaccines (Moderna and Pfizer vaccines)[35-37] and viral vector-based vaccines (AstraZeneca and Sputnik-V vaccines)[38,39] for vaccination against SARS-CoV-2. Nucleic acid-based vaccines showed excellent immunization (generally up to 90%) against COVID-19. In addition to high immunogenicity, these vaccines were designed and developed in a comparatively short time against other platforms, making them a dominant suitable future vaccine platform for new emerging natural infection and bioterrorism attacks.[36,40]

Increase the Effectiveness of General Immunization

Estimates of the effectiveness of general immunization vary from country to country for each vaccine. For example, for pneumococcal and rotavirus vaccines, it is between 35 and 47%. The World Health Organization recommends a more than 90% general immunization estimate. Although various factors affect the estimated efficacy of the vaccine, a person's reluctance to inject the vaccine is one of the main obstacles to broader coverage of immunization worldwide.[21] Pain experienced during vaccination is among most commonly reported causes of doubt about vaccine use. One of the reasons for the success of the oral polio vaccine in bringing the disease closer to eradication (despite recent issues with the vaccine itself) and increasing the uptake of nasal flu vaccine for children shows that non-injectable vaccination routes have a significant impact on improving general immunization effectiveness. New approaches to increase the effectiveness of general immunization include the development of new technologies for the manner and route of vaccine injection and formulations for dermal, oral, and mucosal delivery (Figure 3).[22]

Next Generation Vaccination

This section will discuss why the next generation of vaccines could help prevent infections and how we could accelerate

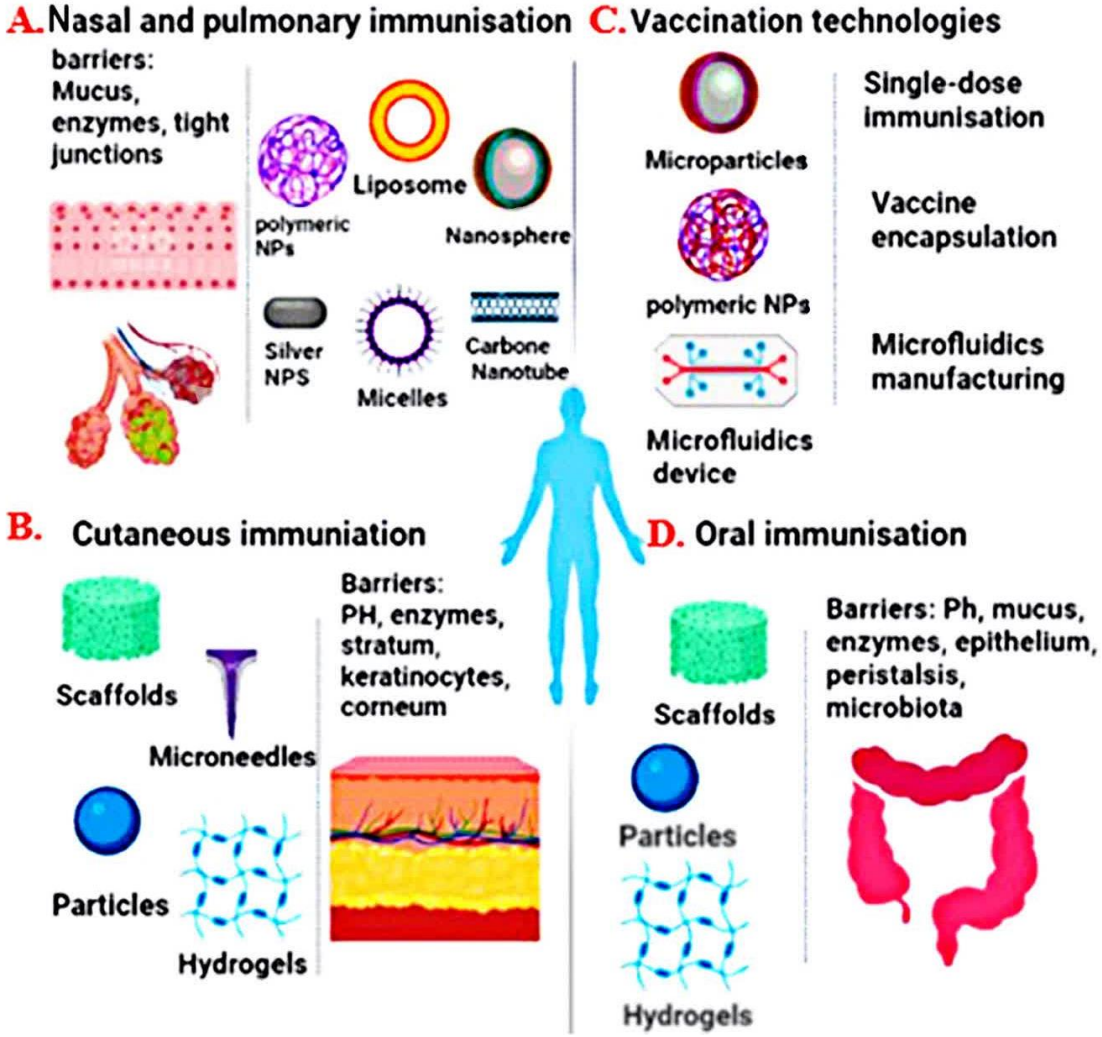

Figure 3. New Methods of Vaccine Design to Overcome Barriers to Vaccine Effectiveness. **A)** Nasal and pulmonary immunization using particle transport systems such as liposomes or nanocapsules; **B)** Oral immunization using modern transmission systems such as hydrogels; **C)** Oral immunization using microparticle delivery systems; **D)** Single-dose immunization using polymer nanoparticles or vaccine encapsulation using emulsions and microfluidic systems.

vaccine development.

Why Vaccination and Why Next Generation Vaccines?

