Advances in Vaccine Development and Delivery Systems

Michael Andrew McKenzie [1*], Dr. Sophia Danielle Sullivan [2]
[1] PhD, Centre for Nanotherapeutics, University of British Columbia, Canada
[2] PhD, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

* Corresponding Author: **Michael Andrew McKenzie**

Article Info

E-ISSN: 3107-393X
Volume: 01
Issue: 06
November-December 2024
Received: 11-09-2024
Accepted: 13-10-2024
Published: 15-11-2024
Page No: 43-58

Abstract
Vaccines represent one of the most successful public health interventions in history, preventing millions of deaths annually and contributing to the control and eradication of infectious diseases worldwide. Despite their remarkable success, traditional vaccine platforms face limitations in terms of production scalability, stability, and immunogenicity against emerging pathogens. This article reviews recent advances in vaccine design and delivery systems that address these challenges and expand the potential of immunization strategies. Key innovations in vaccine design include recombinant protein vaccines, messenger RNA platforms, viral vector systems, and subunit approaches that offer improved safety profiles and targeted immune responses. Parallel developments in delivery systems, particularly nanoparticle carriers, liposomal formulations, and mucosal administration routes, have enhanced vaccine stability, immunogenicity, and patient compliance. These technological advances have demonstrated significant impacts on immune response optimization, reduction of adverse events, and improved accessibility in resource-limited settings. The rapid development and deployment of COVID-19 vaccines exemplified the potential of modern platforms to respond to pandemic threats with unprecedented speed. Looking forward, next generation vaccines incorporating adjuvant technologies, thermostable formulations, and personalized immunization strategies promise to further transform disease prevention. This review synthesizes current knowledge on vaccine innovation and identifies critical areas for future research to achieve universal vaccine coverage and prepare for emerging infectious disease threats.

DOI: https://doi.org/10.54660/IJPIS.2024.1.6.43-58

Keywords: Vaccine development, Vaccine delivery systems, mRNA vaccines, Recombinant vaccines, Nanoparticle delivery, Immunogenicity

1. Introduction

Vaccination stands as one of humanity's greatest achievements in disease prevention, saving an estimated two to three million lives each year and contributing to the control of previously devastating infectious diseases [1]. The concept of immunization dates back to the late eighteenth century with Edward Jenner's pioneering work on smallpox vaccination, which ultimately led to the disease's eradication in 1980 [2]. Since then, vaccine science has evolved dramatically, progressing from simple live attenuated and inactivated whole pathogen preparations to sophisticated molecular designs that harness our understanding of immunology, molecular biology, and materials science [3]. Traditional vaccine platforms, including live attenuated vaccines, inactivated vaccines, and toxoid preparations, have proven highly effective against numerous pathogens such as measles, polio, diphtheria, and tetanus [4]. However, these conventional approaches face inherent limitations that challenge their application to emerging infectious diseases and complex pathogens. Live attenuated vaccines, while often highly immunogenic, carry risks of reversion to virulence and are contraindicated in immunocompromised populations [5]. Inactivated vaccines typically require

multiple doses and adjuvants to achieve protective immunity, and their production is often time-consuming and resource-intensive [6]. Furthermore, traditional platforms have shown limited success against pathogens with high antigenic variability, intracellular persistence, or complex life cycles [7].

The twenty-first century has witnessed a paradigm shift in vaccine development, driven by advances in genomics, structural biology, immunology, and nanotechnology. The sequencing of pathogen genomes has enabled rational vaccine design based on reverse vaccinology, allowing identification of protective antigens without the need to culture organisms [8]. Structural vaccinology approaches have facilitated the engineering of immunogens with enhanced stability and immunogenicity [9]. These scientific breakthroughs have given rise to novel vaccine platforms including recombinant protein vaccines, virus-like particles, viral vectors, nucleic acid vaccines, and synthetic peptide vaccines [10].

Among the most transformative innovations has been the development of messenger RNA vaccine technology, which demonstrated remarkable efficacy and rapid development timelines during the COVID-19 pandemic [11]. The success of mRNA vaccines has validated the concept of using nucleic acids as immunogens and has opened new possibilities for vaccine design against infectious diseases, cancer, and other conditions [12]. Similarly, advancements in viral vector platforms have enabled the creation of vaccines that elicit robust cellular and humoral immunity, as demonstrated by vaccines against Ebola virus disease and COVID-19 [13].

Parallel to innovations in vaccine design, significant progress has been made in delivery systems that enhance vaccine stability, control antigen release, and target specific immune compartments. Nanoparticle-based delivery platforms, including liposomes, polymeric nanoparticles, and virus-like particles, have shown promise in improving vaccine immunogenicity while reducing the required antigen dose [14]. These systems can protect fragile antigens from degradation, facilitate uptake by antigen-presenting cells, and provide sustained release of immunogens [15]. Additionally, exploration of alternative administration routes, particularly mucosal delivery, offers the potential to induce protective immunity at the sites of pathogen entry [16].

The development and deployment of vaccines also face substantial challenges beyond scientific innovation. Global vaccine access remains inequitable, with low- and middle-income countries often experiencing delays in vaccine availability and higher disease burdens [17]. The requirement for cold chain infrastructure limits vaccine distribution in resource-limited settings, where the need is often greatest [18]. Vaccine hesitancy, driven by misinformation and distrust, poses an additional barrier to achieving high coverage rates necessary for population-level protection [19]. Manufacturing capacity constraints and intellectual property considerations further complicate efforts to ensure universal vaccine access [20].

Regulatory frameworks for vaccine evaluation must balance the urgent need for rapid development, particularly during outbreaks, with rigorous safety and efficacy standards [21]. Novel vaccine platforms require updated regulatory guidelines that address their unique characteristics while maintaining public confidence in vaccine safety [22]. The accelerated development timelines demonstrated during the COVID-19 pandemic have prompted discussions about sustainable mechanisms for rapid vaccine development without compromising safety evaluation [23].

This review article aims to provide a comprehensive analysis of recent advances in vaccine development and delivery systems, examining their scientific foundations, clinical applications, and potential to transform disease prevention. The article is organized into sections addressing modern vaccine platforms, innovations in vaccine design and formulation, delivery system technologies, preclinical and clinical evaluation approaches, safety and regulatory considerations, global access challenges, and future directions in vaccine science. By synthesizing current knowledge and identifying research gaps, this review seeks to inform researchers, clinicians, policymakers, and public health professionals working to advance vaccine innovation and implementation.

The subsequent sections will explore in detail the molecular mechanisms underlying modern vaccine platforms, the materials science advances enabling novel delivery systems, the clinical evidence supporting new vaccine technologies, and the translational challenges that must be overcome to realize the full potential of these innovations. Understanding these advances is essential for addressing current and future infectious disease threats, achieving universal immunization coverage, and ultimately reducing the global burden of vaccine-preventable diseases.

2. Overview of Traditional and Modern Vaccine Platforms

Vaccine platforms represent the foundational technologies upon which specific vaccines are designed and manufactured. The evolution from traditional to modern platforms reflects both advances in scientific understanding and the need to address limitations of conventional approaches. This section provides a comparative overview of established and emerging vaccine technologies, highlighting their mechanisms of action, advantages, and applications in disease prevention.

Traditional vaccine platforms have formed the backbone of immunization programs for over a century and continue to play vital roles in global health. Live attenuated vaccines contain weakened forms of pathogens that retain the ability to replicate but have lost most or all virulence properties [24]. The attenuation process typically involves serial passage in non-human hosts or cell cultures, resulting in mutations that reduce pathogenicity while maintaining immunogenicity [25]. Live attenuated vaccines against measles, mumps, rubella, varicella, and yellow fever have demonstrated exceptional efficacy, often providing lifelong immunity after one or two doses [26]. The replicating nature of these vaccines mimics natural infection, stimulating both humoral and cellular immune responses without causing disease in immunocompetent individuals [27]. However, live attenuated vaccines carry risks including reversion to virulence, transmissibility to contacts, and contraindications in immunocompromised populations and pregnant women [28].

Inactivated vaccines contain pathogens that have been killed through chemical or physical treatments such as formaldehyde exposure or heat inactivation [29]. These vaccines cannot replicate in the host, eliminating concerns about reversion to virulence but generally requiring multiple doses and adjuvants to achieve protective immunity [30]. Inactivated vaccines against polio, hepatitis A, rabies, and influenza have proven safe and effective, though immune

responses tend to be shorter-lived compared to live attenuated vaccines [31]. The production of inactivated vaccines requires large-scale pathogen cultivation, which can be challenging for fastidious organisms or those requiring high biosafety containment [32].

Subunit vaccines represent a refinement of traditional approaches, containing only specific antigenic components of pathogens rather than whole organisms [33]. Protein subunit vaccines, such as those against hepatitis B and human papillomavirus, are produced through recombinant DNA technology and offer excellent safety profiles without risk of infection [34]. Polysaccharide and conjugate vaccines against encapsulated bacteria like Streptococcus pneumoniae and Neisseria meningitidis have dramatically reduced the incidence of invasive bacterial diseases, particularly in children [35]. Conjugate vaccines link polysaccharide antigens to carrier proteins, converting T-independent antigens into T-dependent antigens that elicit stronger and longer-lasting immunity [36].

Toxoid vaccines contain inactivated bacterial toxins and represent one of the oldest vaccine types still in widespread use. Vaccines against diphtheria and tetanus use toxoids produced by chemical inactivation of bacterial exotoxins, providing protection against disease caused by toxin-producing organisms [37]. Toxoid vaccines require booster doses to maintain protective antibody levels, and their efficacy depends on eliciting neutralizing antibodies against specific toxins rather than the bacteria themselves [38].

