A review on current status and future prospectus of Oral vaccines

Abstract

The second leading reason of mortality worldwide is communicable diseases. The highly cost effective approach for disease prevention in case of communicable disease is vaccination. It is need of the hour to design and develop novel as well as safe vaccine delivery systems to safeguard against present incurable and emerging diseases. For various factors, the production of orally administered vaccines is superior to conventional injection-based formulations, including increased protection and compliance, and simplified processing and administration. In comparison, the oral route helps humoral and cellular immune responses to be activated at both systemic and mucosal sites to create larger and longer-lasting defence. This review addresses the reasoning for oral vaccines here including important biological and physicochemical implications for the design of oral vaccines for the next decade.

Keywords

Communicable diseases, immune response, oral vaccines, vaccine drug delivery systems

1. Introduction

Vaccines have greatly decreased the problem of communicable diseases by decreasing deaths globally. Immunization is a cost-effective technique that by creating herd immunity, protects not only the vaccinated individuals, but also defend the community through the generation of herd immunity.[1] Vaccine production against a number of illnesses, including diphtheria, tetanus, polio, measles, mumps, rubella, hepatitis B, and meningitis, has decreased the resulting mortality by 97% to 99%. However, along with several active vaccine programmes, communicable diseases are the world's second most key cause of death, affecting children under 5 years of age and people in low-income countries disproportionately. In reality, infective agents are accountable for five of the top ten foremost reasons of death in low-income countries: lower respiratory infections (e.g. pneumonia), HIV/AIDS, diarrheal illness, malaria, and tuberculosis.[2] Although some of these viruses still lack the vaccination needed for disease avoidance, an estimated 20% of these deaths are caused by vaccine-preventable diseases, signifying that vaccine technologies and administration need to be considerably improved. After crossing one of the various defensive mucosal barriers of the body, maximum infections take place. An effective practise to evade infection at the point of interaction between microbes and the host would be the development of an immunologically strong mucosal barrier. The current vaccine technology strategies, however, usually address only pathogens that have already crossed the mucosal barrier.[3] Most appropriate vaccines are offered either by subcutaneous injection or intramuscular injection. As a universal rule, the resultant immune response is limited to systemic humoral immunity (e.g. production of antibodies) against the pathogen or toxin, with limited cellular immunity (e.g. T-cell-mediated) and only poor mucosal surface defence.[4] Vaccination on mucosal surfaces, on the other hand, effectively triggers mucosal

antibodies (IgA) and cell-mediated immune responses while also inducing a systemic antibody response (IgG).