After the application of 2nd generation of vaccines, such as subunit recombinant vaccines, 3rd generation of vaccines, including viral vector and mRNA vaccines, are attracted huge interest. These platforms have many advantages compared to 2nd and first generations of vaccines. This section will discuss why 3rd generation vaccines are beneficial in special situations like epidemics and pandemics. Various epidemics and pandemics occurred throughout the history of human life due to emerging and reemerging infections. The lack of a rapid response in terms of diagnosis, treatment, and prevention has claimed the lives of millions of people and affected the economy, political, and social relations in different countries.[41-43] In addition, with the growth and development of technologies for manipulating organisms and microorganisms, the possibility of political, economic, and racial abuse of these potentially helpful technologies has raised many concerns and suspicions about new emerging infections.[44] The major concern behind the beneficial application of these technologies, such as genetic engineering, synthetic biology, genome editing, and systems biology, is that they can be used to develop new infectious diseases in bioterrorism and anti-human biological attacks.[45] Therefore, new vaccine production platforms must be welcomed today to be prepared for any threat to people's lives to combat natural emerging diseases and the potential abuse of dangerous pathogens. Despite great advances in diagnostics, therapeutics, and vaccines, fast transportation, and high global human communication help infectious diseases spread faster than in the past centuries.[46] For this reason, emerging infectious diseases (EIDs) or natural threats and the potential abuse of pathogens for criminal applications are among the biggest concerns for modern human life.[47] In recent decades, modern human societies have grown very fast. The complexity has raised and made a proper context for emerging unexpected pathogens or reemerging of eradicated pathogens whether naturally in the absence of prior health measures or intentionally for criminal uses against human populations. The vulnerability of modern human life and populations to emerging and reemerging pathogens and their risks of rapidly evolving into devastating outbreaks and pandemics should be considered to regulate healthcare and control potential centers including genetic engineering laboratories.[48-50]

How to Accelerate Vaccine Development?

Generally, attempts for R & D of traditional platforms of vaccines need 5-10 years.[4] Traditional methods do not meet the expectations to fast response and control epidemics and pandemics of an emerging or reemerging pathogen. However, fortunately, the vaccine development route has significantly changed through the COVID-19 pandemic. For instance, the vaccine development period and regulation agencies' response to Ebola and SARS-CoV-2 infections are comparable, only six years apart. The 2014 Ebola epidemic lasted more than 24 months, and the vaccine developed after the pandemic finished.[51] In the context of the COVID-19 pandemic, the period of R & D and the beginning of clinical trials was under one year.[52] This shows that vaccine development could be significantly rapid and effective in urgent situations using new technologies. The Coalition for Epidemic Preparedness Innovations (CEPI) was established to accelerate vaccine development for new epidemics. CEPI priority was multiple EIDs, including the Middle East respiratory syndrome (MERS), Lassa fever, Nipah, Rift Valley fever (RVF), and chikungunya (https://cepi.net/). Vaccine development platforms that could complete design from sequence release to clinical trials in a few weeks rather than months or years, such as mRNA and DNA vaccine platforms, are the priority of CEPI. As mentioned earlier, after the release of the SARS-CoV-2 genome sequence from the Chinese strain, these platforms showed responsive potential during the COVID-19 pandemic. They made a great promise for the future established vaccine development platform. CEPI has to fund Moderna, Inovio, Oxford–AstraZeneca, and Novavax for vaccine development (https://cepi.net/COVAX/). Vaccinations are the basis of controlling infectious disease outbreaks and are the surest strategy to overcome pandemics and epidemics. Experiences of epidemics and outbreaks of infections have shown that the immediate availability of a vaccine, the easier and faster it will be to control the infection.[53] However, as we discussed, the standard and traditional vaccine development platforms and regulations are not suited to the needs of explosive pandemics. New vaccine platform technologies dramatically shorten the period from research to development, clinical trials and vaccination.[53] In Table 1, we provided the most important new vaccine platforms with high responsivity for fast-spreading new infections.

To provide a very concrete example of the effectiveness of these new technologies' effectiveness, we have to look at the recent COVID-19 pandemic. In this pandemic, the mRNA-based vaccine of Moderna was designed and synthesized within a few days after the release of the virus genomic sequence, and its animal trials began in less than a month. Simultaneously, the Pfizer-Biontech vaccine was developed with the same platform. Both vaccines are very effective in clinical trial phases regarding immunization and preventing disease-related mortality. Their efficacy was about 95% for immunogenicity and 100% for preventing mortality, which few vaccines have ever shown.[36,58] On the other hand, the vaccine Sputnik-V uses DNA with two separate vectors for use in two doses. Similar to miRNA-based vaccines, this vaccine also showed very high efficacy in immunogenicity and prevention of disease-related mortality. The FDA has

Table 1. Future Vaccine Technologies

Type	Pathogen	Diseases	Year	Type	Scientific Name	Trade Name	Delivery System	Ref
Viral Vector	Ebola virus	Ebola	2019	Recombinant vesicular stomatitis virus	rVSV-ZEBOV	Ervebo	VSV	55
	SARS-COV-2	COVID-19	2021	Ad5 and Ad26	Gam-COVID-Vac	Sputnik V	Genome Engineered Human Adenovirus serotype 5 and 26	56
	SARS-COV-2	COVID-19	2021	Ad26	Ad26-CoV2-S	Janssen COVID-19 vaccine	Genome Engineered Human Adenovirus serotype 26	57
	SARS-COV-2	COVID-19	2021	chAdoX	chAdOx1-nCOV-19	Vaxzevira	Genome Engineered chimpanzee Adenovirus	58
mRNA Vaccine	SARS-COV-2	COVID-19	2021	Naked mRNA	mRNA-1273	Moderna COVID -19 vaccine	Lipid nanoparticles (LNP)	59
	SARS-COV-2	COVID-19	2021	Naked mRNA	BNT162b2	Comirnaty	Lipid nanoparticles (LNP)	37

