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Biotechnological Advances in the Development of Oral Vaccines: A New Era in Immunization

Dr. Girish R. Pathade,

Dean, Krishna Institute of Allied Sciences, Krishna Institute of Medical Sciences "Deemed to be University," Karad. Email: girishpathade@yahoo.co.in

Dr. Jayant R. Pawar,

Associate professor, Krishna Institute of Allied Sciences, Krishna Institute of Medical Sciences "Deemed to be University," Karad. Email: jayantpawar26@gmail.com

Ms.Aishwarya D.Jagtap,

Assistant professor, Krishna Institute of Allied Sciences, Krishna Institute of Medical Sciences "Deemed to be University," Karad. Email: aishwarya22999@gmail.com

Abstract. Oral vaccines represent a promising avenue for immunization, offering numerous advantages such as ease of administration, improved patient compliance, and potential cost-effectiveness. Biotechnological advancements have significantly contributed to the development of oral vaccines, paving the way for a new era in immunization. This paper reviews the latest biotechnological innovations driving the formulation, delivery, and efficacy of oral vaccines. Key topics include the use of live attenuated and subunit vaccines, antigen encapsulation techniques, mucosal adjuvants, and novel delivery systems such as nanoparticles and microparticles. Additionally, the challenges and future directions in the field of oral vaccine development are discussed, highlighting the potential for biotechnology to revolutionize global vaccination strategies.

Keywords: Oral vaccines, Biotechnology, Immunization, Attenuated vaccines, Subunit vaccines, Antigen encapsulation, Mucosal adjuvants, Nanoparticles, Microparticles.

I. Introduction

Immunization stands as one of the most significant public health interventions, playing a pivotal role in preventing infectious diseases and reducing global morbidity and mortality rates. The advent of vaccines has led to the eradication of deadly diseases like smallpox and near-elimination of others such as polio and measles. Despite these achievements, challenges persist in reaching underserved populations, ensuring vaccine access, and overcoming vaccine hesitancy [1]. Traditional vaccine administration primarily involves parenteral routes, such as intramuscular or subcutaneous injection, which although effective, may pose logistical challenges in resource-limited settings and induce discomfort in patients. Oral vaccines, in contrast, offer a convenient and non-invasive alternative that could potentially revolutionize immunization strategies globally [2]. The concept of oral vaccination dates back centuries, with historical accounts of variolation, a practice where individuals were intentionally exposed to smallpox scabs through ingestion. However, it wasn't until the 20th century that modern oral vaccines began to emerge, most notably with the development of Albert Sabin's oral polio vaccine in the 1960s. Since then, significant progress has been made in harnessing biotechnological innovations to enhance the efficacy, safety, and feasibility of oral vaccination [3].

Advantages of oral vaccines over injectable counterparts are manifold [4]. Firstly, they eliminate the need for needles and trained medical personnel for administration, potentially facilitating mass immunization campaigns, particularly in resource-limited or hard-to-reach areas [5]. This characteristic is of paramount importance during outbreaks or emergency vaccination programs. Secondly, oral vaccines trigger mucosal immune responses, stimulating both systemic and mucosal immunity at sites of pathogen entry, such as the gastrointestinal and respiratory tracts. This dual immune response provides enhanced protection against pathogens like rotavirus, cholera, and influenza, which primarily infect mucosal surfaces [6]. Thirdly, oral vaccines offer improved patient compliance, especially among pediatric and needle-phobic populations, leading to higher vaccination coverage rates and subsequently, better disease control. Biotechnological advancements have played a pivotal role in unlocking the full potential of oral vaccines [7]. By leveraging techniques such as genetic engineering, antigen encapsulation, and novel delivery systems, researchers have overcome longstanding challenges associated with oral vaccine development, including antigen stability, immunogenicity, and targeted delivery [8]. These innovations have ushered in a new era in immunization, where oral vaccines stand poised to address some of the most pressing global health challenges, from infectious disease outbreaks to endemic illnesses [9]. This paper aims to explore the latest biotechnological advances driving the formulation, delivery, and efficacy of oral vaccines. Through a comprehensive review of current literature and research findings, key topics such as the types of oral vaccines, biotechnological approaches for vaccine delivery, immunological considerations, challenges, and future perspectives will be examined [10]. By elucidating the role of biotechnology in shaping the landscape of oral vaccination, this paper seeks to contribute to the growing body of knowledge aimed at optimizing immunization strategies for the benefit of global health.