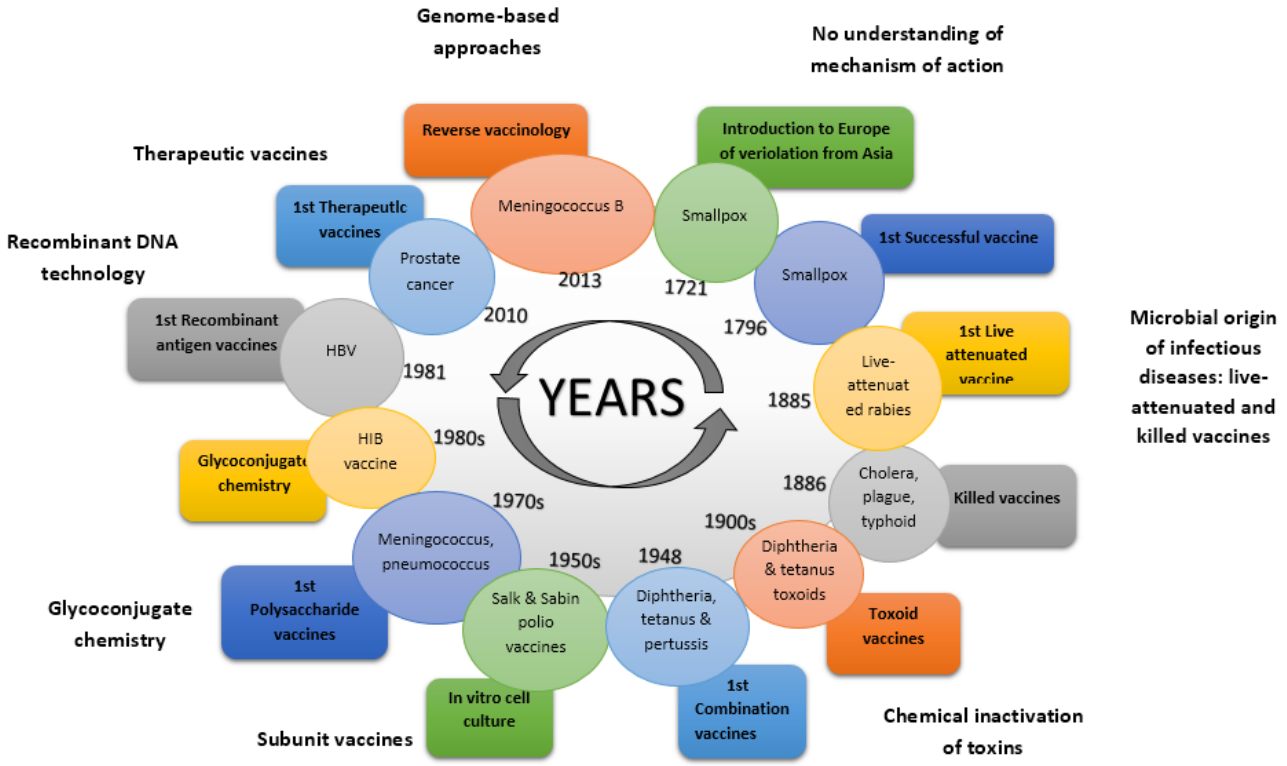

Figure 1: History of vaccine development

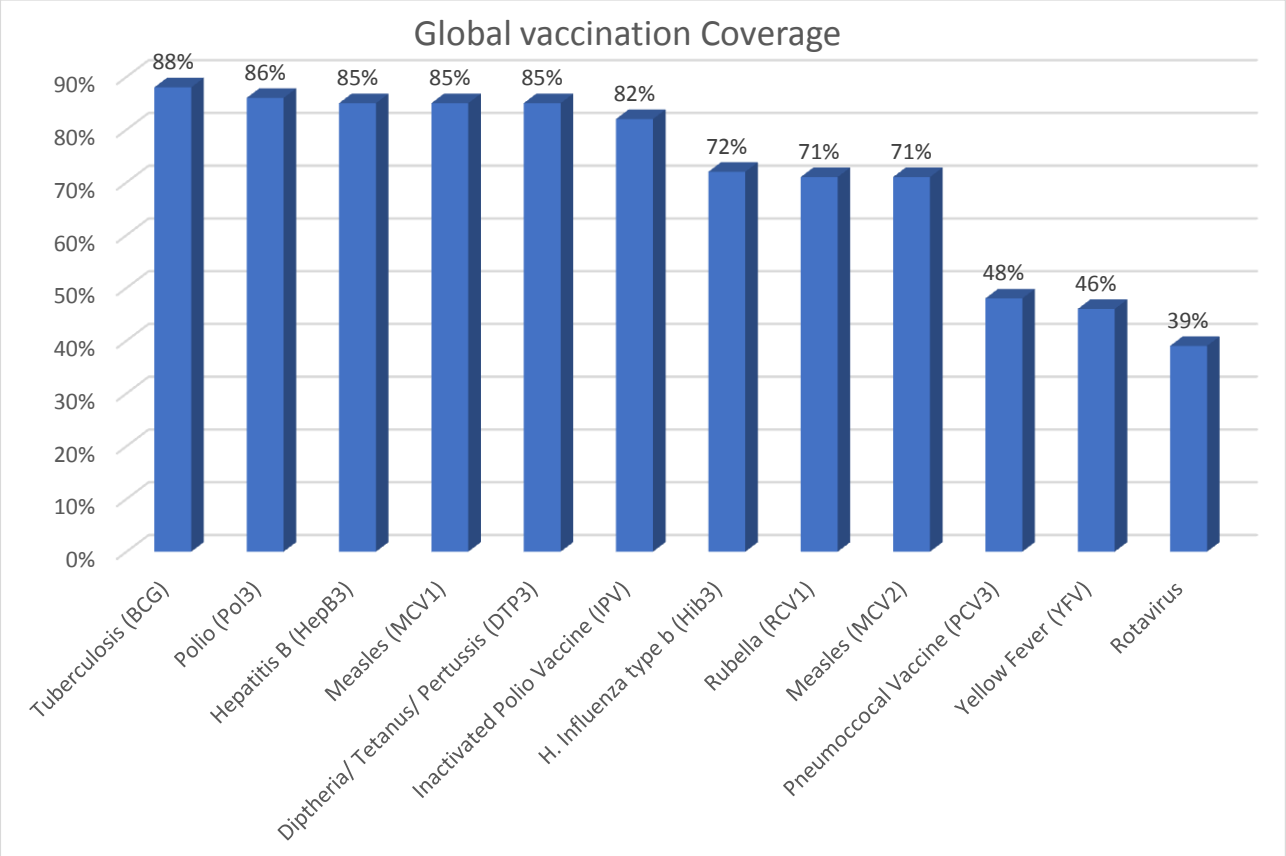

Figure 2: Global vaccination Coverage

2. Challenges of oral administration

The oral transmission of antigens needs to resolve several physicochemical and biological hurdles in the GI tract to prompt a vigorous immune response. Among them is the intestinal epithelium's biological barrier and its mucus secreting layers that aid to digest ingested nutrient absorption content and to guard the body from the incursion of pathogenic threats. The GI tract needs a very acidic environment in the intestine, a wide pH spectrum along the length of the GI tract, and the involvement of proteolytic enzymes responsible for protein degradation in order to accomplish these activities.[5] These features can hinder with the distribution of fragile biomolecules that are extremely vulnerable to degradation and denaturation, such as antigenic proteins or peptides. In addition due to the residence time in the small intestine (3-4 h), where the bulk of absorption processes exist, there is a temporal restriction to the absorption of these formulations.[6]