The limitations of traditional platforms in addressing emerging infectious diseases and complex pathogens have driven the development of modern vaccine technologies. Recombinant protein vaccines utilize genetic engineering to produce specific antigens in heterologous expression systems, including bacteria, yeast, insect cells, and mammalian cells [39]. This approach offers precise control over antigen composition, scalability of production, and elimination of pathogen-associated risks [40]. The recombinant hepatitis B vaccine, first licensed in 1986, demonstrated the feasibility and safety of this platform and paved the way for numerous subsequent vaccines [41]. Recent advances in protein engineering have enabled the design of stabilized antigens with improved immunogenicity, as exemplified by structure-based design of respiratory syncytial virus and HIV vaccine candidates [42].

Virus-like particles represent a sophisticated evolution of subunit vaccines, consisting of self-assembling viral structural proteins that mimic the native virus structure without containing genetic material [43]. These particles present antigens in a highly organized, repetitive array that efficiently activates B cells and generates strong antibody responses [44]. Virus-like particle vaccines against human papillomavirus and hepatitis B have demonstrated exceptional efficacy and safety, and this platform is being explored for vaccines against numerous other pathogens [45].

Viral vector vaccines employ replication-competent or replication-defective viruses to deliver genes encoding antigens of interest into host cells [46]. The vectored antigens are expressed intracellularly and processed through endogenous pathways, eliciting both cellular and humoral immune responses similar to natural infection [47]. Adenoviral vectors, modified vaccinia Ankara, and vesicular stomatitis virus vectors have been successfully used in vaccines against Ebola virus disease and COVID-19 [48]. Viral vector platforms offer the advantages of strong immunogenicity, single-dose efficacy potential, and the ability to induce cell-mediated immunity critical for control of intracellular pathogens. Challenges include pre-existing immunity to common viral vectors and the potential for anti-vector immune responses that may limit booster effectiveness.

Nucleic acid vaccines represent a revolutionary departure from traditional approaches by directly introducing genetic material encoding antigens into host cells. DNA vaccines consist of bacterial plasmids containing genes for target antigens under the control of strong eukaryotic promoters. Following intramuscular injection, DNA is taken up by cells, transcribed, and translated to produce antigens that stimulate immune responses. DNA vaccines have shown promise in veterinary applications but have generally demonstrated lower immunogenicity in humans compared to other platforms. Strategies to enhance DNA vaccine potency include electroporation, gene gun delivery, and optimization of plasmid design.

Messenger RNA vaccines have emerged as one of the most significant innovations in vaccine technology, validated by the rapid development and remarkable efficacy of COVID-19 vaccines. These vaccines consist of *in vitro* transcribed mRNA encoding antigens, formulated in lipid nanoparticles that protect the RNA and facilitate cellular uptake. Following injection, mRNA is translated by host cell ribosomes to produce antigens that elicit immune responses. The advantages of mRNA vaccines include rapid design and manufacturing, no risk of integration into the host genome, and potent activation of innate immunity through recognition of RNA by pattern recognition receptors. The mRNA platform enables unprecedented speed in vaccine development, with COVID-19 vaccines progressing from sequence identification to emergency authorization in less than one year. Challenges include requirements for ultra-cold storage temperatures and optimization of mRNA stability and translation efficiency.

Self-amplifying RNA vaccines represent an evolution of the mRNA platform, incorporating viral replicase genes that enable intracellular amplification of vaccine RNA. This self-amplification results in higher and more sustained antigen expression from lower vaccine doses, potentially improving immunogenicity and reducing manufacturing costs. Self-amplifying RNA vaccines derived from alphaviruses are in clinical development for various infectious diseases. The diversity of modern vaccine platforms provides a rich toolkit for addressing different types of pathogens and clinical scenarios. Platform selection depends on multiple factors including pathogen characteristics, desired immune response profiles, target populations, manufacturing considerations, and regulatory pathways. The successful deployment of multiple different platform types during the COVID-19 pandemic demonstrated that platform diversity enhances resilience and capacity in vaccine development and supply. Understanding the strengths and limitations of each platform enables rational selection and optimization for specific vaccine development challenges.

3. Advances in Vaccine Design and Formulation

Contemporary vaccine design has transcended empirical approaches to embrace rational, structure-guided strategies that leverage detailed molecular understanding of pathogen-host interactions and immune system activation. This section examines key advances in antigen design, stabilization, adjuvant incorporation, and formulation optimization that

have enhanced vaccine performance and expanded the range of targetable diseases.

Rational antigen design represents a fundamental shift from using whole pathogens or crude extracts to engineering specific molecular entities with optimized immunogenic properties. Reverse vaccinology, pioneered in the development of vaccines against Neisseria meningitidis serogroup B, employs genomic and proteomic analyses to identify vaccine candidates without the need to culture organisms or isolate individual antigens through traditional biochemical methods. This approach has accelerated antigen discovery for pathogens that are difficult to culture or highly variable, enabling identification of conserved antigens present across strains. Computational prediction of surface-exposed proteins, secreted factors, and other potential immunogens has streamlined the vaccine development pipeline.

Structural vaccinology has emerged as a powerful approach for engineering antigens with enhanced immunogenic properties based on high-resolution structural information from X-ray crystallography, cryo-electron microscopy, and nuclear magnetic resonance spectroscopy. Knowledge of three-dimensional antigen structure enables identification of neutralizing epitopes and design of immunogens that preferentially present these epitopes to the immune system. Prefusion-stabilized viral fusion proteins represent a notable success of structure-based design, as demonstrated by respiratory syncytial virus vaccine candidates that maintain the metastable prefusion conformation recognized by neutralizing antibodies. Similar approaches have been applied to design improved immunogens for influenza, HIV, and other viruses with unstable surface proteins. Epitope-focused design strategies aim to focus immune responses on specific protective epitopes while minimizing responses to non-protective or potentially problematic regions of antigens. This approach is particularly relevant for pathogens like HIV and influenza where broadly neutralizing antibodies recognize conserved epitopes that are typically subdominant in natural infection. Epitope scaffolding techniques transplant minimal epitopes onto heterologous protein scaffolds that stabilize epitope conformation and present them prominently to B cells. Computational design algorithms have enabled creation of novel protein scaffolds with desired geometric properties for epitope presentation. Protein engineering techniques have expanded the possibilities for optimizing antigen properties beyond native structures. Introduction of specific mutations can stabilize proteins in immunologically relevant conformations, eliminate protease cleavage sites, remove unwanted glycosylation sites, or enhance thermal stability. Directed evolution approaches allow screening of large libraries of antigen variants to identify those with improved expression, stability, or immunogenicity. Chimeric antigens combining elements from different strains or species can broaden immune responses and provide cross-protection against variant pathogens.

Glycoengineering of vaccine antigens represents another dimension of molecular optimization. Many viral and bacterial surface proteins are heavily glycosylated, and glycans can shield epitopes from antibody recognition or modulate immune responses. Site-specific removal of glycans through mutagenesis can expose underlying protein epitopes, while addition or modification of glycosylation patterns can enhance protein stability and alter antigen processing. Production of vaccines in expression systems with humanized glycosylation patterns may improve immunogenicity by reducing immune responses to non-human glycan structures.

Multivalent antigen designs that incorporate multiple epitopes or antigens within a single vaccine construct offer advantages for inducing broad immunity. Mosaic antigens containing elements from diverse strains assembled computationally to maximize epitope coverage have shown promise for HIV and influenza vaccines. Consensus sequences derived from alignment of multiple strains represent another strategy for capturing sequence diversity within a single immunogen. These approaches aim to overcome the limitations of strain-specific immunity that plague vaccines against highly variable pathogens. Adjuvant technology has advanced significantly beyond the aluminum salts that have been used in vaccines for nearly a century. Modern adjuvants are designed to activate specific innate immune pathways that shape subsequent adaptive responses, with selection based on the type of immunity required for protection against particular pathogens. Toll-like receptor agonists such as monophosphoryl lipid A activate innate immune cells and enhance antigen presentation, promoting robust antibody and T cell responses. Saponin-based adjuvants like AS01 and Matrix-M form immune-stimulating complexes with antigens and have demonstrated enhancement of both cellular and humoral immunity in vaccines against malaria, shingles, and other diseases. Oil-in-water emulsion adjuvants such as MF59 and AS03 enhance antibody responses through multiple mechanisms including formation of antigen depot, recruitment of immune cells to injection sites, and activation of innate immunity. These adjuvants have proven particularly valuable for influenza vaccines, enabling dose-sparing and improving responses in elderly populations with immunosenescence. Combination adjuvant systems that incorporate multiple active components can provide synergistic effects and enable fine-tuning of immune response quality.

Nanoparticle antigen presentation has emerged as a powerful strategy for enhancing vaccine immunogenicity by mimicking the size and structure of pathogens. Antigens displayed on nanoparticle surfaces in repetitive arrays efficiently crosslink B cell receptors, triggering strong activation signals. Self-assembling protein nanoparticles derived from ferritin, lumazine synthase, and other scaffolds can be engineered to display vaccine antigens in highly immunogenic configurations. These platforms have shown particular promise for presenting challenging antigens like HIV envelope trimers and influenza hemagglutinin in orientations that focus immune responses on conserved epitopes.

Formulation science plays a critical role in translating promising vaccine candidates into stable, administrable products. Stabilization of protein antigens and RNA molecules requires careful optimization of buffer composition, pH, ionic strength, and excipient selection. Lyophilization enables production of thermostable vaccine formulations that eliminate cold chain requirements, potentially transforming vaccine access in low-resource settings. However, lyophilization can stress proteins and requires inclusion of cryoprotectants and reconstitution optimization.