II. Types of Oral Vaccines

Oral vaccines encompass a diverse array of formulations designed to induce protective immune responses against a wide range of pathogens. This section discusses three main categories of oral vaccines: live attenuated vaccines, subunit vaccines, and recombinant vector vaccines.

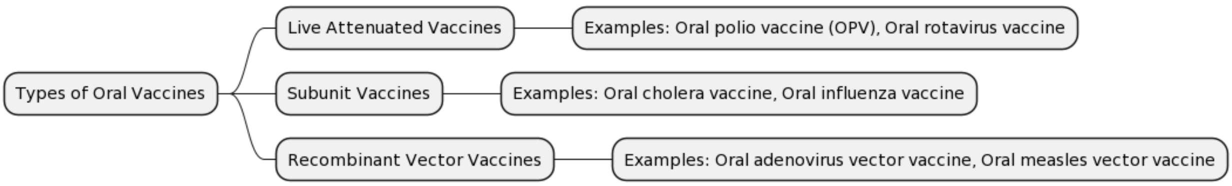

Figure 1. Types of Oral Vaccines

i. Live Attenuated Vaccines

Live attenuated vaccines contain weakened forms of pathogens that retain the ability to replicate and induce immune responses but are rendered avirulent to prevent disease. The process of attenuation involves modifying the pathogen's genetic makeup or adapting it to grow under specific conditions, resulting in reduced virulence while maintaining immunogenicity.

Examples of successful oral live attenuated vaccines include the oral polio vaccine (OPV), developed by Albert Sabin, which played a crucial role in the global eradication of wild poliovirus. OPV consists of three attenuated strains of poliovirus (types 1, 2, and 3) administered orally in drops. Another notable example is the oral rotavirus vaccine, which protects against severe gastroenteritis caused by rotavirus infection. Rotavirus vaccines, such as Rotarix and RotaTeq, contain live attenuated strains of rotavirus delivered orally as liquid formulations.

Despite their effectiveness, live attenuated vaccines carry a small risk of reversion to virulence and can potentially cause vaccine-associated disease in immunocompromised individuals. Additionally, the requirement for cold chain storage and the possibility of shedding live vaccine strains pose logistical challenges for vaccine distribution and implementation.

ii. Subunit Vaccines

Subunit vaccines consist of purified antigens or antigenic components derived from pathogens, rather than whole organisms. These vaccines leverage specific protein subunits, polysaccharides, or recombinant antigens to elicit immune responses targeted against key epitopes involved in pathogen recognition and neutralization.

Production of subunit vaccines often involves recombinant DNA technology or protein expression systems to produce antigens in large quantities. By focusing on immunogenic components while excluding non-essential or potentially harmful elements of the pathogen, subunit vaccines offer improved safety profiles compared to live vaccines.

Examples of oral subunit vaccines include the oral cholera vaccine, which contains inactivated cholera toxin (CT) or a recombinant cholera toxin B subunit (CTB) combined with whole-cell inactivated Vibrio cholerae. These vaccines stimulate mucosal and systemic immune responses against cholera toxin and bacterial antigens, providing protection against cholera infection.

While subunit vaccines offer enhanced safety and stability profiles, they often require adjuvants or delivery systems to enhance immunogenicity and overcome mucosal tolerance mechanisms. Additionally, subunit vaccines may necessitate multiple doses or booster immunizations to achieve robust and durable immunity.

iii. Recombinant Vector Vaccines

Recombinant vector vaccines employ live viral or bacterial vectors engineered to express heterologous antigens from target pathogens. These vectors serve as delivery vehicles to introduce foreign antigens into host cells, thereby eliciting immune responses against both the vector and the encoded antigen.