Additional challenge in the production of oral vaccines is that, compare to conventional parenteral immunizations, a higher dosage of antigen is essential to cause an immune response. The available formulations used as carriers are limited by this function as they must be able to bear the essential antigen dose effectively.[7] Instead of prompting a defensive reaction, greater doses often increase the danger of causing resistance. A number of diseases are frequently introduced to the GI tract. If a vaccine does not prompt adequate risk signals, the body may recognise it as non-pathogenic and inhibit the immune response from being activated, ensuing in immune resistance rather than defence.[8] In the nature of oral vaccine carriers, it is also significant to use strong adjuvants in order to activate the immune system effectively.[9]

3. Types of vaccines

Live attenuation of the early vaccines, signifying that they included a variant of the live microbe that was reduced or altered in the laboratory so as not to prompt substantial infection.[10] Live-attenuated formulations bear a resemblance to natural pathogens most closely, provoking forceful cellular and antibody responses that are likely to deliberate long-lived defensive immunity. Unfortunately, living, weakened vaccines could also pose risks, mostly in immuno-compromised patients, such as infection, unregulated replication, and disease.[11] Furthermore, attenuated pathogens, though exceedingly unusual, have the capability to return to a pathogenic form and cause the disease. Such as, due to the threat of vaccine-associated paralytic poliomyelitis and the availability of a safer alternative in the form of an injected inactivated vaccine, the live oral poliovirus (OPV) vaccine has not been circulated in the United States since 2000.[12] Genetic engineering progresses have reduced the irregularity of laboratory attenuation and improved the protection of live attenuated viruses in a number of ways, including genetic modification or removal required for replication.[13] Whole-cell vaccines that comprise of a disease-causing microbe inactivated by additives, sun, or radiation are a better substitute to live-attenuated vaccines. Inactivated vaccines can also prompt, but cannot replicate, an immune response. Therefore, these vaccines are superior and more stable than live vaccines, but they prompt a weaker immune

response, typically requiring extra doses or booster shots in order to maintain safety.[14] Though the production of vaccines has historically focussed on either live or dead whole organism vaccines, the progress of next-generation vaccines has started to concentrate on much improved and more cost-effective candidates for vaccines: subunit vaccines.[15] Subsequently they do not have any live components of the pathogen, subunit vaccines are considered the best option. They can be categorised into four main categories: protein-based, conjugates, polysaccharides, and toxoids.[16] A specific and isolated protein which is introduced to the immune system as an antigen is used by protein-based subunit vaccines. It is likely to extract and refine these molecules from a cultured microbe or to yield them using recombinant DNA technology. [17]Proteins, though are delicate structures and are readily denatured and degraded by pH modifications or proteolytic enzyme presence. Polysaccharide vaccines bear a resemblance to infectious bacteria-associated polysaccharide capsules, thus producing an immune response.[18] They are not principally immunogenic, similar to protein subunit vaccines, and are therefore related to short-term immunological reactions, not long-term memory. Conjugate vaccines time and again prompt a reaction against the protective polysaccharide capsule of the pathogen; also, they contain, in addition to polysaccharides, a carrier protein to boost the production of long-term protective immunity.[19] Any of the protein carriers generally used comprise toxoids for diphtheria which tetanus, and are also generally used against bacterial infections. In conclusion, toxoid vaccines are used against diseases of which the main cause of disease, such as diphtheria and tetanus, is bacterial toxin. They are inactivated forms of the poisons and are both healthy and stable, thus.[20] On the other hand, for an effective immune response, the widely held toxoid vaccines need the use of adjuvants, such as aluminium or calcium salts.[21] The distinction between both subunit vaccines and inactivated immunizations is that they comprise selected antigenic sections of a pathogen desired to induce a defensive immune response.[22] These formulations have exceptional stability and protection profiles, but it is very time-consuming to find a sufficient combination of the aforementioned antigenic components to create a suitable immune response.[23] In comparison, subunit vaccines appear to be less immunogenic than their equivalents with whole cells. In order to reinforce the immune response by the addition of immunostimulatory molecules or the nature of antigen delivery mechanisms, recent research has focused on the use of adjuvants.[24]

Licensed oral vaccines

4.1. Oral polio vaccine

The first active mucosal vaccine created was the oral polio vaccine (OPV). OPV consists of a mixture of one of the three infectious serotypes of live attenuated poliovirus strains.[25] To yield safety via both humoral and mucosal immunity, three spaced doses are necessary. Serum antibodies block poliovirus from spreading to the nervous system, therefore saving patients from the paralysis of polio.[26] In addition, and precise to OPV, OPV induces a local SIgA immune response in the intestinal mucosa, which is the main site for the entry and replication of poliovirus, similar to the inactivated injectable polio vaccine (IPV).[27] This local intestinal response is extremely positive in averting the spread of wild poliovirus person-to-person. Though a remarkably low but actual chance of reversion to neurovirulence exists with OPV administration, arising in approximately 1 of every 2.5 million cases.[28] The threat of contracting the disease from the wild-type pathogen was minor than that of acquiring

polio from OPV after positive eradication of the disease by extensive vaccine campaigns. In most developed countries, OPV has thus, been substituted by IPV.[29]

