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Review

A guide to oral vaccination: Highlighting electrospraying as a promising manufacturing technique toward a successful oral vaccine development

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ABSTRACT

Most vaccines approved by regulatory bodies are administered via intramuscular or subcutaneous injections and have shortcomings, such as the risk of needle-associated blood infections, pain and swelling at the injection site. Orally administered vaccines are of interest, as they elicit both systemic and mucosal immunities, in which mucosal immunity would neutralize the mucosa invading pathogen before the onset of an infection. Hence, oral vaccination can eliminate the injection associated adverse effects and enhance the person's compliance. Conventional approaches to manufacturing oral vaccines, such as coacervation, spray drying, and membrane emulsification, tend to alter the structural proteins in vaccines that result from high temperature, organic and toxic solvents during production. Electrohydrodynamic processes, specifically electrospraying, could solve these challenges, as it also modulates antigen release and has a high loading efficiency. This review will highlight the mucosal immunity and biological basis of the gastrointestinal immune system, different oral vaccine delivery approaches, and the application of electrospraying in vaccines development.

Abbreviations: APCs, Antigen-presenting cells; GIT, Gastro-intestinal tract; DCs, Dendritic cells; GALT, Gut-associated lymphoid tissue; BALT, Bronchus-associated lymphoid tissue; MALT, Mucosa-associated lymphoid tissue; NALT, Nasopharynx-associated lymphoid tissue; PP, Peyer's patches; MLN, Mesenteric lymph nodes; IL, Interleukin; TGF-β, Transforming growth factor-β; FAE, Follicle-associated epithelium; TLRs, Toll-like receptors; Ig, Immunoglobulin; Secretory, ($SIgA_1$ and $SIgA_2$); PLGA, Polylactide-co-glycolide acid; MNPs, Micro/Nanoparticles; HIV, Human immune virus; WHO, World Health Organization.

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Contents

1. Introduction

Vaccines have been well adopted and utilized to prevent infectious diseases. There are three types of vaccines known for human use: live-attenuated, inactivated, and subunit vaccines (Riese et al., 2013). However, the advances in gene therapy technologies have enabled the utilization of nucleic acid, i.e. mRNA, carried into a carrier system, e.g. liposomes, as a new era in vaccinology similar to the recently developed vaccine for COVID-19 (Pardi et al., 2018). The attenuated and inactivated vaccines contain a pathogen, i.e. viral or bacterial, which can replicate within a host or are pathogenically deactivated, respectively, to trigger an immune response. Some examples of the attenuated vaccines include Measles, Mumps, and Rubella (i.e. MMR), chickenpox, rotavirus, seasonal influenza, and polio (i.e. oral vaccine) vaccines, whereas inactivated vaccines examples are inactivated polio and hepatitis A vaccines (Lee and Nguyen 2015). Both vaccine types are considered safe for clinical use and used for several decades. Nevertheless, the need for multiple dosing, poor immunity, undesirable immunogenicity, inflammation, uncontrolled replication, and the possibility of the weakened microbe reverting to a pathogenic form is considered the main drawbacks of both vaccine types (Lee and Nguyen 2015, Vela Ramirez et al., 2017).

Subunit vaccines are derived from non-living antigens, such as a specific antigen protein or epitopes, which can recognize and attach to antibodies or T-cells (Lee and Nguyen 2015). This type of vaccine is more cost-effective, stable and safer, i.e. less immunogenic with low adverse reactions, candidate to the live-attenuated and inactivated vaccines (Reed et al., 2013, Vela Ramirez et al., 2017). Examples are the hepatitis B and the pertussis vaccines (Lee and Nguyen 2015). Subunit vaccines can be furtherly categorized into protein-based, polysaccharides, conjugates and toxoids. The protein-based category uses a specific protein from the antigen to stimulate an immune response. At the same time, the polysaccharide subunit mimics the polysaccharide capsules of the infectious bacteria, which will trigger the immune system. Both subunit vaccines are susceptible to denaturation and degradation by changes in pH or the presence of proteolytic enzymes (Pawar et al., 2014, Vela Ramirez et al., 2017). The conjugate subunits can elicit a response similar to the polysaccharide subunit vaccine and, in many cases, have a carrier protein to elongate the protective immunity, for instance, diphtheria and tetanus vaccines (Vela Ramirez et al., 2017). Toxoid vaccines are inactivated bacterial toxins that are safe and stable against pathogens, such as diphtheria and tetanus vaccines (Vela Ramirez et al., 2017). All these subunit vaccines tend to have weak immunogenicity than the live-attenuated vaccines.