Vectors commonly used in oral vaccine development include attenuated strains of viruses such as adenovirus, measles virus, and vaccinia virus, as well as bacteria like Salmonella and Lactobacillus. By harnessing the replicative and immunogenic properties of these vectors, recombinant vector vaccines can induce potent cellular and humoral immune responses.

An example of an oral recombinant vector vaccine is the oral polio vaccine type 2 (nOPV2), which utilizes a live attenuated poliovirus type 2 vector engineered to express antigens from other pathogens, such as the coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2). This bivalent vaccine strategy aims to simultaneously target poliovirus and emerging infectious diseases, demonstrating the versatility and potential of recombinant vector platforms.

Despite their promise, recombinant vector vaccines face challenges related to vector immunogenicity, pre-existing immunity, and vector stability. Additionally, regulatory considerations and safety concerns surrounding the use of live vectors necessitate rigorous evaluation and surveillance throughout the vaccine development process.

III. Biotechnological Approaches for Oral Vaccine Delivery

Effective delivery of oral vaccines poses unique challenges due to the harsh environment of the gastrointestinal tract, mucosal tolerance mechanisms, and the need to target antigen-presenting cells for optimal immune stimulation. Biotechnological innovations have thus been instrumental in developing strategies to enhance vaccine stability, improve mucosal adhesion, and facilitate antigen uptake and presentation. This section explores key biotechnological approaches employed in oral vaccine delivery:

i. Antigen Encapsulation Techniques

Encapsulation of vaccine antigens within protective carriers offers several advantages, including improved stability, controlled release kinetics, and enhanced mucosal targeting. Various biocompatible materials, such as polymers, lipids, and proteins, can be employed to encapsulate antigens and protect them from degradation in the gastrointestinal tract.

Polymeric nanoparticles: Nanoparticles composed of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA) or chitosan, have gained traction as oral vaccine delivery vehicles. These nanoparticles can be engineered to encapsulate antigens and adjuvants, providing sustained release and protection against enzymatic degradation. Surface modification of nanoparticles with mucoadhesive polymers enhances their residence time in the mucosal epithelium, facilitating antigen uptake and immune activation.

Liposomes and lipid-based carriers: Liposomes, phospholipid bilayer vesicles, offer versatility in encapsulating hydrophilic and lipophilic antigens within their aqueous core or lipid membrane. Lipid-based carriers, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), provide stable and biocompatible platforms for oral vaccine delivery. Surface modifications with ligands targeting mucosal receptors can further enhance the specificity and efficacy of lipid-based vaccine carriers.

ii. Mucosal Adjuvants

Mucosal adjuvants play a crucial role in enhancing the immunogenicity of oral vaccines by promoting antigen uptake, activating innate immune cells, and modulating mucosal immune responses. Biotechnological advancements have enabled the development of novel adjuvant formulations tailored to stimulate mucosal immunity while minimizing adverse effects.

Types of adjuvants: Mucosal adjuvants encompass a diverse range of compounds, including bacterial toxins (e.g., cholera toxin, heat-labile enterotoxin), bacterial-derived molecules (e.g., flagellin, CpG oligodeoxynucleotides), and synthetic immunomodulators (e.g., polymeric nanoparticles, lipopolysaccharide analogs). These adjuvants stimulate pattern recognition receptors (PRRs) on mucosal epithelial cells and immune cells, triggering pro-inflammatory cytokine production and antigen presentation.

Enhancement of mucosal immune responses: Mucosal adjuvants promote the recruitment and activation of antigen-presenting cells (APCs) in mucosal-associated lymphoid tissues (MALT), leading to the priming of antigen-specific T and B cells. By modulating the balance between regulatory T cells (Tregs) and effector T cells, mucosal adjuvants can skew immune responses toward protective Th1, Th2, or Th17 phenotypes, depending on the desired immune outcome.

iii. Novel Delivery Systems

Advancements in nanotechnology and biomaterial engineering have facilitated the development of innovative delivery systems for oral vaccines, ranging from microparticles to engineered bacterial vectors. These delivery systems offer precise control over vaccine formulation, release kinetics, and targeting specificity, thereby enhancing vaccine efficacy and reducing adverse effects.