Controlled release formulations aim to provide sustained antigen exposure that mimics prime-boost regimens from a

single administration. Biodegradable polymer microspheres can be engineered to release encapsulated antigens according to programmable kinetics determined by polymer composition and particle size. Pulsatile release systems designed to deliver initial and booster doses from a single injection have shown promise in preclinical studies but face challenges in achieving precise control over release timing. Microarray patch delivery represents an innovative formulation approach that incorporates vaccine antigens into dissolvable microneedles for painless, simple administration. These patches can be self-administered, eliminate sharp waste, and may enhance immunogenicity through delivery to dermal immune cells. Thermostable formulations suitable for microarray patches could revolutionize vaccine distribution in resource-limited settings.

The integration of computational modeling into vaccine formulation development has accelerated optimization processes. Molecular dynamics simulations can predict protein stability under different formulation conditions, while machine learning approaches can identify optimal formulation parameters from experimental datasets. These computational tools complement empirical formulation screening and enable rational formulation design. Quality by design principles have been increasingly applied to vaccine formulation development, defining critical quality attributes and establishing design spaces that ensure consistent product quality. This systematic approach to formulation development improves manufacturing reproducibility and facilitates regulatory approval.

4. Innovations in Vaccine Delivery Systems

Contemporary vaccine design has transcended empirical approaches to embrace rational, structure-guided strategies that leverage detailed molecular understanding of pathogen-host interactions and immune system activation. This section examines key advances in antigen design, stabilization, adjuvant incorporation, and formulation optimization that have enhanced vaccine performance and expanded the range of targetable diseases.

Rational antigen design represents a fundamental shift from using whole pathogens or crude extracts to engineering specific molecular entities with optimized immunogenic properties. Reverse vaccinology, pioneered in the development of vaccines against Neisseria meningitidis serogroup B, employs genomic and proteomic analyses to identify vaccine candidates without the need to culture organisms or isolate individual antigens through traditional biochemical methods. This approach has accelerated antigen discovery for pathogens that are difficult to culture or highly variable, enabling identification of conserved antigens present across strains. Computational prediction of surface-exposed proteins, secreted factors, and other potential immunogens has streamlined the vaccine development pipeline.

Structural vaccinology has emerged as a powerful approach for engineering antigens with enhanced immunogenic properties based on high-resolution structural information from X-ray crystallography, cryo-electron microscopy, and nuclear magnetic resonance spectroscopy. Knowledge of three-dimensional antigen structure enables identification of neutralizing epitopes and design of immunogens that preferentially present these epitopes to the immune system. Prefusion-stabilized viral fusion proteins represent a notable success of structure-based design, as demonstrated by respiratory syncytial virus vaccine candidates that maintain the metastable prefusion conformation recognized by neutralizing antibodies. Similar approaches have been applied to design improved immunogens for influenza, HIV, and other viruses with unstable surface proteins. Epitope-focused design strategies aim to focus immune responses on specific protective epitopes while minimizing responses to non-protective or potentially problematic regions of antigens. This approach is particularly relevant for pathogens like HIV and influenza where broadly neutralizing antibodies recognize conserved epitopes that are typically subdominant in natural infection. Epitope scaffolding techniques transplant minimal epitopes onto heterologous protein scaffolds that stabilize epitope conformation and present them prominently to B cells. Computational design algorithms have enabled creation of novel protein scaffolds with desired geometric properties for epitope presentation. Protein engineering techniques have expanded the possibilities for optimizing antigen properties beyond native structures. Introduction of specific mutations can stabilize proteins in immunologically relevant conformations, eliminate protease cleavage sites, remove unwanted glycosylation sites, or enhance thermal stability. Directed evolution approaches allow screening of large libraries of antigen variants to identify those with improved expression, stability, or immunogenicity. Chimeric antigens combining elements from different strains or species can broaden immune responses and provide cross-protection against variant pathogens.

Glycoengineering of vaccine antigens represents another dimension of molecular optimization. Many viral and bacterial surface proteins are heavily glycosylated, and glycans can shield epitopes from antibody recognition or modulate immune responses. Site-specific removal of glycans through mutagenesis can expose underlying protein epitopes, while addition or modification of glycosylation patterns can enhance protein stability and alter antigen processing. Production of vaccines in expression systems with humanized glycosylation patterns may improve immunogenicity by reducing immune responses to non-human glycan structures.

Multivalent antigen designs that incorporate multiple epitopes or antigens within a single vaccine construct offer advantages for inducing broad immunity. Mosaic antigens containing elements from diverse strains assembled computationally to maximize epitope coverage have shown promise for HIV and influenza vaccines. Consensus sequences derived from alignment of multiple strains represent another strategy for capturing sequence diversity within a single immunogen. These approaches aim to overcome the limitations of strain-specific immunity that plague vaccines against highly variable pathogens. Adjuvant technology has advanced significantly beyond the aluminum salts that have been used in vaccines for nearly a century. Modern adjuvants are designed to activate specific innate immune pathways that shape subsequent adaptive responses, with selection based on the type of immunity required for protection against particular pathogens. Toll-like receptor agonists such as monophosphoryl lipid A activate innate immune cells and enhance antigen presentation, promoting robust antibody and T cell responses. Saponin-based adjuvants like AS01 and Matrix-M form immune-stimulating complexes with antigens and have demonstrated enhancement of both cellular and humoral immunity in

vaccines against malaria, shingles, and other diseases. Oil-in-water emulsion adjuvants such as MF59 and AS03 enhance antibody responses through multiple mechanisms including formation of antigen depot, recruitment of immune cells to injection sites, and activation of innate immunity. These adjuvants have proven particularly valuable for influenza vaccines, enabling dose-sparing and improving responses in elderly populations with immunosenescence. Combination adjuvant systems that incorporate multiple active components can provide synergistic effects and enable fine-tuning of immune response quality.

Nanoparticle antigen presentation has emerged as a powerful strategy for enhancing vaccine immunogenicity by mimicking the size and structure of pathogens. Antigens displayed on nanoparticle surfaces in repetitive arrays efficiently crosslink B cell receptors, triggering strong activation signals. Self-assembling protein nanoparticles derived from ferritin, lumazine synthase, and other scaffolds can be engineered to display vaccine antigens in highly immunogenic configurations. These platforms have shown particular promise for presenting challenging antigens like HIV envelope trimers and influenza hemagglutinin in orientations that focus immune responses on conserved epitopes.

Formulation science plays a critical role in translating promising vaccine candidates into stable, administrable products. Stabilization of protein antigens and RNA molecules requires careful optimization of buffer composition, pH, ionic strength, and excipient selection. Lyophilization enables production of thermostable vaccine formulations that eliminate cold chain requirements, potentially transforming vaccine access in low-resource settings. However, lyophilization can stress proteins and requires inclusion of cryoprotectants and reconstitution optimization.

Controlled release formulations aim to provide sustained antigen exposure that mimics prime-boost regimens from a single administration. Biodegradable polymer microspheres can be engineered to release encapsulated antigens according to programmable kinetics determined by polymer composition and particle size. Pulsatile release systems designed to deliver initial and booster doses from a single injection have shown promise in preclinical studies but face challenges in achieving precise control over release timing.

Microarray patch delivery represents an innovative formulation approach that incorporates vaccine antigens into dissolvable microneedles for painless, simple administration. These patches can be self-administered, eliminate sharp waste, and may enhance immunogenicity through delivery to dermal immune cells. Thermostable formulations suitable for microarray patches could revolutionize vaccine distribution in resource-limited settings.

The integration of computational modeling into vaccine formulation development has accelerated optimization processes. Molecular dynamics simulations can predict protein stability under different formulation conditions, while machine learning approaches can identify optimal formulation parameters from experimental datasets. These computational tools complement empirical formulation screening and enable rational formulation design. Quality by design principles have been increasingly applied to vaccine formulation development, defining critical quality attributes and establishing design spaces that ensure consistent product quality. This systematic approach to formulation development improves manufacturing reproducibility and facilitates regulatory approval.

5. Preclinical and Clinical Evaluation of Novel Vaccines

The pathway from vaccine concept to licensed product requires systematic evaluation through increasingly rigorous stages of preclinical and clinical testing. This process ensures that vaccines are safe, immunogenic, and efficacious before widespread deployment. This section outlines the key phases of vaccine evaluation, highlighting methodologies, endpoints, and considerations specific to novel vaccine platforms.

Preclinical evaluation encompasses *in vitro* and *in vivo* studies that establish proof of concept, characterize immune responses, assess safety, and inform clinical trial design. *In vitro* assays evaluate vaccine-induced immune responses using human or animal immune cells, providing initial evidence of immunogenicity and mechanism of action. Dendritic cell activation assays assess uptake of vaccine antigens and adjuvants, maturation marker expression, and cytokine production, predicting *in vivo* immunogenicity. T cell assays measure proliferation, cytokine secretion, and cytotoxic function in response to vaccine antigens, characterizing the cellular immune response.

In vivo studies in animal models are essential for evaluating vaccine safety, immunogenicity, and protective efficacy before human testing. Small animal models, particularly mice, are widely used for initial vaccine assessment due to their genetic homogeneity, well-characterized immune systems, availability of immunological reagents, and cost-effectiveness. However, interspecies differences in immune responses, pathogen susceptibility, and disease manifestations limit the predictive value of murine models for human outcomes. Non-human primate models more closely recapitulate human immune responses and disease pathogenesis for certain pathogens, providing valuable data on vaccine performance, though their use is limited by expense, ethical considerations, and availability.

Immunogenicity assessment in animal models typically includes measurement of antigen-specific antibodies through enzymqe-linked immunosorbent assays, evaluation of antibody functionality through neutralization or opsonization assays, and characterization of T cell responses through enzyme-linked immunospot assays, intracellular cytokine staining, or tetramer staining. Correlates of protection, when known, guide selection of immunological endpoints that predict vaccine efficacy. For many pathogens, correlates of protection remain undefined, necessitating challenge studies where vaccinated animals are exposed to live pathogens to directly assess protective efficacy.