approved all three vaccines for emergency vaccination. Although this pandemic was the first time humans extensively began using nucleic acid-based vaccines, it was a considerable experience. A beautiful lesson from COVID-19 was that these vaccines could be one of the solutions to new diseases that are rapidly spreading, and also, these platforms can be a way to deal with bioterrorism threats. Because in both conditions, the priority is to respond quickly and control disease using a vaccine so that the vaccine can be made with a much shorter platform to build and develop than a traditional vaccine.[40] On the other hand, a platform that can be used for various infections with minimal changes is much preferred. For example, in the case of mRNA and DNA vaccines, all elements for gene delivery, translation, and transcription within the cytoplasm and nucleus of the human cell can be optimized for all possible infections. The subsequent fort is that the sequence of antigen in the form of RNA in the mRNA vaccine and DNA in the DNA vaccine vector can be included in the platform for vaccine development. These fixed elements are one of the reasons for the short development time of these vaccines.[53,59] In contrast, in the case of subunit vaccines, significant optimization is needed for designing, purifying, and producing each antigen from different infections. Similarly, in producing vaccines based on complete virus particles, it is necessary to consider the manufacturing and safety conditions for each infection by different protocols.[16,60] Therefore, it can be concluded that mRNA (Moderna and Pfizer-BioNtech) and DNA-based vaccines based on rAd5 and rAd26 (Sputnik V) or chimpanzee adenovirus-derived ChAdOx (Oxford-Astrazeneca),[57] that were successfully used in the COVID-19 pandemic, are platforms that require only the replacement of one antigen for an infection in the platform.

Fast Development and Manufacturing of Vaccines

Upscaling, downstream analysis (including quality control), and post-manufacturing consideration for biotechnology products (vaccines, recombinant therapeutic proteins, and enzymes) need much more care and equipment than chemicals and industrial products.[61] Scaling up, faster production, and distributing large amounts of vaccine doses for situations like the COVID-19 pandemic or biological attacks depends on various factors. As discussed in the previous section, the success of a fast response to an outbreak largely depends on the rapid development of a vaccine. In addition, the period of vaccine development also depends on the chosen vaccine platform. The platform of the vaccine, single or multiple dosages for immunization, the production capacity, and vaccine storage condition are all important for the rapid development of the vaccine from laboratory to clinical trials and approval.[3,6,62] In the previous section, we discussed vaccine platforms' capability for rapid design and development. The second important factor is how a platform is robust for scaling up and how vaccine manufacturers in nations predominantly low-income countries can provide vaccines for the vaccination of a large population. In this regard, the main goal of the Developing Countries Vaccine Manufacturers Network (DCVMN) is to enhance vaccine large production and availability for low-income countries to protect against epidemics.[63] Creating such organizations and connecting and coordinating the major vaccine manufacturers to expand the vaccine production line in an emergency will be a grating effort. The third important factor, especially for underdeveloped countries, is vaccine storage and transportation condition. In this regard, under the condition of outbreaks, the need for more practical vaccine development and vaccination route, supply, and dosing is much more important. For instance, most mRNA vaccines need ultra-cold chain storage (-70 ºC) conditions, and the equipment required for creating ultra-cold chains is limited in most underdeveloped countries. While traditional technologies, such as whole-organism vaccines, need only a typical cold chain.[64,65] However, through advances in RNA and DNA, stabling technologies, nucleic acid-based vaccines, especially mRNA vaccines, can be stored in milder temperature conditions like other vaccines. So a vaccine's stability is related to a vaccine platform's intrinsic characteristics. A highly stable vaccine, more available in underdeveloped countries. For this reason, new platforms, especially mRNA vaccines, need further modifications to enhancement of their stability.[66,67] The next factor for rapid response is the vaccine immunization dosage. Protection by single or multiple doses could also affect the response duration against an outbreak and bioterrorism attacks.[68] The COVID-19 pandemic has given

us valuable lessons and dramatic advances in the vaccine industry. These valuable lessons and findings should be used to prevent emerging infections and criminal biological purposes. The permanent threat of emerging pathogens and potential biological attacks reminds scientists, politicians, and all human health planning agents to be prepared to deal with and respond quickly to emerging infectious agents. These preparations require comprehensive collaboration between scientists and others involved in social health.

Future Considerations for Vaccination

Generally, vaccines are being formulated to protect heterogeneous populations of humans or animals regarding genotype, sex, age, etc., against infectious diseases. In the past decades, hundreds of studies and clinical trials have shown that vaccines' efficacy may differ in inter-populations or intra-populations of individuals for some known and unknown reasons.[17] For this reason, clearing the factors creating the variability of vaccine efficacy among individuals and developing of more personalized vaccination strategy regarding population genotypes is the next effort. In addition, except for the recently developed mRNA and DNA vaccines, other vaccine platforms usually suffer from low overall immunogenicity.[69-71] The vaccine delivery systems are among the most critical factors, especially for nucleic acid-based vaccines. Advanced bio-material delivery systems significantly enhance vaccine safety, drug safety, and immunogenicity.[28] However, more advanced vaccine delivery systems need to reduce vaccination side effects and increase the immunogenicity of vaccines for those diseases that existing vaccines that do not have high immunogenicity. Therefore, with these systems, potentially stronger vaccines with high capabilities can be developed to replace the existing low-efficient vaccines. Despite many advances in vaccine designing tools, delivery systems, and platforms, there are important infectious diseases for which no effective vaccine is available (for instance, TB, HIV, and malaria). In addition, some at-risk groups show even low responses to the vaccines or the existing vaccine have severe side effects and need further development of suitable or more immunogenic vaccines (e.g., for elderly individuals or infants).[72,73] New vaccines are needed for several important infections to reduce morbidity and mortality (group B *Streptococcus*, RSV, and CMV).[74] In addition, the development of new vaccines is required for hospital-acquired infections, especially for antibiotic-resistant strains such as *Staphylococcus aureus*.[75]