Micro/nanoparticles for targeted vaccine delivery: Micro- and nanoparticles can be functionalized with ligands targeting mucosal receptors or specific cell populations, enabling targeted delivery of vaccine antigens to immune cells in the gastrointestinal tract. Surface modification with bioadhesive polymers enhances particle retention and uptake by mucosal epithelial cells, prolonging antigen exposure and immune activation.

Mucoadhesive formulations and bioadhesive polymers: Mucoadhesive formulations exploit the adhesive properties of polymers, such as carbomers, chitosan, and hyaluronic acid, to enhance mucosal adhesion and prolong residence time at mucosal surfaces. These formulations promote intimate contact between vaccine antigens and mucosal epithelial cells, facilitating antigen uptake and transport to underlying immune cells.

Biotechnological approaches for oral vaccine delivery hold great promise in overcoming the challenges associated with mucosal immunization and expanding the utility of oral vaccines in global immunization programs. By harnessing the synergistic effects of antigen encapsulation, mucosal adjuvants, and novel delivery systems, researchers aim to develop next-generation oral vaccines capable of eliciting robust and durable immune responses against a broad spectrum of infectious pathogens.

IV. Immunological Considerations

The success of oral vaccines relies heavily on their ability to elicit robust immune responses at mucosal surfaces while also inducing systemic immunity. Understanding the complex interplay between mucosal and systemic immune compartments is essential for optimizing vaccine design and enhancing protective efficacy. This section delves into key immunological considerations relevant to oral vaccine development:

i. Mechanisms of Mucosal Immunity

Mucosal surfaces, including the gastrointestinal, respiratory, and urogenital tracts, serve as primary portals of entry for pathogens and represent crucial sites for immune surveillance and defense. Mucosal immunity encompasses a diverse array of specialized immune mechanisms tailored to combat pathogens encountered at mucosal surfaces while maintaining tissue homeostasis.

Mucosal-associated lymphoid tissues (MALT): MALT comprises organized lymphoid structures, such as Peyer's patches in the intestine and nasopharynx-associated lymphoid tissue (NALT) in the respiratory tract, as well as diffuse lymphoid aggregates dispersed throughout mucosal epithelia. These specialized lymphoid tissues serve as sites of antigen sampling, immune activation, and generation of mucosal immune responses.

Secretory IgA (sIgA) antibody responses: Secretory IgA antibodies, produced by plasma cells in the lamina propria and transported across mucosal epithelial cells via the polymeric immunoglobulin receptor (pIgR), constitute the primary effector mechanism of mucosal immunity. sIgA antibodies neutralize pathogens by blocking adhesion, agglutination, and immune exclusion, thereby preventing microbial invasion and colonization of mucosal surfaces.

Innate immune defenses: Mucosal epithelial cells secrete antimicrobial peptides, mucus, and surfactant proteins that contribute to the physical barrier function of mucosal surfaces and inhibit pathogen growth and dissemination. Innate immune cells, including dendritic cells, macrophages, and innate lymphoid cells, detect microbial products through pattern recognition receptors (PRRs) and initiate innate immune responses characterized by cytokine production, phagocytosis, and antigen presentation.

ii. Induction of Systemic and Mucosal Immune Responses

Oral vaccines must elicit both systemic and mucosal immune responses to provide comprehensive protection against pathogens. The route of vaccine administration, antigen formulation, and adjuvant selection critically influence the balance and magnitude of immune responses elicited.

Oral tolerance versus immune priming: The gastrointestinal tract is endowed with mechanisms to maintain immune tolerance to harmless dietary antigens and commensal microorganisms while mounting protective immune responses against pathogens. Oral vaccines must navigate these tolerance mechanisms to induce antigen-specific immune priming without triggering systemic or local tolerance.

Induction of mucosal IgA and systemic antibody responses: Effective oral vaccines stimulate the production of secretory IgA antibodies at mucosal surfaces, conferring localized protection against mucosal pathogens. Concurrently, systemic antibody responses, including serum IgG antibodies, play a critical role in neutralizing circulating pathogens and preventing systemic dissemination.