Safety evaluation in preclinical studies includes acute and chronic toxicity testing, assessment of local reactogenicity at injection sites, and evaluation of systemic adverse events. Biodistribution studies track the fate of vaccine components, particularly for novel platforms like mRNA vaccines, to ensure that they do not accumulate in unintended tissues. Reproductive and developmental toxicity studies are conducted if vaccines are intended for use in women of childbearing age. Safety assessment must be particularly rigorous for novel platforms lacking established safety profiles.

Phase I clinical trials represent the first administration of vaccines to humans and primarily focus on safety evaluation while also gathering preliminary immunogenicity data. These

trials typically enroll twenty to one hundred healthy adult volunteers who receive escalating doses of the vaccine to identify safe dose ranges and characterize adverse events. Participants are closely monitored for local and systemic reactions, with frequent clinical assessments and laboratory testing to detect any safety concerns. Phase I trials also collect blood samples for immunological analyses, providing first evidence of vaccine immunogenicity in humans and informing dose selection for subsequent trials. Phase II clinical trials enroll larger cohorts of several hundred participants and focus on optimizing vaccine dose, schedule, and formulation while expanding safety databases. These trials may include populations similar to those targeted for eventual vaccine use, including different age groups, and provide more robust immunogenicity data. Dose-ranging studies compare immune responses across different vaccine doses to identify optimal dosing regimens. Schedule optimization examines intervals between prime and boost doses, with longer intervals sometimes generating superior immunity. Phase II trials may incorporate exploratory endpoints examining mechanisms of immune response or biomarkers predictive of efficacy.

Phase III clinical trials are large-scale efficacy studies enrolling thousands to tens of thousands of participants, powered to detect statistically significant reductions in disease incidence. These randomized, controlled trials compare vaccine recipients to placebo recipients or recipients of a licensed comparator vaccine, following participants over months to years to detect vaccine-preventable disease outcomes. Phase III trials provide definitive evidence of vaccine efficacy, defined as the percentage reduction in disease incidence in vaccinated versus unvaccinated groups. These trials also generate comprehensive safety databases sufficient to detect relatively uncommon adverse events and to evaluate safety in diverse populations. Efficacy endpoints in Phase III trials must be clearly defined and clinically meaningful. For some vaccines, prevention of laboratory-confirmed infection serves as the primary endpoint, while for others, prevention of severe disease, hospitalization, or death may be more appropriate. Secondary endpoints may include prevention of asymptomatic infection, reduction in disease severity, or prevention of transmission. Selection of appropriate endpoints requires understanding of disease natural history and public health priorities. Immunobridging studies offer an alternative pathway for vaccine licensure when large efficacy trials are not feasible or ethical. These studies demonstrate that vaccines induce immune responses in new populations or settings that are comparable to responses in populations where efficacy was previously established. Immunobridging has been used to extend vaccine approvals to pediatric populations based on adult data, or to approve new formulations based on established vaccines. The validity of immunobridging depends on established correlates of protection that reliably predict clinical outcomes.

Challenge studies, where healthy volunteers are intentionally exposed to pathogens after vaccination, can accelerate vaccine development by enabling efficacy assessment in small numbers of participants under controlled conditions. Human challenge models exist for several pathogens including influenza, malaria, typhoid, and cholera. These studies require careful ethical review, informed consent, and ability to treat resulting infections, limiting their application to pathogens with effective treatments and low risks of severe outcomes.

Accelerated development pathways implemented during public health emergencies can compress timelines while maintaining rigorous evaluation standards. Overlapping clinical trial phases, adaptive trial designs that allow modification based on accumulating data, and rolling submissions of data to regulatory agencies enabled the rapid development of COVID-19 vaccines. Platform technologies that use established delivery systems with only antigen changes may qualify for abbreviated development pathways based on existing safety data

Real-world effectiveness studies following vaccine licensure provide evidence of vaccine performance in diverse populations under routine use conditions. These observational studies complement clinical trial data by including populations typically excluded from trials, assessing longer-term outcomes, detecting rare adverse events, and evaluating effectiveness against emerging pathogen variants. Test-negative case-control designs comparing vaccination rates among persons testing positive versus negative for a disease offer efficient approaches for estimating vaccine effectiveness.

Phase IV post-licensure surveillance monitors vaccine safety in large populations receiving vaccines through routine immunization programs. Passive surveillance systems collect reports of adverse events, while active surveillance systems systematically follow vaccine recipients to detect safety signals. Large-linked database studies can identify rare adverse events that may not be detected in pre-licensure trials. Risk-benefit analyses integrate effectiveness and safety data to inform vaccination policies.

The evaluation of novel vaccine platforms requires adaptation of traditional assessment paradigms. Regulatory agencies have developed guidance documents addressing specific considerations for mRNA vaccines, viral vector vaccines, and other innovative platforms. These guidelines balance the need for thorough evaluation with recognition that entirely novel data packages may be needed for unprecedented technologies.

6. Safety, Immunogenicity, and Regulatory Considerations

Ensuring the safety of vaccines is paramount given their administration to healthy individuals, often including children, for disease prevention rather than treatment. This section examines safety assessment frameworks, immunogenicity evaluation strategies, and regulatory pathways that govern vaccine development and licensure. Safety evaluation begins in preclinical studies and continues through all phases of clinical development and post-licensure monitoring. The types of adverse events associated with vaccines range from common local reactions such as pain, redness, and swelling at injection sites to rare but serious events including allergic reactions, neurological complications, or autoimmune phenomena. Reactogenicity refers to the expected, often transient inflammatory responses to vaccination, while adverse events encompass any untoward medical occurrences following vaccination regardless of causality.

Systematic collection and analysis of safety data in clinical trials follows standardized protocols defining adverse events of special interest, grading scales for severity, and timeframes for relatedness assessment. Solicited adverse events that participants actively report using diaries capture common

reactions, while unsolicited adverse events and serious adverse events are reported throughout study follow-up. Independent data and safety monitoring boards review accumulating safety data during trials and can recommend modifications or halting if safety concerns arise. Causality assessment distinguishes adverse events caused by vaccines from temporally associated but unrelated events that would have occurred regardless of vaccination. Bradford Hill criteria including temporal relationship, biological plausibility, consistency across studies, and strength of association inform causality determinations. However, definitively establishing causation is often challenging, particularly for rare events where limited data exist. Some adverse events initially suspected to be vaccine-caused, such as autism following measles-mumps-rubella vaccination, have been conclusively disproven through extensive epidemiological studies.

Rare adverse events may not be detected until vaccines are administered to millions of people, necessitating robust post-licensure surveillance systems. The Vaccine Adverse Event Reporting System in the United States and similar systems globally collect spontaneous reports of adverse events, generating signals that warrant further investigation. The Vaccine Safety Datalink and other large linked database systems enable active surveillance and rapid epidemiological studies to confirm or refute safety signals. Global coordination of safety monitoring through the World Health Organization facilitates detection of adverse events that may be rare in any single country but apparent when data are aggregated.

Signal detection algorithms applied to surveillance databases use statistical methods to identify adverse events occurring more frequently than expected after vaccination. These algorithms must balance sensitivity to detect true signals with specificity to avoid false alarms that can undermine vaccine confidence. Confirmed safety signals lead to updates in vaccine labeling, recommendations for risk mitigation, or in rare cases, withdrawal of vaccines from the market. Risk communication regarding vaccine safety must convey complex information accurately while maintaining public trust. Transparent acknowledgment of known risks, explanation of risk-benefit balances, and prompt investigation of safety concerns are essential for maintaining confidence in immunization programs. Failure to communicate effectively about vaccine risks has contributed to vaccine hesitancy and declining coverage for some vaccines.

Novel vaccine platforms require special safety considerations based on their unique characteristics. Nucleic acid vaccines raise theoretical concerns about integration into the host genome, though mRNA vaccines cannot integrate and DNA vaccines have not shown integration in preclinical studies. The lipid nanoparticles used in mRNA vaccines have been associated with rare anaphylactic reactions, likely due to polyethylene glycol components. Viral vector vaccines using replication-competent vectors require assessment of shedding and potential transmission risks. Platform-specific safety assessments inform regulatory requirements and post-licensure monitoring strategies.

Immunogenicity evaluation characterizes the ability of vaccines to elicit immune responses and establishes immune correlates that predict protection when possible. Humoral immunity assessment focuses on antibody responses measured through quantitative assays such as enzyme-linked immunosorbent assays that detect binding antibodies, and functional assays including neutralization assays, opsonophagocytosis assays, and complement-mediated killing assays. The magnitude, breadth, durability, and functionality of antibody responses all contribute to vaccine-induced protection.

Cellular immunity assessment evaluates T cell responses through assays measuring proliferation, cytokine production, cytotoxic function, and memory phenotypes. Enzyme-linked immunospot assays detect antigen-specific T cells secreting interferon-gamma or other cytokines, providing sensitive measures of cellular immunity. Flow cytometry-based assays enable multi-parameter characterization of T cell populations including subset identification, functional capabilities, and memory differentiation states. For pathogens where cellular immunity is critical for protection, such as Mycobacterium tuberculosis and many viruses, T cell assays are essential for vaccine evaluation.

Immune correlates of protection are immunological parameters that predict vaccine efficacy and can serve as surrogate endpoints for licensure. Established correlates include neutralizing antibody titers for many viral vaccines and serum bactericidal antibody titers for meningococcal vaccines. Identification of correlates requires demonstrating statistical relationships between immune responses and protection across multiple studies and populations. Mechanistic correlates represent immune responses directly responsible for protection, while non-mechanistic correlates are associated with protection but may not be causal. Systems vaccinology approaches apply high-throughput technologies and computational analyses to comprehensively characterize vaccine-induced immune responses.

Transcriptomic profiling identifies gene expression signatures in blood cells that predict subsequent antibody responses, enabling early assessment of vaccine immunogenicity. Proteomic and metabolomic analyses provide complementary data on immune activation and metabolism. Integration of multi-omics data using machine learning can identify complex signatures predictive of vaccine efficacy.