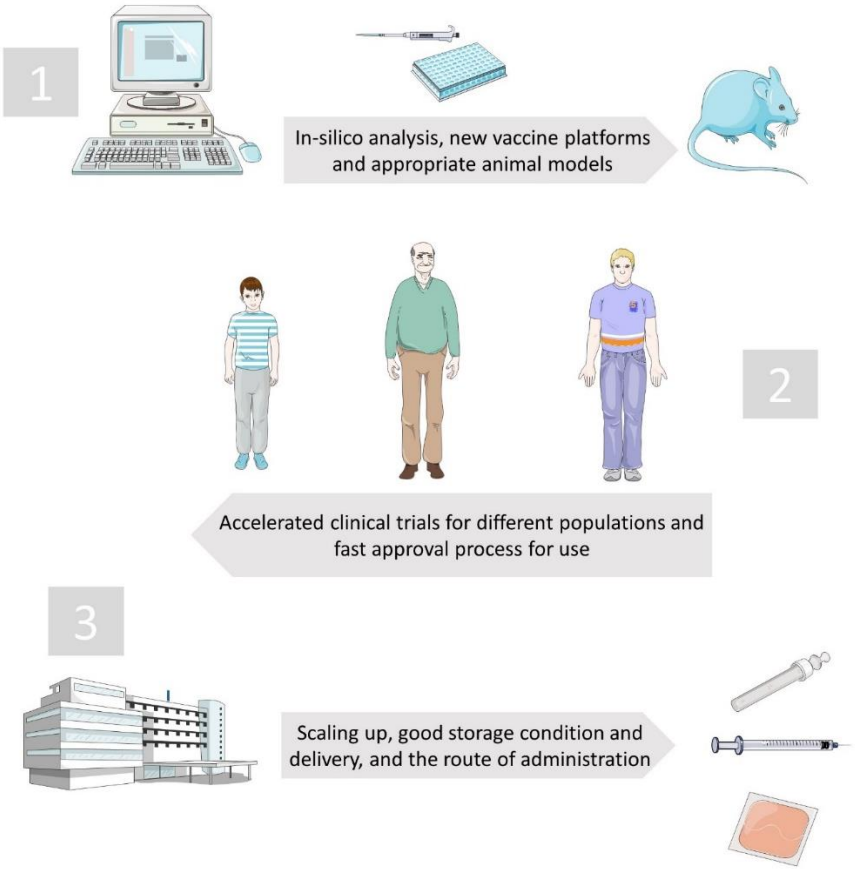

Figure 4. Schematic View of the Steps of Vaccine Development and Critical Steps for Fast Vaccine Development. **1)** *In-silico* analysis by new tools, choosing a fast-developing vaccine platform, and pre-clinical studies by appropriate animal models reflecting a good human immune system; **2)** Accelerated and facilitated clinical trials for side effects, dosage, and timing in different populations for approval; **3)** Large-scale manufacturing and good post-manufacturing conditions (e.g., storage condition, delivery, routes of administration such as oral administration or microneedle patches).

Effective Vaccination and Rapid Response against Biological Threats

Before the COVID-19 pandemic, a vaccine development period takes more than five years. The recent COVID-19 pandemic showed that vaccine development and vaccination programs should be more flexible and facile for production and scaling up than before (Figure 4).

New vaccine technologies for overcoming pandemic situations or biological threats should use platforms for improved antigen delivery, enhanced antigen design, and suitable adjuvants to improve immunogenicity, ease, and production speed. New vaccine development technologies, such as viral vector and RNA-based vaccines, have multiple benefits against traditional platforms. In addition, cell-based vaccines such as dendritic cells,[76] T cells,[77] and bacterial vectors[78] are promising and need further research among vaccine development platforms, viral vector, and nucleic acid-based vaccines significantly increase the time of development and production processes.[79] DNA or RNA of pathogens antigens could allow for the induction of humoral and cellular immune responses. A considerable advantage of these vaccines is that they are highly versatile, quick, and easy to develop and adapt for any emerging pathogen. DNA or RNA vaccines should be taken up by host cells (person who was vaccinated) and should be delivered directly into cells, which requires efficient delivery systems (discussed earlier). In this regard, developing highly efficient methods for delivering these vaccines is one of the most important for future vaccine platforms. Current delivery systems include liposomes,[80] polymeric particles,[81-82] dendrimers,[82] extracellular vesicles and exosomes,[83-84] lipid nanoparticles,[86] and self-assembling protein nanoparticles[87] for drug and vaccine delivery show high efficiency. New minimally invasive vaccine administration methods such as microneedle patches[88] have the advantages of minimal pain, ease of extensive and safer vaccination. In addition, besides intramuscular, sub-cutaneous, and intradermal routes of vaccine administration, mucosal administration routes such as intranasal, oral, sublingual, or skin-using microneedle or needle-free devices have many advantages, such as more accessible and more comfortable for vaccine recipients and may improve vaccine acceptance and uptake, especially by those who fear needles pain.[89] In addition, mucosal administration could induce both systemic and mucosal immune responses, especially at the sites of pathogen entry. Vaccination by mucosal route has been successfully used for OPV, cholera vaccine,[90] and rotavirus vaccines for the oral route, and the live attenuated influenza vaccine for the nasal route.[91,92] Mucosal route vaccination could elicit T- and B-cell immune responses in the local and distal mucosal surfaces, such as the respiratory tract, and establish effective immunity in the first line of defense against air-borne or food-borne pathogens and aerosol transmission of bioterrorism agents. Among various mucosal delivery routes, oral vaccines may be the most suitable and effective for mass and rapid vaccination in epidemics and biological attacks.[93] In addition, the oral route is the best choice for enteric pathogens. On the other hand, besides methods of vaccination and vaccine platforms, we should understand more about immune response and long-lasting immunity against emerging pathogens.[11] Unfortunately, in most cases, while vaccine candidate development is perfect, the immunity disappears after a while.[94] In this regard, new tools and interdisciplinary sciences like bioinformatics, systems biology, personalized medicine, and genome analyzing tools could solve the unknowns of the nature of host-pathogen interaction. New vaccines and future vaccination concepts should improve current vaccines with low efficacy and solve the problems with hard-to-vaccination pathogens like dengue, *S. pneumoniae*, TB, HIV, and malaria. Finally, many vaccine candidates may be induced adequate immunity in common animal models but fail to show similar results in large animals or humans. Vaccines that study animal models, including murine, cannot be naturally infected by most of the emergent pathogens mentioned above. The pathogenesis of these pathogens, vaccine dosing for immunization, and types and durability of induced or shown immune responses may not reflect the human immune response. Therefore, a more versatile and appropriate screening process should be acquired in the early stages of vaccine development.[95,96] The discussed new technologies, methods, and systems have shown great promise for addressing current limitations in vaccine technologies against emerging infectious agents and biological threats. They should be strongly considered for public health preparedness, countermeasures against potential outbreaks, and bioterror threats.