4.2. Live oral typhoid vaccine (Ty21a)

Salmonella typhi, an infectious enteric bacterium generally consumed by infected food or drink, causes typhoid fever. Typhoid fever is too very rare in advanced nations, but still common in less developed areas where access to treated water sources and sanitation is generally missing.[30] An oral live attenuated Ty21a vaccine, formed through chemical mutagenesis of the Ty2 S. Typhi strain, is one of the two approved typhoid fever vaccines. None of the vaccines offered are 100 percent effective and the safety of Ty21a differs based on the dosage of the vaccine, the number of doses and the spacing between doses.[31] The formulation is presently offered as either a liquid suspension or an entero-coated capsule and is provided on alternating days in three or four doses. Seven days after the last injection, the vaccine confers protection, with up to 62 percent protection for a follow-up duration of seven years.[32] While Ty21a is linked with the progress of serum IgG, intestinal sIgA, as well as numerous cell-mediated immune responses such as T cell proliferation and Th1-type cytokines, the degree to which this vaccine mediates defence by systemic immunity or gut mucosal immunity is anonymous.[33] However serum antibodies are certainly helping to attain S defence. New trials in Typhi seek to clarify the leading immune system in order to attain long-term efficacy.[34]

4.3. Cholera vaccines

Cholera is an enormously virulent and acute diarrheal disease spread through fecal contamination of food and water.[35] Of the many enteric pathogens related with diarrheal disease, V. cholera causes the most severe epidemic outbursts, most frequently related with natural disasters that interrupt access to clean water.[36] It remains endemic in regions with deprived hygiene in needy and overcrowded areas. Developed through ingestion of polluted food and water, the bacteria V. cholerae colonizes the epithelial lining of the gut, causing profuse watery diarrhea that can kill within hours if untreated.[37] For numerous years, the only vaccine presented was a killed full cell cholera vaccine given by injection, but safety was insufficient, short-lived, and related to painful side effects.[38] For general public health use, it was found unacceptable and has since been changed by two enhanced oral vaccines.[39] A recombinant cholera toxin B (CTB) subunit and inactivated whole cell V. cholera O1 called Dukoral® is the most frequently used, developed by Crucell (Leiden, The Netherlands) and delivered fourteen days apart in two doses.[40] The recombinant cholera toxin B vaccine offers safety against multiple serotypes, is safe and stable and for 2 years, offers approximately 65% safety against cholera, including substantial safety for herds. Local creation of both antitoxic and antibacterial SIgA antibodies in the gut mediates the protection. In addition, the CTB vaccine portion offers significant cross-protection against ETEC, which holds a heat-labile toxin that is structurally and functionally identical.[41] Though since Dukoral® is administered with a buffer solution that includes 150 mL of clean water for adults, in comparison to crisis areas where clean water is often scarce, it is mostly used for travellers.[42] An oral live cholera attenuated vaccine, CVD-10-HgR, having a genetically modified V. cholera O1 Inaba strain, is the second and current globally approved vaccine (Vaxchora, PaxVax, USA). It is a reformulation of the previous CVD 103-HgR vaccine (Orochol; Mutachol), which for economic reasons, was taken off the market.[43] It is available as a single dosage, but is currently only approved for adults 18-64 travelling to areas

affected by cholera and is planned to be given at least 10 days prior to likely exposure to V. cholera.[44]

4.4. Rotavirus

The foremost cause of diarrheal mortality in babies and children under 5 years of age is rotavirus. The virus is a triple-layered particle displaying different forms of antigens.[45] The bulk of human rotavirus infections are triggered by five serotypes. A monovalent attenuated human rotavirus (RotaRix) and a pentavalent bovine-human rotavirus vaccine are now available in two oral vaccine formulations: (RotaTeq). While the composition differs, their potency and mode of action are similar.[46] Both are effective (N90 percent) in evading serious rotavirus gastroenteritis, but less effective (60-75 percent) against moderate infections.[47] From a social viewpoint, in view of the need for less doses, and thus less storage capacity, as well as confirmed thermostability, RotaRix has an increased cost-effectiveness ratio.[48]

4.5. Oral adenovirus vaccine

Acute respiratory infection triggered by type 4 and type 7 adenoviruses used to be the primary cause of hospitalisation in the United States.[49] Medical symptoms, including high fever, cough, chest pressure, fatigue, and congestion lasting 3-10 days, are like to the flu.[50] Oral administration permits for selective asymptomatic inflammation in the lower intestinal tract, while the upper respiratory tract offers immunity.[51] The presence of serotype-specific serum neutralising antibodies is reliable with defence, but there is no indication that the main source of protection is neutralising antibodies.[52]