Regulatory approval of vaccines requires demonstration of safety and efficacy through well-controlled clinical trials or, for some situations, on immunogenicity data with established correlates of protection. Regulatory agencies including the United States Food and Drug Administration, European Medicines Agency, and national authorities in other countries review comprehensive dossiers containing preclinical data, manufacturing information, clinical trial results, and proposed labeling. The review process evaluates whether benefits of vaccination outweigh risks for intended populations.

Accelerated regulatory pathways are available for vaccines addressing unmet medical needs or public health emergencies. Emergency Use Authorization in the United States and comparable mechanisms in other jurisdictions allow conditional approval based on preliminary evidence when potential benefits outweigh potential risks during emergencies. These authorizations may be granted before Phase III trials are complete if available data suggest favorable benefit-risk profiles. Full licensure follows upon completion of trials and comprehensive review. International coordination of regulatory standards through mechanisms like the World Health Organization prequalification program facilitates global vaccine access by

enabling countries to rely on evaluations conducted by stringent regulatory authorities. Harmonization of regulatory requirements reduces duplication of effort and accelerates vaccine availability in low- and middle-income countries. Lot release testing ensures that each manufactured vaccine lot meets quality specifications for safety, potency, and purity before distribution. Regulatory authorities or designated laboratories perform or review lot release tests, providing an additional quality control layer. Consistency of manufacturing over time is monitored through ongoing analysis of lot release data.

Post-licensure requirements may include completion of deferred pediatric studies, long-term safety follow-up, effectiveness studies in special populations, or additional clinical trials if accelerated approval pathways were used. Risk evaluation and mitigation strategies may be implemented for vaccines with identified safety concerns that require specific risk management approaches. Good manufacturing practices govern vaccine production, ensuring consistent quality through validated processes, quality control testing, and documentation. Manufacturing changes require regulatory approval and demonstration that product quality is maintained. The complexity of biological products and novel platforms like mRNA vaccines presents unique manufacturing challenges that must be addressed through robust quality systems.

Vaccine hesitancy, influenced by misinformation, distrust, and complacency, poses significant challenges to immunization programs despite strong safety records for licensed vaccines. Addressing vaccine hesitancy requires multi-faceted approaches including education, community engagement, healthcare provider communication training, and policy interventions. Maintaining public confidence depends on transparent communication about both benefits and risks of vaccination.

7. Challenges in Global Vaccine Access and Distribution

Despite remarkable progress in vaccine technology, inequitable access to vaccines remains a critical global health challenge. This section examines barriers to vaccine access in low- and middle-income countries and explores strategies to achieve universal immunization coverage.

The stark disparities in vaccine availability between high-income and low- and middle-income countries became particularly evident during the COVID-19 pandemic, when wealthy nations secured vaccine supplies through advance purchase agreements while many low-income countries waited months or years for access. This vaccine nationalism, driven by national self-interest, contravened principles of global health equity and prolonged the pandemic by allowing continued viral transmission and evolution in unvaccinated populations.

Manufacturing capacity constraints limit global vaccine supply, particularly during pandemics or disease outbreaks requiring rapid scale-up. Most vaccine manufacturing capacity is concentrated in a small number of high-income countries and a few middle-income countries like India and China. Building manufacturing capacity in low- and middle-income countries requires substantial investment in infrastructure, technology transfer, workforce training, and regulatory systems. Technology transfer from originator companies to manufacturers in developing countries has historically been slow and incomplete, though recent initiatives aim to accelerate this process.

Intellectual property rights, including patents and trade secrets, can impede vaccine access by limiting competition and maintaining high prices. During the COVID-19 pandemic, proposals to waive intellectual property protections for vaccines generated intense debate between advocates who argued that public health imperatives justified intellectual property waivers and pharmaceutical companies who contended that intellectual property protection incentivizes innovation. Resolution came through negotiated agreements on technology transfer and voluntary licensing rather than mandatory waivers.

Vaccine pricing remains a significant barrier to access, with newer vaccines often costing substantially more than traditional vaccines. While global vaccine procurement mechanisms like Gavi, the Vaccine Alliance, negotiate reduced prices for low-income countries, middle-income countries often pay higher prices and may struggle to afford expensive vaccines. Tiered pricing strategies that charge higher prices in wealthier countries while subsidizing access in poorer countries aim to balance affordability with incentives for research and development, but implementation is complex.

Cold chain infrastructure requirements pose formidable logistical challenges in settings with unreliable electricity, limited transportation infrastructure, and hot climates. Many vaccines require storage between two and eight degrees Celsius, necessitating continuous refrigeration from manufacture to administration. Ultra-cold chain requirements for some mRNA COVID-19 vaccines initially limited their use in many countries, though later formulation improvements enabled storage at standard refrigerator temperatures. Investment in cold chain equipment, training of health workers in vaccine storage and handling, and development of thermostable vaccines are all essential for improving vaccine access.

Health system capacity affects the ability to deliver vaccines even when they are available. Weak health systems with insufficient healthcare workers, limited infrastructure, and competing health priorities struggle to achieve high immunization coverage. Conflict, political instability, and displacement disrupt immunization services, leaving vulnerable populations unprotected. Strengthening primary healthcare systems benefits vaccine delivery and broader health outcomes.

Vaccine hesitancy and misinformation undermine demand for vaccines even when they are accessible and affordable. Cultural beliefs, religious concerns, distrust of government or pharmaceutical companies, and exposure to misinformation contribute to vaccine refusal or delay. Social media amplifies anti-vaccine messaging, making it challenging to counter with accurate information. Community engagement, culturally appropriate health communication, and involvement of trusted local leaders are essential for building vaccine acceptance.

Programmatic challenges including weak routine immunization systems, inadequate surveillance for vaccine-preventable diseases, and insufficient data systems limit the effectiveness of immunization programs. Many countries lack complete birth registries or health information systems to track which children have been vaccinated, resulting in missed opportunities and inequitable coverage. Strengthening immunization information systems and integrating vaccination services with other health interventions can improve coverage.

Emergency preparedness and response capabilities proved inadequate during the COVID-19 pandemic despite warnings from previous outbreaks. The lack of advance agreements for equitable vaccine allocation, insufficient global manufacturing capacity, and limited coordination mechanisms resulted in chaotic initial responses. The Coalition for Epidemic Preparedness Innovations and the COVID-19 Vaccines Global Access initiative represented efforts to address these gaps through advance investment in vaccine development and procurement mechanisms, though challenges remained in implementation.

Regulatory capacity in low- and middle-income countries varies substantially, with some national regulatory authorities lacking resources for thorough vaccine evaluation. Reliance on World Health Organization prequalification or approvals by stringent regulatory authorities helps ensure vaccine quality but can delay access. Strengthening national regulatory capacity enables countries to make independent decisions about vaccine approval while maintaining safety standards.

Surveillance systems for vaccine-preventable diseases are essential for assessing disease burden, guiding vaccine introduction decisions, and monitoring vaccine impact. Many countries lack robust disease surveillance, particularly for conditions that do not require laboratory confirmation or that are rarely seen due to vaccination. Investment in laboratory capacity and disease reporting systems supports evidence-based vaccination policies.

Financing mechanisms for immunization vary across countries, with Gavi providing support to the poorest countries while middle-income countries must increasingly finance vaccines domestically as they graduate from Gavi eligibility. Sustainable domestic financing requires political commitment, adequate health budgets, and prioritization of immunization within health spending. Innovative financing mechanisms including advance market commitments and international bonds have been proposed to increase resources for global immunization.

Vaccine delivery strategies must be adapted to local contexts, considering geography, health system organization, cultural factors, and population distribution. Strategies including fixed facility delivery, outreach services, mobile clinics, and mass vaccination campaigns each have roles depending on context. Integration of vaccination with other health services such as maternal and child health visits or nutrition programs can improve efficiency and coverage.

Equity within countries is as important as equity between countries, with marginalized populations including ethnic minorities, migrants, refugees, and residents of remote or conflict-affected areas often having lower vaccination coverage than national averages. Targeted efforts to reach these populations require understanding barriers they face and designing appropriate interventions.

Research and development for vaccines addressing diseases primarily affecting low- and middle-income countries receives insufficient investment due to limited commercial markets. Product development partnerships bringing together public, private, and philanthropic sectors aim to address this market failure by financing development of vaccines for diseases like malaria, tuberculosis, and neglected tropical diseases. Advance purchase commitments guarantee markets for successful vaccines, encouraging investment in development.

Global cooperation and solidarity are essential for achieving universal vaccine access and preparedness for future pandemics. The pandemic treaty currently under negotiation by World Health Organization member states aims to establish frameworks for equitable access to medical countermeasures including vaccines during health emergencies. Success depends on political will, adequate financing, and genuine commitment to global health equity.

8. Future Directions in Vaccine Technology and Personalized Immunization

The future of vaccine development promises transformative advances that will expand the range of preventable diseases, improve vaccine performance, and enable personalized immunization approaches tailored to individual characteristics. This section explores emerging technologies and concepts that will shape the next generation of vaccines.

Universal vaccines that provide broad protection against multiple strains or even multiple pathogens within a family represent a major goal for vaccine research. Universal influenza vaccines targeting conserved regions of viral proteins aim to eliminate the need for annual reformulation and provide protection against pandemic strains. Leading approaches include vaccines targeting the hemagglutinin stalk domain, internal proteins like nucleoprotein and matrix protein, and multi-antigen combinations. Clinical trials of universal influenza vaccine candidates have shown promising results in generating broad antibody responses, though demonstration of clinical efficacy remains.