Conclusion

Despite many advances in vaccination and protecting the lives of millions of people, as we mentioned, there are important knowledge unknowns and challenges faced in vaccination, from labs to regulatory agencies. In the laboratory, unknown understandings of immunity, immune response, and antigenic variation are the biggest challenges for developing vaccines for highly variable pathogens such as influenza and HIV and immune system escaping pathogens like TB. In addition to pathogen variation, variation in host response is an important factor that should be addressed. Besides the scientific challenges, sociopolitical barriers and limitations stand in the way of safe and effective vaccination for all human populations. Access to vaccines for all human populations is one of the most important factors for efficient protection against emerging pathogens and biological threats. A most important lesson from the COVID-19 pandemic is that, in the case of an emergency, organizations with different activities can coordinate and connect to develop vaccines rapidly and safely. In addition to emerging natural

pathogens, we should take the potential abuse of new technologies seriously. In biodefence, most of the discussed factors for the future, high-speed and rapid manufacturing of vaccines, and pre-manufactured and prepared vaccines for potentially dangerous pathogens for criminal use, like anthrax and smallpox, should be taken into account.

Authors' Contributions

The conceptualization was done by HEG. The resource and writing-original draft preparation were carried out by HEG, MF, ASH, RD, AMMF, and MAH. The writing review and editing were performed by HEG and MF. The supervision was done by HEG. The whole manuscript was read and approved by all authors.

Ethics Approval

This study was executed under the supervision of the ethical committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ethical code: IR.BMSU.REC.1399.272).

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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References

1. Maltezou HC, Theodoridou K, Ledda C, Rapisarda V, Theodoridou M. Vaccination of healthcare workers: is mandatory vaccination needed? Expert Rev Vaccines. 2019;18(1):5-13. doi:10.1080/14760584.2019.1552141
2. Jefferson T. Bioterrorism and compulsory vaccination. BMJ. 2004;329(7465):524-5. doi:10.1136/bmj.329.7465.524
3. Saleh A, Qamar S, Tekin A, Singh R, Kashyap R. Vaccine Development Throughout History. Cureus. 2021;13(7). doi:10.7759/cureus.16635
4. Hardt K, Bonanni P, King S, Santos JI, El-Hodhod M, Zimet GD, Preiss S. Vaccine strategies: Optimising outcomes. Vaccine. 2016;34(52):6691-9. doi:10.1016/j.vaccine.2016.10.078
5. Shaker R, Fayad D, Dbaibo G. Challenges and opportunities for meningococcal vaccination in the developing world. Hum Vaccin Immunother. 2018;14(5):1084-97. doi:10.1080/21645515.2018.1434463
6. Muraskin W. The global alliance for vaccines and immunization: Is it a new model for effective public–private cooperation in international public health? Am J Public Health. 2004;94(11):1922-5. doi:10.2105/AJPH.94.11.1922
7. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. Ann Med. 2018;50(2):110-20. doi:10.1080/07853890.2017.1407035
8. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. Expert Rev Vaccines. 2016;15(3):313-29. doi:10.1586/14760584.2016.1124762
9. Slifka MK, Amanna I. How advances in immunology provide insight into improving vaccine efficacy. Vaccine. 2014;32(25):2948-57. doi:10.1016/j.vaccine.2014.03.078
10. Mascola JR, Fauci AS. Novel vaccine technologies for the 21st century. Nat Rev Immunol. 2020;20(2):87-8. doi:10.1038/s41577-019-0243-3
11. Sell S. How vaccines work: immune effector mechanisms and designer vaccines. Expert Rev Vaccines. 2019;18(10):993-1015. doi:10.1080/14760584.2019.1674144
12. Boehm T, Swann JB. Origin and evolution of adaptive immunity. Annu Rev Anim Biosci. 2014;2(1):259-83. doi:10.1146/annurev-animal-022513-114201
13. Mok DZ, Chan KR. The effects of pre-existing antibodies on live-attenuated viral vaccines. Viruses. 2020;12(5):520. doi:10.3390/v12050520
14. Bandyopadhyay AS, Garon J, Seib K, Orenstein WA. Polio vaccination: past, present and future. Future Microbiol. 2015;10(5):791-808.
15. Haber P, Moro PL, Ng C, Dores GM, Perez-Vilar S, Marquez PL, et al. Safety review of tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccines (Tdap) in adults aged≥ 65 years, Vaccine Adverse Event Reporting System (VAERS), United States, September 2010–December 2018. Vaccine. 2020;38(6):1476-80. doi:10.1016/j.vaccine.2019.11.074
16. Moyle PM, Toth I. Modern subunit vaccines: development, components, and research opportunities. ChemMed Chem. 2013;8(3):360-76. doi:10.1002/cmdc.201200487
17. Kocourkova A, Honegr J, Kuca K, Danova J. Vaccine ingredients: components that influence vaccine efficacy. Mini Rev Med Chem. 2017;17(5):451-66.
18. Moise L, Gutiérrez AH, Khan S, Tan S, Ardito M, Martin WD, et al. New immunoinformatics tools for swine: designing epitope-driven vaccines, predicting vaccine efficacy, and making vaccines on demand. Front Immunol. 2020;11:563362. doi:10.3389/fimmu.2020.563362
19. Jafari D, Malih S, Gomari MM, Safari M, Jafari R, Farajollahi MM. Designing a chimeric subunit vaccine for influenza virus, based on HA2, M2e and CTxB: a bioinformatics study. BMC Mol Cell Biol. 2020;21(1):89. doi:10.1186/s12860-020-00334-6
20. He Y, Xiang Z. Databases and in silico tools for vaccine design. Methods Mol Biol. 2013;993:115-27. doi:10.1007/978-1-62703-342-8_8
21. Lewnard JA, Lo NC, Arinaminpathy N, Frost I, Laxminarayan R. Childhood vaccines and antibiotic use in low-and middle-income countries. Nature. 2020;581(7806):94-9. doi:10.1038/s41586-020-2238-4
22. Lemoine C, Thakur A, Kraji nik D, Guyon R, Longet S, Razim A, et al. Technological approaches for improving vaccination compliance and coverage. Vaccines. 2020;8(2):304. doi:10.3390/vaccines8020304
23. Chua BY, Sekiya T, Jackson DC. Opinion: making inactivated and subunit-based vaccines work. Viral Immunol. 2018;31(2):150-8. doi:10.1089/vim.2017.0146
24. Tombacz I, Weissman D, Pardi N. Vaccination with messenger RNA: a promising alternative to DNA vaccination. Sousa B. (eds) DNA Vaccines. Methods Mol Biol. 2021. pp. 13-31. doi:10.1007/978-1-0716-0872-2_2
25. Alameh MG, Weissman D, Pardi N. Messenger RNA-based vaccines against infectious diseases. Yu D, Petsch B. (eds) mRNA Vaccines. Curr Top Microbiol Immunol. Springer, Cham. 2020. doi:10.1007/82_2020_202
26. Voigt EA, Kennedy RB, Poland GA. Defending against smallpox: a focus on vaccines. Expert Rev Vaccines. 2016;15(9):1197-211. doi:10.1080/14760584.2016.1175305