5. Oral vaccine strategies

5.1. Polymeric/particulate vaccine design

For the production of subunit-based vaccines, polymeric microparticles (MPs) and nanoparticles (NPs) have been carefully studied.[53] The components of the proteins, DNA, and polysaccharide vaccine are fragile molecules that may be structurally degraded during transition through the gastrointestinal tract or the substrate of the mucosa, resulting in reduced bioactivity.[54] Entrapment or encapsulation within polymeric particles of the antigenic payload gives protection, while also evading dilution of antigen across the broad surface area of the GI tract.[55] Particles may have control over the release site and profile for better distribution of stable antigens, in addition to robust structural stability.[56] In addition, by passive or active targeting to stimulate cellular and humoral responses, NP carriers have exhibited the ability to successfully transmit an antigenic payload directly to phagocytic APCs.[57] Particulate delivery systems passively, merely by means of APC detection and internalisation, offer characteristics of adjuvant activity to weakly immunogenic subunit vaccines.[58] NPs, however, allow improved adjuvant strategies to be executed by co-delivery of immunomodulators or by modulation of surface properties for enhanced or targeted immune cell uptake.[59] In addition, both synthetic and natural materials with favourable physicochemical properties are diverse and are capable of reacting to physiological changes, making polymeric particles a flexible choice for the design of legitimate vaccines.[60]

5.2. Lipid-based vehicles

Some of the most widely used vehicles for oral administration are lipid-based vaccine delivery carriers. These include liposomes, bilosomes, and ISCOMs, among others.[61] They

are based on a distinct encapsulation using lipid bilayers of hydrophilic and lipophilic agents.[62]

5.2.1. Liposomes

Liposomes are spherical vesicles formed from cholesterol and other non-toxic lipids synthesised by one or more phospholipid bilayers.[63] Based on their structure (i.e. their scale, charge, and protein compatibility), the properties of these structures differ and can be improved by adjusting their manufacturing parameters.[64] This liposomal systems also provide the ability to distribute multiple active agents with dramatically different properties, as they can be mounted in different carrier compartments.[65] Specifically, in the inner layer of these vehicles, water-soluble molecules such as proteins, RNA, carbohydrates or peptides are encapsulated; meanwhile, lipophilic compounds may be found in the outer portion of the composition.[66] To target a wide range of virus and bacterial infections, a number of liposome-based vaccines for oral administration have previously been synthesised. For instance, a viral influenza A vaccine was developed using a DNA build vaccine encapsulated in cationic liposomes with a pcDNA 3.1(+) plasmid.[67] In addition to increasing cytokine production, oral immunisation with this formulation has triggered humoral and cellular immune responses. Liposomes, including Salmonella Enteritidis, have also been used to eliminate bacterial infections.[68] Using a liposome-associated carrier with a recombinant SefA protein by Pang and collaborators, a vaccine was developed to prevent this disease.[69] This oral vaccine was able to produce defensive immunity in poultry, and after an oral competition with 2×10^6 CFUs of live Salmonella Enteriditis, a substantial reduction in intestinal bacterial load was observed. Liposomes, including DNA, peptides, and proteins, have proven their ability to deliver different antigens. For instance, after three oral immunizations, encapsulation of a DNA-based antigen (Mycobacterium pcDNA3.1$^+$/Ag85A) in liposomal formulations increased its presence in the epithelium, M cells, DCs and PPs inside the small intestine of C57BL/6 mice.[70] The system's ability to cause antigen-specific mucosal immunity has rendered this formulation a possible carrier of the vaccine.[71] In another analysis, the delivery inside the liposomes of antigenic peptides and CTL epitopes allowed their successful transport to APCs and improved the host response to these antigens. Furthermore, these formulations' adjuvant capabilities have been tested using sample antigens (e.g. ovalbumin, bovine serum albumin).[72] Such studies have shown that liposomes can load and release stable proteins efficiently. They can also evoke Th1/Th2 immunity, expressed by the generation of responses from mucosal and systemic antibodies.[73] Finally, to improve their effectiveness, these structures may also be adorned with targeting molecules (e.g. carbohydrates). Lectinized liposomes have been able to effectively attack M cells in the PPs in oral immunisation studies, resulting in elicited mucosal responses with high antibody titers.[74] In the production of oral vaccine delivery systems, the ability of conventional liposomal vehicles to evoke immune responses is important. These platforms, however, need to be more engineered to be stable in the GI tract under harsh conditions and to shield delicate antigens.[75] In addition, the encapsulation efficiency of proteins inside liposomes is highly dependent on the charge and size of the antigen, which may restrict their ability if strong immunity is needed to evoke large protein doses.[76] Overall, for vaccine delivery uses, liposomes have demonstrated promising properties. They need more analysis and optimization to lead to successful human vaccine formulations.[77]