T-cell-mediated immunity: Oral vaccines promote the generation of antigen-specific T-cell responses, including CD4+ T helper (Th) cells and CD8+ cytotoxic T lymphocytes (CTLs), which orchestrate cellular immunity against intracellular pathogens. Th cells provide help for B-cell antibody production and activation of macrophages, while CTLs directly kill infected cells and suppress pathogen replication.

iii. Factors Influencing Vaccine Efficacy at Mucosal Surfaces

Several factors influence the efficacy of oral vaccines in inducing protective immune responses at mucosal surfaces, including vaccine antigen design, delivery system properties, mucosal adjuvants, and host factors.

Antigen stability and immunogenicity: Vaccine antigens must withstand the harsh environment of the gastrointestinal tract, including low pH, enzymatic degradation, and mucosal clearance mechanisms, to retain their immunogenicity and elicit protective immune responses. Formulation strategies, such as antigen encapsulation within biodegradable carriers or stabilization with mucosal adjuvants, can enhance antigen stability and promote effective immune recognition.

Delivery system properties: The physicochemical properties of oral vaccine delivery systems, including particle size, surface charge, and mucoadhesive properties, influence their interaction with mucosal epithelia and immune cells, thereby modulating antigen uptake, transport, and immune activation. Optimal delivery systems should promote prolonged antigen residence time at mucosal surfaces while minimizing off-target effects and systemic dissemination.

Mucosal adjuvants and immune modulation: Adjuvants play a crucial role in enhancing vaccine immunogenicity by promoting antigen uptake, activation of antigen-presenting cells, and modulation of mucosal immune responses. The selection of appropriate adjuvants and their formulation with vaccine antigens can tailor immune responses toward desired phenotypes, such as Th1, Th2, or Th17, to confer protection against specific pathogens.

Host factors and immune competency: Individual variations in mucosal immune competency, influenced by factors such as age, nutritional status, microbiota composition, and underlying medical conditions, can impact vaccine responsiveness and efficacy. Strategies to enhance mucosal immunity in vulnerable populations, such as infants, elderly individuals, and immunocompromised patients, are critical for achieving optimal vaccine outcomes.

Understanding the intricacies of mucosal immunology and the factors influencing vaccine efficacy at mucosal surfaces is essential for the rational design and optimization of oral vaccines. By harnessing biotechnological innovations to tailor antigen formulations, delivery systems, and adjuvant strategies, researchers aim to develop next-generation oral vaccines capable of eliciting robust and durable mucosal immune responses against a wide range of infectious pathogens.

V. Challenges and Future Perspectives

Despite significant progress in oral vaccine development, several challenges remain to be addressed to fully realize the potential of this immunization approach. This section examines key challenges facing the field of oral vaccines and explores future perspectives for overcoming these hurdles:

i. Regulatory Hurdles and Safety Concerns

Regulatory approval for oral vaccines often requires stringent evaluation of safety, efficacy, and manufacturing consistency, which can pose significant barriers to vaccine licensure and commercialization. Unlike injectable vaccines, which have well-established regulatory pathways, oral vaccines may face additional challenges related to stability, shelf life, and quality control, particularly for live attenuated and recombinant vector platforms.

Safety concerns surrounding oral vaccines include the risk of reversion to virulence or vaccine-associated adverse events, especially in immunocompromised individuals or vaccine recipients with underlying health conditions. Addressing these safety concerns requires thorough preclinical and clinical evaluation, including assessment of vaccine shedding, transmission potential, and long-term immunogenicity.

ii. Strategies for Overcoming Barriers to Oral Vaccine Development

To overcome regulatory hurdles and safety concerns, researchers are exploring innovative strategies to enhance the safety, stability, and immunogenicity of oral vaccines. These strategies include:

Genetic engineering and rational attenuation of live vaccine strains to improve safety profiles and reduce the risk of reversion to virulence.

Development of novel adjuvants and delivery systems tailored to enhance mucosal immune responses while minimizing off-target effects and systemic toxicity.

Integration of advanced manufacturing technologies, such as microfluidics and bioreactors, to streamline vaccine production and ensure batch-to-batch consistency.