27. Tarighi P, Eftekhari S, Chizari M, Sabernavaei M, Jafari D, Mirzabeigi P. A review of potential suggested drugs for coronavirus disease (COVID-19) treatment. Eur J Pharmacol. 2021;895:173890. doi:10.1016/j.ejphar.2021.173890
28. Eslami SS, Jafari D, Montazeri H, Sadeghizadeh M, Tarighi P. Combination of curcumin and metformin inhibits cell growth and induces apoptosis without affecting the cell cycle in LNCaP prostate cancer cell line. Nutr Cancer. 2021;73(6):1026-39. doi:10.1080/01635581.2020.1783327
29. Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. Front Immunol. 2018;9:1963. doi:10.3389/fimmu.2018.01963
30. Eto A, Saito T, Yokote H, Kurane I, Kanatani Y. Recent advances in the study of live attenuated cell-cultured smallpox vaccine LC16m8. Vaccine. 2015;33(45):6106-11. doi:10.1016/j.vaccine.2015.07.111
31. Delrue I, Verzele D, Madder A, Nauwynck HJ. Inactivated virus vaccines from chemistry to prophylaxis: merits, risks and challenges. Expert Rev Vaccines. 2012;11(6):695-719. doi:10.1586/erv.12.38
32. Angsantikul P, Fang RH, Zhang L. Toxoid vaccination against bacterial infection using cell membrane-coated nanoparticles. Bioconjug Chem. 2017;29(3):604-12. doi:10.1021/acs.bioconjchem.7b00692
33. Pai VB, Heyneman CA, Erramouspe J. Conjugated heptavalent pneumococcal vaccine. Ann Pharmacother. 2002;36(9):1403-13. doi:10.1345/aph.1A048
34. Le TK, Paris C, Khan KS, Robson F, Ng WL, Rocchi P. Nucleic acid-based technologies targeting coronaviruses. Trends Biochem Sci. 2021;46(5):351-65. doi:10.1016/j.tibs.2020.11.010
35. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med. 2020;383(20):1920-31. doi:10.1056/NEJMoa2022483
36. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603-15. doi:10.1056/NEJMoa2034577
37. Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature. 2020;586(7830):594-9. doi:10.1038/s41586-020-2814-7
38. Ikegame S, Siddiquey MN, Hung CT, Haas G, Brambilla L, Oguntuyo KY, et al. Neutralizing activity of Sputnik V vaccine sera against SARS-CoV-2 variants. Nat Commun. 2021;12(1):4598. doi:10.1038/s41467-021-24909-9
39. Hwang J, Lee SB, Lee SW, Lee MH, Koyanagi A, Jacob L, et al. Comparison of vaccine-induced thrombotic events between ChAdOx1 nCoV-19 and Ad26. COV. 2. S vaccines. J Autoimmun. 2021;122:102681. doi:10.1016/j.jaut.2021.102681
40. Logunov DY, Dolzhikova IV, Shcheblyakov DV. Data discrepancies and substandard reporting of interim data of Sputnik V phase 3 trial–Authors' reply. Lancet. 2021;397(10288):1883-4. doi:10.1016/S0140-6736(21)00894-1
41. Тнuves C, Crubйzy E, Biagini P. History of smallpox and its spread in human populations. Paleomicrobiol Hum. 2016. pp. 161-72. doi:10.1128/9781555819170.ch16
42. Mokrani K, Fournier PE, Dalichaouche M, Tebbal S, Aouati A, Raoult D. Reemerging threat of epidemic typhus in Algeria. J Clin Microbiol. 2004;42(8):3898-900. doi:10.1128/JCM.42.8.3898-3900.2004
43. Kagaayi J, Serwadda D. The history of the HIV/AIDS epidemic in Africa. Curr HIV/AIDS Rep. 2016;13(4):187-93. doi:10.1007/s11904-016-0318-8
44. Jansen HJ, Breeveld FJ, Stijnis C, Grobusch MP. Biological warfare, bioterrorism, and biocrime. Clin Microbiol Infect. 2014;20(6):488-96. doi:10.1111/1469-0691.12699
45. Ahteensuu M. Synthetic biology, genome editing, and the risk of bioterrorism. Sci Eng Ethics. 2017;23(6):1541-61. doi:10.1007/s11948-016-9868-9
46. Tatem AJ, Rogers DJ, Hay SI. Global transport networks and infectious disease spread. Adv Parasitol. 2006;62:293-343. doi:10.1016/S0065-308X(05)62009-X
47. Fan Y, Moon JJ. Particulate delivery systems for vaccination against bioterrorism agents and emerging infectious pathogens. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2017;9(1):e1403. doi:10.1002/wnan.1403
48. Jones TF, Yackley J. Foodborne disease outbreaks in the United States: a historical overview. Foodborne Pathog Dis. 2018;15(1):11-5. doi:10.1089/fpd.2017.2388
49. Principi N, Esposito S. Mumps outbreaks: A problem in need of solutions. J Infect. 2018;76(6):503-6. doi:10.1016/j.jinf.2018.03.002
50. Smith KF, Goldberg M, Rosenthal S, Carlson L, Chen J, Chen C, et al. Global rise in human infectious disease outbreaks. J R Soc Interface. 2014;11(101):20140950. doi:10.1098/rsif.2014.0950
51. Bezbaruah R, Borah P, Kakoti BB, Al-Shar'I NA, Chandrasekaran B, Jaradat DS, et al. Developmental landscape of potential vaccine candidates based on viral vector for prophylaxis of COVID-19. Front Mol Biosci. 2021:96. doi:10.3389/fmolb.2021.635337
52. Walsh EE, Frenck Jr RW, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med. 2020;383(25):2439-50. doi:10.1056/NEJMoa2027906
53. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261-79. doi:10.1038/nrd.2017.243
54. Bache BE, Grobusch MP, Agnandji ST. Safety, immunogenicity and risk–benefit analysis of rVSV-ΔG-ZEBOV-GP (V920) Ebola vaccine in Phase I–III clinical trials across regions. Future microbiol. 2020;15(2):85-106. doi:10.2217/fmb-2019-0237
55. Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatulin AI, Shcheblyakov DV, Dzharullaeva AS, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet. 2020;396(10255):887-97. doi:10.1016/S0140-6736(20)31866-3
56. Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, et al. Interim results of a phase 1–2a trial of Ad26. COV2. S Covid-19 vaccine. N Engl J Med. 2021;384(19):1824-35. doi:10.1056/NEJMoa2034201
57. Voysey M, Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397(10269):99-111. doi:10.1016/S0140-6736(20)32661-1
58. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-16. doi:10.1056/NEJMoa2035389
59. Lee J, Arun Kumar S, Jhan YY, Bishop CJ. Engineering DNA vaccines against infectious diseases. Acta Biomater. 2018;80:31-47. doi:10.1016/j.actbio.2018.08.033
60. Tripathi NK, Shrivastava A. Recent developments in recombinant protein–based dengue vaccines. Front Immunol. 2018;9:1919. doi:10.3389/fimmu.2018.01919