5.2.2. Bilosomes

Bilosomes are a different lipid-based carrier being investigated for oral immunisation. In their composition, these non-ionic surfactant vesicles have adjuvant functionalities and contain bile salts.[78] Monopalmitoyl glycerol (MPG), cholesterol (CH) and dicetyl phosphate (DCP) are usually synthesised with bilosomes and surfactants such as sodium deoxycholate (SDC) or sorbitan tristearate (STS).[79] Bilosomes also have a bilayer of polar and non-polar ends, similar to liposomes, allowing vaccine components with substantially different properties to be combined.[80] Standard liposomal vesicles may be damaged by bile salts, however, if vesicles are fabricated in the presence of bile sales, such as bilosomes, they are no longer affected by their activity and remain intact.[81] These mechanisms are capable of enhancing humoral and cellular immune responses, and bile salt incorporation helps the cargo to be shielded from the harsh environment of the GI tract.[82] The increased stability that they can impart on fragile antigens is one of the key benefits of bilosomal formulations. In previous research, a number of fragile antigens, including tetanus toxoid (TT), A/Panama (influenza A immunogen), diphtheria toxoid, Bac-VP1 toxoid, have been shown to capture and stabilise bilosomes (hand, foot and mouth disease vaccine candidate).[83] Additionally, bilosome adjuvant and drug release experiments were performed using model antigens such as bovine serum albumin (BSA) and subunit B cholera toxin.[84] Using different disease models, their immunogenic capacities have also been explored. Previously, systemic and local immunity, including in the mucosa, was developed by mannosylated bilosomes targeting DCs for oral immunisation against the hepatitis B virus.[85] The development of soluble immunoglobulin A at both local and distal GI tract sites was induced by the use of these formulations. High antibody titers and cellular responses were also elicited from a separate series of trials carried out using a subunit influenza vaccine in an orally administered formulation.[86] Specifically, responses to Th1 and Th2 were successfully developed. These findings are particularly promising, as these mechanisms have shown the ability to stimulate healthy mucosal and systemic immunity.[87] Despite this, it is nevertheless important to further research their capacity to impart long-term immunity and defence against lethal challenges.[88] As outlined here, the above advantages offered to multiple antigens due to bilosomal trapping make this method a viable oral immunisation vaccine delivery tool. The next step in the production of bilosomal oral vaccines is further evaluation using clinical trials.[89]

5.2.3. ISCOMs

Immune-stimulating complexes (ISCOMs) are liposomes of the second generation, known as both a transporter and an immunostimulant for the delivery of vaccines.[90] Synthesized with colloidal saponin (often derived from the Quillaja saponaria tree by QuilA), cholesterol and other phospholipids (usually phosphatidylethanolamine or phosphatidylcholine), these self-adjuvant nano-sized vectors (~40 nm) are arranged into open-caged frameworks.[91] To promote vaccines against certain infections, these vehicles have been used to capture bacterial and viral envelope proteins.[93] In the presence of a non-ionic detergent that is withdrawn after synthesis, classical ISCOMs are self-assembling structures manufactured. It has been demonstrated that these formulations have a wide variety of uses, adding antigens to deter infections with herpes simplex virus 1, hepatitis B, respiratory syncytial virus, Escherichia coli, Brucella abortus, and Plasmodium falciparum.[94] In multiple animal models, ISCOM-based vaccines have proved to be highly immunogenic, inducing healthy humoral and cellular responses.[95] Components of both the innate and adaptive immune systems are interested in the properties of this mechanism. This function renders this platform a highly

attractive implementation technique, although its complex modes of operation remain to be completely clarified.[96] However, elicitation of mucosal immunity (i.e. secretory IgA) to prevent enteric infections is also essential for oral applications. To maximise the adjuvant properties of this platform, the production of ISCOM-based vaccines needs further evaluation in pre-clinical studies.[97]

5.3. Adenoviral vectors

Traditional vaccinations is focused around the usage of killed or attenuated pathogens, however as previously mentioned, owing to the possible reversal of their pathogenicity, there are dangers of immunising susceptible communities of those platforms.[98] However, with advancements in genetic engineering and molecular virology, without their adverse side effects, there are several alternatives to the use of such microbial systems.[99] Adenoviruses are double-stranded DNA viruses that are species-specific that have distinct serotypes with a ~40 kb genome.[100] Although this platform was originally devised for gene delivery, it became less desirable for clinical use due to its highly immunogenic nature.[101]