Gene-based vaccine genetically encodes a foreign antigen to be delivered to a host to stimulate the body's immune response, such as T-cell responses, both CD4 and CD8, after being presented to the immune cells (Weiner and Nabel 2018). This type of vaccine includes viral vectors (i.e. viral recombinant system) or nucleic acid (i.e. nonviral RNA- or DNA-based systems) vaccines (Pushparajah et al., 2021). Gene-based vaccines have attracted significant attention owing to their excellent safety and stability profiles, rapid and ease of production, potent protective immunity, high specificity, and cost-effectiveness (Rauch et al., 2018, Liu 2019). Viral vector vaccines involved recombinant viruses, generally attenuated, engineered to express a particular antigen at a high level inside a host body, resulting in immune response (Ura et al., 2014). The advantage of this vaccine relies on the high gene transduction abilities that mimic a pathogenic virus ability; however, the potentiality of the virus to reverse to virulence virus is considered a safety concern (Choi and Chang 2013, Ura et al., 2014, Sebastian and Lambe 2018). The need for high doses, due to replication deficiency, and the diverse storage conditions that depend on the properties of the recombinant virus, which could affect the long-term stability, are the significant limitations of viral vector vaccines (Jones et al., 2007, Crommelin et al., 2021).

On the other hand, nucleic acid vaccines are considered a relatively novel vaccine approach to induce a protective immune response and have been recently used and approved for emergency use against the COVID-19 pandemic through utilizing the mRNA delivery technology (Pushparajah et al., 2021). Since this approach does not involve viral vectors, its safety is considered higher than its viral counterparts (Choi and Chang 2013). The RNA-based vaccines have a higher immunogenic response than the DNA-based ones, particularly in humans (Suschak et al., 2017). This might be due to the lack of a transcription process in the RNA-based vaccine (Schlake et al., 2012). However, delivering a naked nucleic acid, including mRNA, is always challenging, associated with stability (Zhang et al., 2019, Wadhwa et al., 2020). Therefore, the use of suitable carrier systems, such as polymeric- and lipid-based formulations, could overcome such challenge by protecting the nucleic acids from physiological, i.e. enzymatic, degradation similar to the Pfizer COVID-19 vaccine. Storing nucleic acids is essential for maintaining the molecule's chemical integrity. They are susceptible to temperature; however, DNA is known for its robust stability than RNA, and the proper storage conditions should be considered (Pushparajah et al., 2021).

Most vaccines are developed as injection-based formulations and can be administered through intramuscular (IM) or subcutaneous (SC) injections. The advantage of a faster absorption of drugs through the abundance of blood vessels present in the muscles makes it a preferred route of administration. However, IM injections have several limitations, such as needle-associated pain and phobia, muscle atrophy, injuries to nerves, unsafe needle use, improper needle disposal, and trained healthcare personnel's requirement to be available (Rodger and King 2000). The oral route of vaccine administration can be preferable owing to the ease of

Table 1
Commercially available oral vaccines and vaccines under clinical trials for oral administration.

Disease	Vaccine type	Challenge(s)	Manufacturer/Trade name	References
Poliomyelitis	Poliovirus vaccine inactivated	Although very rare, neuro-virulence reversal risk occurs in approximately 1 in every 2.5 million people	Sanofi Pasteur, Paris, France/ IPOL®	(Strebel et al., 1992)
Rotavirus	Live attenuated monovalent human rotavirus Live Pentavalent bovine-human rotavirus	Both are less effective (60 – 75%) against mild infections Rotarix requires fewer doses and more thermos-stability than the Pentavalent vaccine	GSK, Brentford, UK/Rotarix® MSD, Kenilworth, NJ, USA/ RotaTeq®	(van Hoek et al., 2012, Vesikari 2012)
Cholera	Cholera toxin B Inactivated *V. cholera* 01 whole cell Live attenuated *V. cholera* 01 strain (CVD 103-HgR)	Currently only used in adults traveling to cholera endemic areas. Required to be administered only at least ten days before potential exposure to the bacteria	VALNEVA, Lyon, France/ Dukoral® Eurobiologics, Seoul, South Korea/Euvichol® Incepta, Dhaka, Bangladesh/ Cholvax® SANOFI, Paris, France/Shanchol® Orochol, (Crucell)	(Freedman 2016)
Typhoid	Ty21a live attenuated vaccine Ty21a live oral typhoid vaccine	Not 100% effective Vaccine formulation technique, number of administered doses and time interval between doses may affect the protection given by the Ty21a vaccine	PaxVax, Redwood City, CA, USA/ Vivotif®	(Ferreccio et al., 1989, Black et al., 1990, Levine et al., 1999)
Influenza	Monovalent tablet	Phase 2 clinical trial	VXA-A1.1	(Liebowitz et al., 2020)
COVID19	Tablet	Phase 1 clinical trial	VXA-CoV2-1	(Johnson et al., 2021)
Gastroenteritis	live attenuated *Shigella sonnei* vaccine	Phase 1 clinical trial	WRSS1	(Raqib et al., 2019)
	Adenoviral-Vector Based Norovirus Vaccine	Phase 1 clinical trial	VXA-G1.1-NN	(Kim et al., 2018)