61. Jafari D, Malih S, Eini M, Jafari R, Gholipourmalekabadi M, Sadeghizadeh M, et al. Improvement, scaling-up, and downstream analysis of exosome production. Crit Rev Biotechnol. 2020;40(8):1098-112. doi:10.1080/07388551.2020.1805406
62. Wang YB, Wang LP, Li P. Perspectives on novel vaccine development. Pol J Vet Sci. 2018;21(3):643-9. doi:10.24425/124302
63. Jadhav S, Datla M, Kreeftenberg H, Hendriks J. The Developing Countries Vaccine Manufacturers' Network (DCVMN) is a critical constituency to ensure access to vaccines in developing countries. Vaccine. 2008;26(13):1611-5. doi:10.1016/j.vaccine.2008.01.034
64. Chiodini J. Vaccine storage: A hot topic for cool practice. Travel Med Infect Dis. 2019;31:101477. doi:10.1016/j.tmaid.2019.101477
65. Pascolo S. Messenger RNA-based vaccines. Expert Opin Biol Ther. 2004;4(8):1285-94. doi:10.1517/14712598.4.8.1285
66. Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the Cold Reality of mRNA Vaccine Stability. J Pharm Sci. 2021;110(3):997-1001. doi:10.1016/j.xphs.2020.12.006
67. Cardoso FMC, Petrovajová D, Horňáková T. Viral vaccine stabilizers: status and trends. Acta Virol. 2017;61(3):231-9. doi:10.4149/av_2017_301
68. Rhodes SJ, Knight GM, Kirschner DE, White RG, Evans TG. Dose finding for new vaccines: The role for immunostimulation/immunodynamic modelling. J Theor Biol. 2019;465:51-5. doi:10.1016/j.jtbi.2019.01.017
69. Gonzalez-Dias P, Lee EK, Sorgi S, de Lima DS, Urbanski AH, Silveira EL, et al. Methods for predicting vaccine immunogenicity and reactogenicity. Hum Vaccin Immunother. 2020;16(2):269-76. doi:10.1080/21645515.2019.1697110
70. Porter KR, Raviprakash K. DNA vaccine delivery and improved immunogenicity. Curr Issues Mol Biol. 2017;22(1):129-38. doi:10.21775/cimb.022.129
71. Xu S, Yang K, Li R, Zhang L. mRNA vaccine era—mechanisms, drug platform and clinical prospection. Int J Mol Sci. 2020;21(18):6582. doi:10.3390/ijms21186582
72. Saso A, Kampmann B. Vaccine responses in newborns. Semin Immunopathol. 2017;39(6):627-642. doi:10.1007/s00281-017-0654-9
73. Cowling BJ, Perera RAPM, Valkenburg SA, Leung NHL, Iuliano AD, Tam YH. Comparative Immunogenicity of Several Enhanced Influenza Vaccine Options for Older Adults: A Randomized, Controlled Trial. Clin Infect Dis. 2020;71(7):1704-14. doi:10.1093/cid/ciz1034
74. Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J, et al. Maternal colonization with group B Streptococcus and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis. 2017;65(suppl_2):S100-11. doi:10.1093/cid/cix658
75. Bekeredjian-Ding I. Challenges for clinical development of vaccines for prevention of hospital-acquired bacterial infections. Front Immunol. 2020;11:1755. doi:10.3389/fimmu.2020.01755
76. Santos PM, Butterfield LH. Dendritic cell-based cancer vaccines. J Immunol. 2018;200(2):443-9. doi:10.4049/jimmunol.1701024
77. Korber B, Fischer W. T cell-based strategies for HIV-1 vaccines. Hum Vaccin Immunother. 2020;16(3):713-22. doi:10.1080/21645515.2019.1666957
78. Ding C, Ma J, Dong Q, Liu Q. Live bacterial vaccine vector and delivery strategies of heterologous antigen: A review. Immunol Lett. 2018;197:70-7. doi:10.1016/j.imlet.2018.03.006
79. Ewer KJ, Lambe T, Rollier CS, Spencer AJ, Hill AV, Dorrell L. Viral vectors as vaccine platforms: from immunogenicity to impact. Curr Opin Immunol. 2016;41:47-54. doi:10.1016/j.coi.2016.05.014