However, they have become fascinating possibilities as vaccine delivery carriers, based on the advancement and optimization in the synthesis of adenoviral vectors.[102] The adenoviral genome is well studied and can be easily modified, allowing nonpathogenic vectors to be synthesised.[103] Another benefit of these mechanisms is that most of these viruses only cause minor illnesses in immunocompetent human adults in their original form.[104] In order to nullify their replication process, these mechanisms may also be changed, further reducing their ability to infect a host. The use of adenoviral vectors as vaccine delivery mechanisms for the treatment of viral diseases has been motivated by the previously described features.[105] Adenoviral vector vaccines have been used to target a wide variety of some of the most difficult pathogens, including HIV, pneumonia, rabies, botulism, dengue, SARS, and Ebola.[106] They can develop vigorous immune responses, both cellular and humoral. It has been shown that oral immunisation with the most common adenoviral vector vaccine (AdHu5) induces potent $CD8^+$ T cell responses and antibody responses, but does not include $CD4^+$ T cell responses.[107] The multiple isotypes generated by such vectors (e.g. IgG2a, IgG1) suggest the elicitation of a Th1/Th2 response, but it is mostly distorted. In addition, by expressing pathogen-associated molecular patterns (PAMPs) on their surface, adenoviral vectors stimulate innate immune pathways, inducing the secretion of pro-inflammatory cytokines, activation of complements, and differentiation of APCs.[108] During the creation of novel delivery vehicles, one of the essential factors is their potential to elicit strong responses in specific clinical implementation models.[109] These technologies have been used in various animal models for the administration of vaccines, including rats, sheep, non-human primates and, most notably, in human clinical trials.[110] Adenoviral vectors provide an alternative to killed or attenuated vaccines by taking advantage of their immunogenic properties, and their further use and optimization remains a valuable tool for distribution vehicles that imitate pathogenicity.[111]

6. Approaches to enhance oral vaccination

Advance of targeting approaches may lead to more balanced oral vaccine design being developed. To attain passive targeting of preferred cells, the physicochemical characteristics of antigen delivery systems, including height, form, surface charge, and hydrophobicity, can be modified. Active targeting systems for more specifically direct particulate delivery

systems have, however, been examined, thus theoretically reducing the dose needed to evoke an immune response. Using a range of ligands, including bacterially derived moieties, lectins, PAMPs, and antibodies, receptors on intestinal epithelial cells, M cells and APCs have all been explored to target vaccine distribution.

6.1. M cell targeting

M cells are a vital system of particle transfer from the intestinal lumen into the GALT. These advanced transcytotic cells internalise and transfer particulate matter (e.g. bacteria, viruses) to the underlying Peyer's Patches effectively and are thus highly attractive oral vaccine design goals.[112] M cells express special receptors for carbohydrates that provide selective targets for the delivery of mucosal vaccines. Lectins, made up of proteins and glycoproteins that can attached reversibly to particular carbohydrate residues, are among the highly investigated bioadhesives.[113] The alpha-L-fucose occurs typically expressed on M cells in both Ulex europaeus agglutinin-1 (UEA-1) and Aleuria auranitia target. In comparison to untargeted particles, oral immunisation of the surface of particles adorned with any lectin results in substantially greater SIgA.[114] In addition, enhanced cellular immunity was also shown by these particles, shown by large increases in Th1-cytokines IL-2 and IFN-γ. These results show the ability to boost mucosal immune response through lectin-targeted strategies.[115] It is also to be remembered, though, that certain lectins are poisonous and can be potentially immunogenic. In the use of lectins as mucosal adjuvants, the immunostimulatory potential could be useful, but it also presents the risk of eliciting a reaction against the targeting molecule and finally avoiding uptake.[116] For selective transmission, other protein receptors expressed on M cells have been exploited. RGD, for example, is a ubiquitous peptide for cellular binding, but it has also been used to target M cell-mediated transport due to overexpression of the β1 integrin on the apical side of M cells, increasing humoral response with decreased antigen doses.[117] Claudin 4 is a strongly expressed close junction transmembrane protein in M cells which can be targeted to mediate improved SIgA response with surface-conjugated peptides. Additionally, with a novel monoclonal antibody (NKM 16-2-4) that separated M cells from goblet cells for a highly effective vaccine capable of defending against lethal challenge, elucidation of markers unique to M cells could enable the production of antibody-mediated targeting.[118] In conclusion, techniques ranging from bacterial adhesins and toxins to viral proteins have been borrowed from enteric pathogens, which target M cells to achieve host entry. Glycoprotein 2 (GP2) is a M cell receptor that interacts with FimH, an external membrane portion associated with type I piliated bacteria expressed in humans and mice (E. coli, Yersinia, Salmonella).[119] A method to hijack M-cell-mediated bacterial transcytosis and subsequent activation of mucosal immune response may be represented by FimH or other GP2 ligands.[120] The β1 integrin also binds to Yersinia. Invasion protein conjugation, and more recently, recombinant bacterial strains expressing the invasion of Yersinia to target M cells have been investigated.[121] Although M cell targeting strategies in animal models have been shown to be successful, challenges remain, including finding M cell target receptors that will convert from mice to humans, as well as ensuring that immunity is mediated instead of tolerance.[122] Using an in vitro M cell culture model consisting of Caco-2 human colon adenocarcinoma cells and Raji B human cell line, and later with the inclusion of HT29-MTX mucus secreting goblet cells, work is being conducted to better understand M cell biology.[123] This model has the ability to elucidate the antigen transport pathways through M

cells, speed up the discovery of particular receptors for M cells and improve the design of rational oral vaccines.[124]