Similarly, universal coronavirus vaccines that protect against multiple coronaviruses including future pandemic threats are in development following the COVID-19 pandemic. These vaccines target conserved epitopes in spike protein receptor binding domains, S2 subunits, or internal viral proteins. The challenge lies in identifying epitopes sufficiently conserved across diverse coronaviruses while being accessible to immune responses.

Therapeutic vaccines that treat existing infections or diseases represent an expansion beyond traditional preventive vaccines. Therapeutic vaccines for chronic viral infections like HIV and hepatitis B aim to boost immune responses to control or clear established infections. Cancer vaccines that target tumor-associated antigens or neoantigens seek to generate anti-tumor immunity. While cancer vaccines have shown limited success historically, the advent of personalized neoantigen vaccines informed by tumor genome sequencing has renewed optimism.

Next-generation adjuvants that more precisely modulate immune responses will enable optimization of vaccine immunogenicity for specific pathogens and populations. Adjuvants targeting specific pattern recognition receptors including various toll-like receptors, stimulator of interferon genes, and nucleotide-binding oligomerization domain-like receptors are being developed to tailor innate immune activation. Combination adjuvants that activate multiple innate pathways may provide synergistic effects. Age-appropriate adjuvants optimized for infants, elderly adults, or immunocompromised individuals could improve vaccine responses in populations with altered immunity.

Mucosal vaccine technologies continue to evolve with novel adjuvants, delivery systems, and administration routes that enhance mucosal immunity. Improved intranasal vaccines

incorporating immunostimulatory molecules like cytokines, pattern recognition receptor agonists, or bacterial toxin derivatives can generate robust mucosal and systemic immunity. Sublingual vaccines that exploit the tolerogenic environment beneath the tongue while maintaining immunogenicity are in development for various indications.

Self-amplifying RNA vaccines represent an evolution of mRNA vaccine technology, incorporating genes encoding viral replicases that enable intracellular amplification of vaccine RNA. This amplification allows use of much lower doses while potentially extending duration of antigen expression. Self-amplifying RNA vaccines have shown promising preclinical results and are advancing through clinical development for multiple infectious diseases.

Circular RNA vaccines offer potential advantages over linear mRNA including enhanced stability, prolonged protein expression, and reduced innate immune activation. Circular RNAs lack free ends that trigger degradation pathways and innate immune sensors, potentially enabling sustained antigen expression without excessive inflammation. Translation efficiency of circular RNAs requires optimization through internal ribosome entry sites or other elements.

DNA vaccines continue to evolve with improved delivery methods and adjuvants that enhance immunogenicity. Electroporation devices that apply electrical pulses following DNA injection dramatically increase vaccine uptake and immune responses. Novel plasmid designs incorporating optimized promoters, enhancer elements, and immunostimulatory sequences improve expression and immunogenicity. DNA vaccines offer advantages of thermostability and ease of manufacturing that may prove valuable for veterinary and pandemic applications.

Prime-boost strategies using different vaccine platforms for initial priming and subsequent boosting can elicit superior immunity compared to homologous regimens. Heterologous prime-boost approaches combining different viral vectors, or combining viral vectors with protein subunit vaccines, have shown enhanced T cell and antibody responses in clinical trials. The optimal combinations and intervals for prime-boost regimens vary depending on the pathogen and desired immune response.

Personalized vaccine approaches that tailor immunization to individual genetic backgrounds, prior exposures, and immune status represent a frontier in precision medicine. Genetic variants in immune response genes influence vaccine immunogenicity, and pharmacogenomic approaches might eventually guide vaccine selection or dosing. Assessment of pre-existing immunity to antigens or vaccine vectors could inform decisions about which vaccine platforms to use. Personalized cancer vaccines based on individual tumor mutations exemplify how personalization might be implemented.

Artificial intelligence and machine learning are increasingly applied to vaccine design, development, and optimization. Machine learning algorithms can predict immunogenic epitopes, optimize protein stability, design novel adjuvants, and identify correlates of protection from complex datasets. Artificial intelligence-guided antigen design has produced vaccine candidates with improved properties compared to rationally designed or naturally derived antigens.

High-throughput screening combined with machine learning enables rapid optimization of vaccine formulations.

Synthetic biology approaches enable design of entirely novel antigens and vaccine platforms not constrained by natural sequences or structures. Computational design of proteins with desired immunological properties has produced antigens that elicit broadly neutralizing antibodies against HIV and influenza. Synthetic gene circuits that control antigen expression in response to cellular signals could enable self-regulating vaccines that optimize immune stimulation.

Needle-free delivery technologies beyond microneedle patches are advancing, including jet injectors, inhalable powders, and transdermal patches using electrical or ultrasonic enhancement. These technologies aim to improve vaccine acceptability, simplify logistics, and potentially enhance immunogenicity through novel delivery to immune-rich compartments. Development requires balancing technological sophistication with reliability, cost, and usability in diverse settings.

Thermostable vaccine formulations that eliminate cold chain requirements would transform vaccine access in low-resource settings. Spray drying, lyophilization with optimal excipients, silk fibroin encapsulation, and other stabilization technologies have achieved impressive thermostability for various vaccine types. Some vaccines have been formulated to remain stable at elevated temperatures for extended periods without refrigeration. Widespread adoption of thermostable vaccines requires regulatory approval of new formulations and sufficient cost-effectiveness.

Rapid response vaccine platforms that enable development of vaccines against novel pathogens within weeks or months will be essential for pandemic preparedness. The mRNA platform demonstrated this capability during COVID-19, with vaccine candidates entering clinical trials within months of viral sequence identification. Establishing pre-approved manufacturing processes and regulatory pathways for platform technologies will further accelerate responses to future outbreaks.

Vaccines against non-communicable diseases including cancer, cardiovascular disease, and neurodegenerative disorders represent expanding frontiers. Cancer vaccines targeting shared tumor antigens or patient-specific neoantigens aim to generate anti-tumor immunity. Vaccines against proteins involved in Alzheimer's disease, though challenging due to risks of excessive inflammation, remain under investigation. Vaccines targeting self-proteins involved in disease processes require careful design to break tolerance without causing autoimmunity.

Microbiome-modulating vaccines that shape microbial communities to promote health represent a novel concept. Vaccines could target specific pathogenic microbes within microbial communities or promote beneficial species. Understanding of host-microbiome interactions continues to evolve, and translation to vaccines remains in early stages.

Pregnancy vaccines that protect both mothers and newborns through passive transfer of maternal antibodies are receiving increased attention. Maternal vaccination against pertussis, influenza, and respiratory syncytial virus has demonstrated effectiveness in protecting infants too young for direct vaccination. Additional maternal vaccines are in development for Group B Streptococcus, cytomegalovirus,

and other pathogens that threaten newborns.

Combination vaccines that protect against multiple diseases from a single administration reduce the number of injections, improve compliance, and simplify immunization schedules. Development of combination vaccines requires ensuring that immune responses to each component are non-inferior to separate administration. Interference between vaccine components must be addressed through formulation optimization.

Global vaccine manufacturing capacity is expanding, with technology transfer initiatives establishing production facilities in low- and middle-income countries. Regional manufacturing hubs can improve vaccine access, security of supply, and pandemic preparedness. However, achieving economies of scale, maintaining quality standards, and securing sustainable markets remain challenges for new manufacturers.

Equity considerations must be integrated into vaccine development from the earliest stages to ensure that innovations benefit all populations. Engagement with communities in low- and middle-income countries during clinical development, pricing strategies that ensure affordability, and manufacturing partnerships that enhance access are essential. The lessons from COVID-19 vaccine inequity should inform future pandemic preparedness efforts.

9. Conclusion

Vaccines stand among humanity's most powerful tools for disease prevention, with contemporary advances in vaccine development and delivery systems poised to dramatically expand their impact on global health. The evolution from traditional whole pathogen vaccines to sophisticated molecular platforms including mRNA, recombinant proteins, viral vectors, and engineered nanoparticles has opened unprecedented possibilities for preventing infectious diseases and addressing other health challenges. These technological innovations, combined with improved understanding of immunology and materials science, enable rational design of vaccines that are safer, more immunogenic, and more adaptable than previous generations.

The rapid development and deployment of multiple highly effective COVID-19 vaccines demonstrated the remarkable potential of modern vaccine platforms to respond to pandemic threats with speed and scale previously thought impossible. This achievement validated years of foundational research and established new paradigms for vaccine development that will inform responses to future emerging pathogens. The success of mRNA vaccines in particular has catalyzed investment in nucleic acid vaccine platforms for applications ranging from infectious diseases to cancer immunotherapy. However, this success also highlighted persistent inequities in global vaccine access that must be addressed through strengthened manufacturing capacity, technology transfer, and international cooperation.

Innovations in vaccine delivery systems, particularly nanoparticle carriers, alternative administration routes, and thermostable formulations, are addressing longstanding challenges in vaccine stability, immunogenicity, and accessibility. Microneedle patches, mucosal vaccines, and other needle-free delivery technologies promise to improve vaccine acceptability and simplify logistics while potentially enhancing immune responses.

The development of thermostable vaccines that eliminate cold chain requirements could transform vaccine delivery in resource-limited settings where cold chain infrastructure is inadequate. Integration of novel antigens, adjuvants, and delivery systems into optimized formulations requires interdisciplinary collaboration and careful evaluation to ensure safety and effectiveness.

The pathway from vaccine concept to licensed product demands rigorous evaluation through preclinical studies and clinical trials that establish safety, immunogenicity, and efficacy. Novel vaccine platforms require adapted evaluation frameworks that address their unique characteristics while maintaining high standards for protection of research participants and eventual vaccine recipients. Regulatory agencies worldwide have demonstrated flexibility in accelerating vaccine development during public health emergencies while preserving essential safety evaluations. Post-licensure surveillance systems that monitor vaccine safety and effectiveness in large populations provide ongoing assurance of favorable benefit-risk profiles.