Implementation of quality control measures and stability testing protocols to assess vaccine potency, purity, and stability under diverse storage conditions.

Engagement with regulatory agencies and public health stakeholders to establish clear guidelines and standards for oral vaccine development, licensure, and post-marketing surveillance.

iii. Integration of Oral Vaccines into Global Immunization Programs

The successful integration of oral vaccines into global immunization programs requires concerted efforts to address logistical, financial, and cultural barriers to vaccine access and acceptance. Key strategies for enhancing vaccine uptake and coverage include:

Strengthening health systems and infrastructure to support the delivery, storage, and administration of oral vaccines, particularly in resource-limited settings.

Conducting community engagement and education campaigns to raise awareness about the importance of vaccination and dispel myths and misconceptions surrounding oral vaccines.

Implementing innovative delivery strategies, such as mobile vaccination units, community health worker networks, and school-based immunization programs, to reach underserved populations and marginalized communities.

Leveraging partnerships between governments, non-governmental organizations (NGOs), philanthropic organizations, and vaccine manufacturers to ensure sustainable vaccine procurement, distribution, and financing mechanisms.

iv. Emerging Technologies and Potential Breakthroughs

Looking ahead, emerging technologies hold promise for advancing the field of oral vaccines and addressing existing challenges. Key areas of innovation and potential breakthroughs include:

Development of next-generation adjuvants and delivery systems, such as synthetic nanoparticles, virus-like particles (VLPs), and self-amplifying RNA (saRNA) vaccines, capable of eliciting potent and durable immune responses.

Integration of artificial intelligence (AI) and machine learning algorithms to predict vaccine efficacy, optimize vaccine design, and identify novel vaccine targets.

Application of CRISPR-Cas9 gene editing technology to engineer designer probiotics and commensal microbes capable of delivering vaccines and modulating mucosal immunity.

Exploration of alternative vaccine platforms, such as plant-based vaccines, edible vaccines, and insect-based vaccines, to overcome production constraints and enhance vaccine accessibility and acceptability.

In conclusion, while oral vaccines hold great promise for revolutionizing global immunization efforts, overcoming regulatory, safety, and logistical challenges remains critical for their widespread adoption and impact. By harnessing biotechnological innovations, fostering interdisciplinary collaborations, and leveraging emerging technologies, researchers can pave the way for a new era in oral vaccine development, characterized by improved safety, efficacy, and accessibility for all populations.

VI. Conclusion

The development of oral vaccines represents a significant advancement in the field of immunization, offering a non-invasive and convenient approach to protecting against infectious diseases. Biotechnological innovations have played a pivotal role in driving the formulation, delivery, and efficacy of oral vaccines, ushering in a new era in global health. Through the utilization of live attenuated, subunit, and recombinant vector platforms, researchers have overcome numerous challenges associated with oral vaccine development, including antigen stability, immunogenicity, and targeted delivery. These advancements have paved the way for the development of vaccines against a wide range of pathogens, from poliovirus and rotavirus to emerging infectious diseases like COVID-19. Despite the progress made, challenges remain on the path to realizing the full potential of oral vaccines. Regulatory hurdles, safety concerns, and logistical barriers continue to pose significant obstacles to vaccine licensure, distribution, and acceptance. Addressing these challenges will require collaboration between governments, regulatory agencies, academia, industry, and civil society to develop clear guidelines, implement robust quality control measures, and ensure equitable access to vaccines for all populations. Looking ahead, emerging technologies and innovative strategies hold promise for overcoming existing barriers and unlocking new opportunities in oral vaccine development. By harnessing the power of artificial intelligence, gene editing, and alternative vaccine platforms, researchers can accelerate progress toward safer, more effective, and accessible oral vaccines capable of addressing global health challenges. The biotechnological advances in oral vaccine development mark a significant milestone in the quest to improve public health and combat infectious diseases. By continuing to innovate, collaborate, and prioritize equitable vaccine access, we can usher in a future where oral vaccines play a central role in safeguarding the health and well-being of individuals and communities worldwide.

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