6.2. Next generation adjuvants

Due to their distinct relationship with lymphoid tissues, M cells are such promising candidates for the design of oral vaccines. M cells, however, constitute b5 per cent of the FAE.[125] In addition, due to a very mutable proportion and phenotype of organisms, assessment of targeting strategies can be made enormously difficult in vivo.[126] Thus, a different approach to M-cell targeting is to target receptors expressed on normal gut epithelial cells. For example, a number of lectins are also expressed by epithelial cells and can be used to rise transepithelial transport.[127] For oral drug delivery, ligands such as wheat germ agglutinin (WGA) that targets N-acetyl-D-glucosamine and sialic acid residues expressed throughout the GI tract by enterocytes have been well considered.[128] Pattern recognition receptors (PRRs) are frequently expressed on different types of cells, including epithelial cells which APCs, and identify molecular patterns associated with microorganisms (MAMPs) to strengthen microorganism phagocytosis.[129] Therefore, by inducing key innate immune signals, PRR ligands possess innate adjuvant properties. TLR agonists comprise the majority of PRR ligands used in oral vaccine production as supplemental adjuvants or targeting moieties, most of which are derived from pathogens.[130] Co-delivery of toll-like receptor agonists has been shown to effectively boost transport through intestinal lumen, particularly TLR-2 and TLR-4. A common factor in bacterial and viral DNA that is recognised by TLR9 and has good immunomodulatory properties is CpG oligodeoxy-nucleotides (ODN).[131] TLR5, which is expressed by epithelial cells, B cells, and dendritic cells among others, as well as a nod-like receptor (NLRC4) to potentially enable two PRR systems to increase immune response, is recognised by flagellin, a large bacteria-associated protein.[132] Effective humoral reaction to a model antigen, as well as maturation of intestinal DCs and activation of helper T cell response in vivo, have been shown by flagellin loaded particles. LPS is another endotoxin derived from bacteria that functions as TLR4 agnostic and can be encapsulated or immobilised on particle surfaces, resulting in preferential DC uptake and high humoral and cellular immunity production. For vaccine formulation, though, the intrinsic toxicity associated with LPS may be troublesome.[133] MPLA is a Salmonella lipid A derivative and an alternative TLR4 agonist that is considered better, but also less effective, than LPS. Likewise, Vibrio Cholerae's cholera toxin (CT) and ETEC's heat-labile enterotoxin (LT) are two of the most promising mucosal adjuvants derived from bacteria, but restricted by the possible problems involved with the use of native toxins.[134] Both consist of toxicity-mediating enzymatically active A subunits and cell-penetrating B subunits. The derivation of toxin mutants that either remove or decrease the toxicity of the A subunits while still having mucosal adjuvant capability has been devoted to considerable work.[135]

7. Future directions

Cautious design of delivery vehicles and addition of molecules that can potentiate their outcome to arouse robust and stable immune responses are desirable to produce actual oral vaccines using subunit antigens. There are some advantages of using the oral route to improve the efficacy of vaccination, as specified in this study, but there are problems, including the safety of these delicate proteins, their release and the adjuvant potential of their carriers. In this work, the features of some of these approaches were briefly stated, but there are still options that should be discussed in order to attain optimum oral vaccine

systems. Although the GI system's physiological and biological structure has been broadly documented, apprehensions remain unanswered about biomaterial interactions with the GALT. A number of tests using other mucosal routes have revealed that there is a focus on long-term protective immunity between prolonged immunogen presentation and development. The GI tract poses a harder challenge due to its design and function, as this can instead persuade tolerance. In order to help in the identification of biomaterial candidates to be used as effective delivery mechanisms, a deeper understanding of the dosage and antigen release kinetics well suited for orally administered vaccines is necessary. It has been revealed that differences in the immunity produced by oral vaccines depend on the diet and health of the patient's GI system. Tropical enteropathy, in particular, may prompt childhood malnutrition, intestinal absorption, and inflammatory disorders that reduces the efficacy of oral immunisation. In these formulas, the existence of such chemicals, such as co-factors (i.e. retinoic acid), will improve the vaccinated individual's answer. A substantial component of oral immunizations will be the presence of these nutrients, particularly for their use in low-income countries. The conclusive purpose of vaccines is to yield defensive immunity. There are a myriad of adjuvant/carrier structures that are presently being investigated for this use, as presented in this study. In addition, biomolecules that may target or improve their effectiveness include next-generation vaccines. Despite this, the only approved drugs for these uses in the US are live and attenuated vaccines. The combination of two or three of these methods will increase their individual abilities in order to provide pathogen-mimicking abilities and provoke comparable responses to microbial infections. Gathering these systems would make it easier to take benefit of their strengths if they function in tandem, while mitigating their weaknesses.

Since its initiation, vaccine technology has been recurrently progressive. Among extra things, progresses in genetic and metabolic engineering have simplified the development of novel molecules that are stable and can yield immune responses. In order to design active delivery vehicles for certain antigens, it is time for the biomaterial sector to catch up with physico-chemical and biological tools. In the production of oral subunit vaccines for mucosal diseases, the design and application of these carriers, which may include immune potentiators, mucus-penetrating techniques, adjuvants and other methods, would definitely assist.

8. **References**

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Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.