administration, non-invasiveness, convenience for self-administration, and increased immune response in vaccine use (Shreya et al., 2019, Homayun et al., 2019, Hua 2020). Other advantages of oral vaccinations may include wide acceptability, requiring little or no technical skills, less pain, and high adaptive temperature for storage. The world health organization (WHO) has approved the commercial oral vaccines for typhoid, cholera, measles, and oral polio vaccines globally (Wershil and Furuta 2008, World Health Organization 2010, Finsterer 2012, World Health Organization 2013, World Health Organization 2014). Table 1 presents the clinically approved oral vaccines and those still under clinical trials. Developing an effective oral vaccine can be challenging due to the significant physicochemical and immunological challenges associated with their development. The drawbacks of the oral delivery of biological therapeutics, in general, include the acidic and proteolytic degradation in the gastrointestinal tract (GIT) environment, low absorption and bioavailability, and stimulation of antigen-specific lymphocytes (Jahan et al., 2019).

Enterocytes in the intestine, particularly the microfold cells (M cells), covering the Peyer's patches (PPs) and the lymphoid segment of the small intestine, make the intestine very absorptive (Levine et al., 1999, Maharjan et al., 2016). Nevertheless, the composition of the GI fluid and the differences in pH along the GIT are two main challenges in the oral delivery of vaccines. Most protein-based molecules, particularly vaccines, are pH-sensitive, possibly denaturing their chemical structures in the acidic pH. The GI fluid comprises water, bile salts, and enzymes, such as pepsin, trypsin, chymotrypsin and peptidase, which increase the risk of enzymatic degradation of orally administered biological products (Mudie et al., 2010, Pawar et al., 2014). Pepsin, the primary enzyme in the stomach responsible for protein digestion and is primarily active in the acidic pH, can cleave the amide bonds in protein molecules down to polypeptides. Other enzymes in the small intestine, like trypsin, chymotrypsin, and peptidase, could facilitate the absorption of digested proteins by further breaking them down to tri- and dipeptides and single amino acids.

Previous studies on vaccines mode of delivery, advanced adjuvants, controlled release of antigens, oral and mucosal delivery, and formulation are significant for safe administration. Lycke (Lycke 2012) and Lemoine *et al.* (Lemoine et al., 2020) have discussed potential technologies to improve oral vaccines development. Polymerization and the addition of adjuvant could reduce the adverse events associated with the immune response and enhance the shelf-life of oral vaccines for rapid worldwide coverage. However, finding a means of manufacturing oral vaccines that can eliminate all the abovementioned challenges and other challenges related to the production of vaccines, such as high temperature and the use of toxic organic solvents, would hold great potential in developing vaccines soon. Electrohydrodynamic process, i.e. electrospraying, was previously proven to be effective in developing biological-based products, owing to its ability to eliminate the use of temperature and toxic solvents and to use biodegradable, biocompatible and mechanical strong polymers that can circumvent the direct exposure of such therapeutics to the harsh GI environment.

In this review, an overview of oral delivery of vaccines, means of their manufacture, mucosal immunity, and the biological basis of the GI immune system, focusing on utilizing electrospraying technology in vaccines development.