Despite remarkable scientific progress, significant challenges remain in achieving universal vaccine coverage and equitable access. Disparities in vaccine availability between high-income and low- and middle-income countries persist due to limited manufacturing capacity, inadequate cold chain infrastructure, insufficient financing, and intellectual property barriers. Vaccine hesitancy driven by misinformation and distrust threatens immunization programs even in settings where vaccines are readily available. Addressing these challenges requires sustained political commitment, increased investment in health systems, technology transfer to expand manufacturing capacity, effective risk communication, and genuine global solidarity.

Looking forward, next-generation vaccine technologies including universal vaccines, personalized immunization approaches, and rapid response platforms promise to further transform disease prevention. Artificial intelligence and machine learning are accelerating vaccine design and optimization, while synthetic biology approaches enable creation of entirely novel antigens with enhanced immunological properties. Therapeutic vaccines for chronic infections and cancer represent expanding applications beyond traditional infectious disease prevention. Integration of these advances with improved delivery systems and manufacturing processes will determine whether the promise of vaccine innovation is realized for all populations.

The future of vaccinology depends not only on scientific innovation but also on societal choices regarding research priorities, financing mechanisms, intellectual property frameworks, and commitment to health equity. The COVID-19 pandemic demonstrated both the extraordinary capabilities of modern vaccine science and the profound consequences of failure to ensure equitable access. Building on lessons learned, the global community must invest in pandemic preparedness infrastructure, strengthen regulatory systems, expand manufacturing capacity in diverse regions, and establish mechanisms for equitable vaccine allocation during health emergencies. Success in these endeavors will enhance global health security while saving countless lives through prevention of infectious diseases.

Vaccines have proven transformative for public health over the past century, and contemporary advances ensure their continued and expanded impact. Realizing the full potential of vaccine innovation requires sustained scientific inquiry, adequate investment, effective translation from laboratory to clinic, robust regulatory oversight, and unwavering commitment to ensuring that all people, regardless of geography or economic status, benefit from these lifesaving interventions. The advances reviewed in this article provide reason for optimism that vaccines will play an even more central role in promoting health and preventing disease in the decades ahead.

10. Tables

Table 1: Comparison of Traditional and Modern Vaccine Platforms with Their Mechanisms and Applications

Platform	Mechanism of Action	Key Advantages	Representative Applications	Limitations
Live Attenuated	Weakened pathogen replicates, mimicking natural infection	Strong, durable immunity; single-dose potential; induces cellular and humoral responses	Measles, mumps, rubella, varicella, yellow fever, oral polio	Risk of reversion; contraindicated in immunocompromised; requires cold chain
Inactivated	Killed whole pathogens stimulate immune response without replication	Safety profile; no risk of reversion; suitable for immunocompromised	Injectable polio, hepatitis A, rabies, seasonal influenza	Multiple doses required; weaker cellular immunity; adjuvants often needed
Subunit/Recombinant Protein	Purified or recombinant antigens elicit targeted immune responses	Defined composition; excellent safety; no infectious material	Hepatitis B, human papillomavirus, herpes zoster, pertussis	Multiple doses required; adjuvants necessary; expensive production
Conjugate	Polysaccharides linked to carrier proteins induce T-dependent responses	Converts T-independent to T-dependent antigens; effective in infants	Haemophilus influenzae type b, Streptococcus pneumoniae, Neisseria meningitidis	Limited to polysaccharide antigens; complex manufacturing
Toxoid	Inactivated bacterial toxins induce neutralizing antibodies	Well-established safety; effective against toxin-mediated diseases	Diphtheria, tetanus	Requires boosters; protects only against toxin, not colonization
Viral Vector	Replication-competent or defective virus delivers antigen genes	Strong cellular and humoral immunity; single-dose potential; rapid development	Ebola, COVID-19 (adenovirus, VSV-based)	Pre-existing immunity to vectors; potential anti-vector responses
mRNA	Synthetic mRNA encoding antigens translated by host cells	Rapid design and production; non-infectious; strong immunogenicity	COVID-19 (SARS-CoV-2), emerging for influenza and other diseases	Cold chain requirements; relatively new platform; higher cost
DNA	Plasmid DNA encoding antigens taken up by cells	Thermostable; easy manufacturing; no cold chain; no anti-vector immunity	Veterinary applications; human trials ongoing for multiple diseases	Lower immunogenicity in humans; requires delivery enhancement
Virus-Like Particles	Self-assembling viral proteins form non-infectious particles	Highly organized antigen presentation; strong B cell activation; excellent safety	Human papillomavirus, hepatitis B, malaria (RTS,S)	Complex manufacturing; may require adjuvants for optimal responses
Self-Amplifying RNA	RNA with replicase genes amplifies intracellularly	Lower doses required; prolonged antigen expression; potent immunity	Clinical development for various infectious diseases	Cold chain needs; inflammatory potential; emerging technology

Table 2: Key Vaccine Delivery Systems and Associated Advantages

Delivery System	Composition/Technology	Primary Advantages	Applications/Status	Challenges
Lipid Nanoparticles	Ionizable lipids, phospholipids, cholesterol, PEG-lipids	Protects nucleic acids; facilitates cellular uptake; tunable biodistribution	mRNA COVID-19 vaccines; clinical use for multiple mRNA vaccines	Cold chain requirements; potential allergic reactions to PEG; formulation optimization
Polymeric Nanoparticles	PLGA or other biodegradable polymers	Controlled antigen release; protection from degradation; adjuvant properties	Preclinical and clinical development for various antigens	Particle size control; scaling manufacturing; achieving optimal release kinetics
Liposomes	Phospholipid vesicles	Biocompatible; can carry hydrophilic and hydrophobic cargo; co-deliver antigens and adjuvants	Virosomal influenza vaccines licensed; various candidates in development	Stability; sterility requirements; potential for rapid clearance
Virus-Like Particles	Self-assembling viral structural proteins	Highly immunogenic; repetitive antigen display; no infectious material	HPV vaccines; HBV vaccines; malaria vaccine RTS, S	Complex production; requires protein engineering; may need adjuvants
Gold Nanoparticles	Colloidal gold conjugated with antigens/adjuvants	Precise size control; multivalent display; imaging capabilities	Preclinical development; some veterinary applications	Cost; potential toxicity concerns at high doses; limited clinical data
Microneedle Patches	Dissolvable or coated microneedles containing vaccine	Painless; thermostable; self-administration potential; targets dermal immune cells	Clinical trials for influenza, measles, polio; WHO prequalification pathway	Manufacturing scale-up; regulatory pathways; acceptability studies needed
Intranasal Delivery	Liquid sprays or powder formulations with mucosal adjuvants	Needle-free; induces mucosal immunity; non-invasive	Live attenuated influenza vaccine licensed; others in development	Dose consistency; avoiding neurological access; optimal adjuvants needed
Oral Delivery	Enteric-coated capsules or particles	Needle-free; mucosal immunity; ease of administration	Polio, rotavirus, cholera, typhoid vaccines licensed	Acid and enzyme degradation; variable absorption; immune tolerance
Sublingual Delivery	Tablets or liquids placed under tongue	Rich vasculature; bypass first-pass metabolism; needle-free	Allergy immunotherapy established; vaccines in development	Ensuring sufficient antigen uptake; optimal formulations; limited data
Jet Injectors	High-pressure liquid stream penetrates skin	Needle-free; rapid administration; potential dose sparing	Used in mass campaigns; modern devices address safety concerns	Device cost; potential for cross-contamination if not single-use; acceptability
Electroporation	Electrical pulses enhance DNA/RNA uptake	Dramatically increases nucleic acid vaccine immunogenicity	Clinical trials for DNA vaccines against HIV, Zika, cancer	Requires device; trained personnel; pain/discomfort; site reactions

Table 3: Clinical Evaluation Stages and Parameters for Novel Vaccines

Phase	Primary Objectives	Typical Sample Size	Duration	Key Endpoints/Parameters	Regulatory Milestones
Preclinical	Safety, immunogenicity, proof-of-concept, dose-range finding	Animal models (varies by model)	Several months to years	Toxicity, biodistribution, immune responses, challenge protection in animal models	Investigational New Drug application submitted if satisfactory
Phase I	Safety, reactogenicity, preliminary immunogenicity, dose escalation	20 to 100 healthy adults	Several months	Adverse events (solicited and unsolicited), antibody responses, T cell responses, dose selection	Safety review before advancing; protocol amendments as needed
Phase II	Immunogenicity, dose and schedule optimization, expanded safety	100 to 500 participants (may include target populations)	6 to 12 months	Antibody titers, seroconversion rates, dose-response relationships, reactogenicity profile	Dose/schedule selection for Phase III; data and safety monitoring board reviews
Phase IIb	Proof-of-concept efficacy in controlled settings	100 to 1000 participants	6 to 18 months	Preliminary efficacy against infection or disease; expanded immunogenicity and safety data	Decision point for proceeding to Phase III; refinement of endpoints
Phase III	Efficacy, large-scale safety, confirmatory immunogenicity	1000 to 50,000+ participants (powered for efficacy)	1 to 4 years	Disease incidence, vaccine efficacy, serious adverse events, subgroup analyses	Basis for licensure application; rolling submission possible; regulatory approval decision
Phase IV	Post-licensure surveillance, real-world effectiveness, rare adverse events	Millions through routine use	Ongoing indefinitely	Vaccine effectiveness, rare adverse events, long-term immunity, impact on disease epidemiology	Ongoing safety monitoring; label updates; possible withdrawal if safety concerns arise
Immunobridging Studies	Demonstrate comparable immunogenicity to efficacy population	Varies (typically hundreds)	Months to 1 year	Non-inferiority of immune responses compared to reference population; correlate of protection	Basis for approval in new populations/formulations if correlates established

Table 4: Challenges, Limitations, and Innovations in Vaccine Development and Global Deployment