80. Wang N, Chen M, Wang T. Liposomes used as a vaccine adjuvant-delivery system: From basics to clinical immunization. J Control Release. 2019;303:130-50. doi:10.1016/j.jconrel.2019.04.025
81. Allahyari M, Mohit E. Peptide/protein vaccine delivery system based on PLGA particles. Hum Vaccin Immunother. 2016;12(3):806-28. doi:10.1080/21645515.2015.1102804
82. Bahadoran A, Moeini H, Bejo MH, Hussein MZ, Omar AR. Development of tat-conjugated dendrimer for transdermal DNA vaccine delivery. J Pharm Pharm Sci. 2016;19(3):325-38. doi:10.18433/J3G31Q
83. Jafari D, Shajari S, Jafari R, Mardi N, Gomari H, Ganji F, et al. Designer exosomes: a new platform for biotechnology therapeutics. BioDrugs. 2020;34:567-86. doi:10.1007/s40259-020-00434-x
84. Jafari D, Malih S, Eslami SS, Jafari R, Darzi L, Tarighi P, et al. The relationship between molecular content of mesenchymal stem cells derived exosomes and their potentials: opening the way for exosomes based therapeutics. Biochimie. 2019;165:76-89. doi:10.1016/j.biochi.2019.07.009
85. Rahimi B, Panahi M, Saraygord-Afshari N, Taheri N, Bilici M, Jafari D, et al. The secretome of mesenchymal stem cells and oxidative stress: challenges and opportunities in cell-free regenerative medicine. Mol Biol Rep. 2021;48(7):5607-19. doi:10.1007/s11033-021-06360-7
86. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Therapeutic delivery. 2016;7(5):319-34. doi:10.4155/tde-2016-0006
87. Yang Y, Neef T, Mittelholzer C, Garcia Garayoa E, Bläuenstein P, Schibli R, et al. The biodistribution of self-assembling protein nanoparticles shows they are promising vaccine platforms. J Nanobiotechnol. 2013;11(1):36. doi:10.1186/1477-3155-11-36
88. Li J, Zeng M, Shan H, Tong C. Microneedle Patches as Drug and Vaccine Delivery Platform. Curr Med Chem. 2017;24(22):2413-22. doi:10.2174/0929867324666170526124053
89. Kim SH, Jang YS. The development of mucosal vaccines for both mucosal and systemic immune induction and the roles played by adjuvants. Clin Exp Vaccine Res. 2017;6(1):15-21. doi:10.7774/cevr.2017.6.1.15
90. Davod J, Fatemeh DN, Honari H, Hosseini R. Constructing and transient expression of a gene cassette containing edible vaccine elements and shigellosis, anthrax and cholera recombinant antigens in tomato. Mol Biol Rep. 2018;45:2237-46. doi:10.1007/s11033-018-4385-3
91. Boyaka PN. Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems. J Immunol. 2017;199(1):9-16. doi:10.4049/jimmunol.1601775
92. Bernasconi V, Norling K, Bally M, Höök F, Lycke NY. Mucosal vaccine development based on liposome technology. J Immunol Res. 2016;2016:5482087. doi:10.1155/2016/5482087
93. Li M, Wang Y, Sun Y, Cui H, Zhu SJ, Qiu HJ. Mucosal vaccines: Strategies and challenges. Immunol Lett. 2020;217:116-25. doi:10.1016/j.imlet.2019.10.013
94. Kang SM, Compans RW. Host responses from innate to adaptive immunity after vaccination: molecular and cellular events. Mol Cells. 2009;27(1):5-14. doi:10.1007/s10059-009-0015-1
95. Golding H, Khurana S, Zaitseva M. What is the predictive

value of animal models for vaccine efficacy in humans? The importance of bridging studies and species-independent correlates of protection. Cold Spring Harb Perspect Biol. 2018;10(4):a028902. doi:10.1101/cshperspect.a028902

96. Hirschberg HJ, van Riet E, Oosterhoff D, Bouwstra JA, Kersten GF. Animal models for cutaneous vaccine delivery. Eur J Pharm Sci. 2015;71:112-22. doi:10.1016/j.ejps.2015.02.005