2. Mucosal immunology and intestinal mucosal immune system

Mucosal immunity is the primary defense system against pathogens entering through the mucosa, such as the influenza virus. At the initial mucosal sites, pathogens are transported into the bloodstream to colonize in internal tissues until the failure of the body's immune system, particularly among children, the elderly, and the immunocompromised population (Bloom et al.,

2017, Smith et al., 2019, Guo et al., 2020). A healthy adaptive mucosal immune system can activate antibodies, produce chemical mediators, phagocytose and inhibit the replication of harmful pathogens. Otczyk and Cripps have reported cellular responses from $CD8^+$, restricted cytotoxic T-lymphocytes, and $CD4^+$ T helper (Th) lymphocytes to prevent mucosa infections (Otczyk and Cripps 2010). Secretory immunoglobulin-A ($SIgA_1$ and $SIgA_2$) fights pathogens associated with nasal and bronchial mucosa, while serum-derived IgG contributes to the lower respiratory tract and genitourinary mucosal immunity (Brandtzaeg et al., 2008, Pabst et al., 2008). The mucosa-associated lymphoid tissue (MALT) is a region-specific lymphoid node tissue with B-cell follicles, plasma cells, and various antigen-presenting cells (APCs) such as macrophages, B-lymphocytes and dendritic cells (DCs). MALT possesses intrinsic lymphoid tissues, such as bronchus-associated lymphoid tissue (BALT), nasopharynx-associated lymphoid tissue (NALT), and concerning the intestines specifically, gut-associated lymphoid tissue (GALT) (Kiyono and Fukuyama 2004, Brandtzaeg et al., 2008).

The intestinal immune system is the largest and most complicated immune system in the body (Allez et al., 2002, Mowat 2003, Vossenkämper et al., 2013). The intestinal immune system encounters more antigen exposure than any other part of the body due to being burdened with a prominent resident and heterogeneous microbiota population, in addition to constant external antigen threats. It also has to distinguish between non-harmful and pathogenic antigens constantly; hence, the GALT must provide a quick and efficient immune response (Mowat 2003). However, an autoimmune attack on resident microbial flora and direct activation of the GALT can lead to chronic gut inflammation such as Crohn's disease, ulcerative colitis and other immune bowel diseases (Koboziev et al., 2010).

The GALT is a component of the mucosal immune system which consist of aggregated (organized) lymphoid tissue, including PPs and solitary lymphoid follicles, and non-aggregated (more diffusely scattered) cells in the lamina propria, intestinal epithelial cells (IECs), intraepithelial lymphocytes (IELs), and mesenteric lymph nodes (MLNs) (Ruth and Field 2013). PPs are lymphatic tissues found in the submucosal layer of the small intestine and made of numerous B cell-rich follicles surrounded by an interfollicular T-cell region (Wershil and Furuta 2008). The formation of PPs is dependent on the interleukin-7 (IL-7) and tumor necrosis factor (TNF) receptor family members (Fu and Chaplin 1999). PPs are covered with epithelial, called follicle-associated epithelium (FAE). The FAE consists of M cells, essential short microvilli with a thin mucous layer that allows trans-epithelial transport of antigens from the intestinal lumen into the sub-epithelial lymphoid tissues. This enables a sampling phenotype, whereby suspected antigens from intestinal contents are presented to the resident immune cells, such as B-cells, macrophages, dendritic cells and T-cells (Newberry and Lorenz 2005). Fig. 1 demonstrates the main component of the intestinal mucosal immune system.

Furthermore, PPs possess DCs, in which their significant role involves an immediate uptake of antigens from M cells for presentation to the mucosal T- and B-cells to initiate antigen-specific immune responses (Kunkel et al., 2003, Niess and Reinecker 2006, Kunisawa et al., 2012). DCs in conjunction with T-cells and cytokines, such as transforming growth factor-β (TGFβ) and IL-10, are involved in cellular signalling, which triggers IgA B-cell development at PPs (Matsumoto et al., 1989, Jang et al., 2004, Niess and Reinecker 2006, Tezuka et al., 2007). The plasma cells and the activated $CD4^+$ T-helper cells migrate back into the systemic circulation through the thoracic duct to reach the intestinal tissue, specifically the lamina propria, along with the cytotoxic $CD8^+$ T-cells in the epithelial layer, to provide an efficient host immunity