Challenge/Limitation	Impact on Vaccine Programs	Current/Emerging Innovations	Implementation Barriers	Future Outlook
Limited Manufacturing Capacity	Delayed access during pandemics; insufficient supply for new vaccines in LMICs	Technology transfer to regional hubs; modular manufacturing facilities; mRNA platform scalability	Capital investment; workforce training; quality assurance; regulatory approval	Expanded distributed capacity if sustained investment and political will
Cold Chain Requirements	Excludes remote areas; vaccine wastage; infrastructure costs in LMICs	Thermostable formulations; lyophilization; controlled temperature chain policies; microneedle patches	Regulatory approval of new formulations; cost-effectiveness; acceptability; distribution networks	Reduced cold chain dependence for increasing number of vaccines
Vaccine Hesitancy	Declining coverage; outbreaks of vaccine-preventable diseases; loss of herd immunity	Tailored communication; community engagement; social media monitoring; trusted messengers	Misinformation spread; political polarization; distrust; measuring effectiveness	Ongoing challenge requiring sustained, multifaceted approaches
Intellectual Property Barriers	Limited access to affordable vaccines; delayed technology transfer; patent thickets	Voluntary licensing; patent pools; TRIPS flexibilities; advance purchase commitments	Industry resistance; legal complexities; technology secrets beyond patents	Balance between innovation incentives and access remains contentious
Inequitable Global Access	Delayed vaccination in LMICs; prolonged pandemics; increased disease burden	COVAX and similar mechanisms; Gavi support; WHO prequalification; tiered pricing	Vaccine nationalism; insufficient funding; logistical challenges; governance issues	Requires strengthened multilateral cooperation and financing mechanisms
Short Immunity Duration	Frequent booster requirements; programmatic complexity; compliance challenges	Adjuvants enhancing durability; nanoparticle sustained release; novel prime-boost regimens	Identifying optimal intervals; population adherence; healthcare system capacity	Improved understanding of long-lived immunity may enable better vaccines
Pathogen Antigenic Variation	Need for annual reformulation; vaccine mismatch; reduced effectiveness	Universal vaccines targeting conserved epitopes; broadly neutralizing antibody induction; rapid updating platforms	Complex development; correlates of protection uncertain; clinical trial design	Universal vaccines could transform control of variable pathogens
Weak Health Systems	Low coverage; missed opportunities; inadequate surveillance; poor data	Health system strengthening; integration with primary care; digital immunization registries; mobile outreach	Financing; political commitment; conflict/instability; workforce shortages	Essential for achieving universal coverage; requires sustained investment
Regulatory Capacity Gaps	Delayed approvals; inconsistent standards; duplicative reviews; limited safety monitoring	WHO prequalification; regulatory harmonization; capacity building; reliance on stringent authorities	Resource constraints; maintaining independence; scientific expertise; political interference	Strengthened national authorities enhance sovereignty and timeliness
Insufficient R&D Funding for LMIC Diseases	Neglected diseases remain without vaccines; limited pipeline; market failure	Product development partnerships; advance purchase commitments; push/pull funding mechanisms	Competing priorities; donor fatigue; uncertain markets; technical challenges	Continued public/philanthropic investment essential for neglected diseases
High Vaccine Costs	Unaffordable for individuals and health systems; delayed introductions; limited competition	Biosimilars; generic manufacturing; competition; negotiated pricing; volume guarantees	Patent protections; regulatory barriers; economies of scale; pricing transparency	Downward pressure on prices as technologies mature and markets expand

References

1. World Health Organization. Immunization coverage [Internet]. Geneva: WHO; 2024 [cited 2026 Feb 1[1]]. Available from: https://www.who.int/teams/immunization-vaccines-and-biologicals/immunization-coverage.
2. Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent). 2005;18(1):21-25.
3. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21(2):83-100.
4. Plotkin SA. Vaccines: past, present and future. Nat Med. 2005;11(4 Suppl):S5-11.
5. Minor PD. Live attenuated vaccines: historical successes and current challenges. Virology. 2015;479-480:379-392.
6. Sanders B, Koldijk M, Schuitemaker H. Inactivated viral vaccines. In: Nunnally BK, Turula VE, Sitrin RD, editors. Vaccine Analysis: Strategies, Principles, and Control. Berlin: Springer; 2015. p. 45-80.
7. Rappuoli R, Black S, Lambert PH. Vaccine discovery and translation of new vaccine technology. Lancet. 2011;378(9788):360-368.
8. Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E. Reverse vaccinology 2.0: human immunology instructs vaccine antigen design. J Exp Med. 2016;213(4):469-481.
9. Correia BE, Bates JT, Loomis RJ, *et al*. Proof of principle for epitope-focused vaccine design. Nature. 2014;507(7491):201-206.
10. Vartak A, Sucheck SJ. Recent advances in subunit vaccine carriers. Vaccines (Basel). 2016;4(2):12.

11. Polack FP, Thomas SJ, Kitchin N, *et al*. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383(27):2603-2615.
12. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261-279.
13. 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.
14. Zhao L, Seth A, Wibowo N, *et al*. Nanoparticle vaccines. Vaccine. 2014;32(3):327-337.
15. Smith DM, Simon JK, Baker JR Jr. Applications of nanotechnology for immunology. Nat Rev Immunol. 2013;13(8):592-605.
16. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. Nat Med. 2005;11(4 Suppl):S45-53.
17. Wouters OJ, Shadlen KC, Salcher-Konrad M, *et al*. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. Lancet. 2021;397(10278):1023-1034.
18. Kumru OS, Joshi SB, Smith DE, Middaugh CR, Prusik T, Volkin DB. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. Biologicals. 2014;42(5):237-259.
19. MacDonald NE; SAGE Working Group on Vaccine Hesitancy. Vaccine hesitancy: definition, scope and determinants. Vaccine. 2015;33(34):4161-4164.
20. Usher AD. COVID-19 vaccines for all? Lancet. 2020;395(10240):1822-1823.
21. Lurie N, Saville M, Hatchett R, Halton J. Developing Covid-19 vaccines at pandemic speed. N Engl J Med. 2020;382(21):1969-1973.
22. Kremer M. Advance market commitments: a policy to stimulate investment in vaccines for neglected diseases. Health Aff (Millwood). 2005;24(3):653-665.
23. Krause PR, Fleming TR, Longini IM, *et al*. SARS-CoV-2 variants and vaccines. N Engl J Med. 2021;385(2):179-186.
24. Lauring AS, Jones JO, Andino R. Rationalizing the development of live attenuated virus vaccines. Nat Biotechnol. 2010;28(6):573-579.
25. Vignuzzi M, Stone JK, Arnold JJ, Cameron CE, Andino R. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. Nature. 2006;439(7074):344-348.
26. Halsey NA, Salmon DA. Measles at the turn of the millennium. Pediatr Infect Dis J. 2000;19(3):279-281.
27. Pulendran B, Ahmed R. Immunological mechanisms of vaccination. Nat Immunol. 2011;12(6):509-517.
28. Belshe RB. The need for quadrivalent vaccine against seasonal influenza. Vaccine. 2010;28 Suppl 4:D45-53.
29. Sanders B, Koldijk M, Schuitemaker H. Inactivated viral vaccines. In: Nunnally BK, Turula VE, Sitrin RD, editors. Vaccine Analysis: Strategies, Principles, and Control. Berlin: Springer-Verlag; 2015. p. 45-80.
30. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. Ann Med. 2018;50(2):110-120.
31. Salk D, Salk J. Vaccinology of poliomyelitis. Vaccine. 1984;2(1):59-74.
32. Budowsky EI, Friedman EA, Zheleznova NV, Nosova NG. Principles of selective inactivation of viral genome. VI. Inactivation of the infectivity of the influenza virus by the action of beta-propiolactone. Vaccine. 1993;11(3):343-348.
33. Moyle PM, Toth I. Modern subunit vaccines: development, components, and research opportunities. ChemMedChem. 2013;8(3):360-376.
34. Schiller JT, Lowy DR. Understanding and learning from the success of prophylactic human papillomavirus vaccines. Nat Rev Microbiol. 2012;10(10):681-692.
35. Rappuoli R. Glycoconjugate vaccines: principles and mechanisms. Sci Transl Med. 2018;10(456):eaat4615.
36. Pollard AJ, Perrett KP, Beverley PC. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat Rev Immunol. 2009;9(3):213-220.
37. Collier RJ. Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. Toxicon. 2001;39(11):1793-1803.
38. Cook TM, Protheroe RT, Handel JM. Tetanus: a review of the literature. Br J Anaesth. 2001;87(3):477-487.
39. Ferreira TB, Alves PM, Aunins JG, Carrondo MJ. Use of adenoviral vectors as veterinary vaccines. Gene Ther. 2005;12 Suppl 1:S73-83.
40. Noad R, Roy P. Virus-like particles as immunogens. Trends Microbiol. 2003;11(9):438-444.
41. McAleer WJ, Buynak EB, Maigetter RZ, Wampler DE, Miller WJ, Hilleman MR. Human hepatitis B vaccine from recombinant yeast. Nature. 1984;307(5947):178-180.
42. McLellan JS, Chen M, Leung S, *et al*. Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. Science. 2013;340(6136):1113-1117.
43. Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines. 2010;9(10):1149-1176.
44. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol. 2010;10(11):787-796.
45. Harper DM, Franco EL, Wheeler CM, *et al*. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet. 2006;367(9518):1247-1245.
46. Ura T, Okuda K, Shimada M. Developments in viral vector-based vaccines. Vaccines (Basel). 2014;2(3):624-641.
47. Draper SJ, Heeney JL. Viruses as vaccine vectors for infectious diseases and cancer. Nat Rev Microbiol. 2010;8(1):62-73.
48. Zhu FC, Guan XH, Li YH, *et al*. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2020;396(10249):479-488.