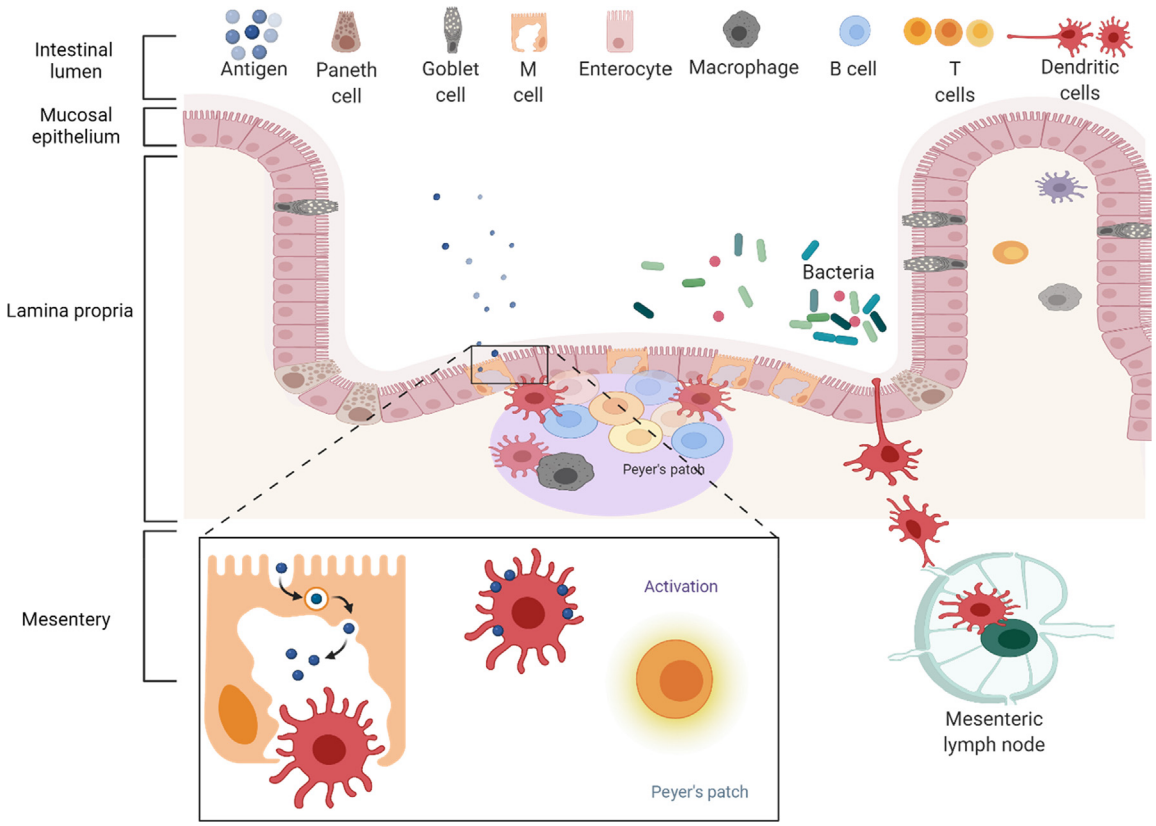

Fig. 1. The intestinal mucosal immune system illustrates its main components. Created with Biorender.com.

(Frizzell 2019), (Kang et al., 2018). Meanwhile, DCs, in conjunction with other antigen-presenting cells, travel from the PPs or the epithelium to the MLNs, where they activate naive T-cells (Frizzell 2019). The MLNs are the most prominent lymph nodes in the human body, and they play a significant role in modulating peripheral and mucosal recirculation pathways (Mowat 2003).

The lamina propria consists mainly of the IgA secreting B-cells and effector T-cells and an abundance of mast cells, all of which are heavily involved in the mucosal immune response post-antigen presentation (McGhee et al., 1992, Koon and Pothoulakis 2006, Ruth and Field 2013). Other components of the non-aggregated lymphoid tissue include the IELs that are a heterogeneous population of mucosal lymphocytes consisting predominantly of effector memory cells, such as γδ T-cell receptor (TCR) $CD8^+$ T-cells and two distinct subsets of αβ TCR $CD4^+$ or $CD8^+$ T-cells residing in the intestinal epithelium (Cheroutre 2004). IELs play a vital role in maintaining intestinal homeostasis and immune response and in cancer surveillance (Cheroutre 2004, Wershil and Furuta 2008).

The IECs are coated with glycoproteins that entrap microbes in the mucus, where they lie at the border between the intestinal lumen and the MALT (Dahan et al., 2007). The essential role of IECs is considered to be the activation of immune responses with the help of a plethora of specialized cell types. One particular example is Paneth cells found deep within the crypts and responsible for producing antimicrobial peptides, including defensins, lysozymes and phospholipase A_2 (Mowat and Agace 2014). IECs can identify and present antigens with the help of pattern recognition molecules, such as Toll-like receptors (TRLs) and Nucleotide-binding oligomerization domain (NODs) (Shao et al., 2005, Dahan et al., 2007). These cells can produce cytokines and chemokines, thus, initiating a cascade of immune response pathways, for example, T-cell activation and the recruitment of leukocytes (Allez et al., 2002; Dahan et al., 2007).

Understanding mucosal immunology and the mechanism of stimulating immunological responses can be the key to developing a successful oral vaccine. Nonetheless, finding a suitable oral delivery approach to overcome the physiological and immunological challenges of oral vaccination remains a concern.

3. The importance of mucosal pathogens in intestinal mucosal immune system

Mucosal pathogens cause diseases at the mucosal membranes lining the intestines, respiratory, or urogenital tracts. Mucosal pathogens may distribute to distant systemic sites through the bloodstream; nonetheless, some microorganisms are limited to the development of disease only at the site of mucosal invasion (Sekirov et al., 2010, Pavot et al., 2012). Mucous membranes lay on the surface of internal organs and consist of epithelial cells, primarily of endodermal origin and connect to the skin through various body openings, including the ears, eyes, inside the mouth, inside the inside mouse, and vagina. Some mucosa secretes a thick protective fluid, i.e. mucus, to avoid pathogens from invading the body and help hydrate the bodily tissues.

Pathogens, like bacteria and viruses, which live in the mucus membrane of the nose and back of the mouth, can cause several severe conditions such as pneumonia, meningitis and sepsis, leading to many deaths worldwide due to the deterioration of an individual's immune system. The primary entry point for various human pathogens occurs at the GIT, for instance, poliovirus, *Escherichia coli, Salmonella Shigella*, *Vibrio cholera*, and *Helicobacter pylori*, the respiratory tract, for example, influenza virus, adenovirus, coronavirus and *Mycobacterium tuberculosis*, or genital tract, such as Human papillomavirus, *Chlamydia trachomatis* and *Neisseria gonorrhea* (Czerkinsky and Holmgren 2010). Pathogens of the GI tract have evolved to infect and spread to the niche of human and animal GI mucus and mucosa. This niche is highly complex, with unique properties that rely heavily on local carbohydrate biology (Kim and Ho 2010, Johansson and Denning, 2012, Johansson et al., 2013).

The human GI mucosa is made up of epithelial cells that are linked together by tight junctions and are scattered with immune cells. This mucosal membrane is shielded by a complex layer of mucus, which forms protective barriers and generates advantaged surroundings that help defend against microorganisms and harmful substances (Bergstrom et al., 2010; Cornick et al., 2015). The GI mucosal layer acts as a physical scaffold enriched with antimicrobial peptides and secretory IgA (Johansson et al., 2011, Dupont et al., 2014, Martens et al., 2018). The gastrointestinal mucus is a dynamic matrix composed of several layers of mucin glycoproteins produced by specialized goblet cells in the intestinal epithelium (Birchenough et al., 2015). Mucus properties and the density and diversity of colonization of resident bacteria are different over the intestinal tract (Ermund et al., 2013). The colon, which has the thickest mucus layer, has the highest concentration of resident bacteria, i.e. microbiota (Atuma et al., 2001).

The mucus in the small intestine is loose and discontinuous, whereas the mucus in the stomach and large intestine is divided into two distinct layers (Atuma et al., 2001, Hansson and Johansson 2010, Johansson et al., 2011, Bansil and Turner 2018). The inner layer, which is close to the epithelium, is more viscoelastic and physiologically sterile, whereas the outer layer towards the GI lumen is less viscoelastic and easily suctioned away (Atuma et al., 2001, Hansson and Johansson 2010). Typically, the outer layer contains many resident microorganisms, whereas the inner layer is relatively impervious to bacteria (Johansson et al., 2014, Li et al., 2015). When mucosal homeostasis or dysbiosis is disrupted, the mucus becomes more permeable, allowing many bacteria to invade the inner layer (Johansson et al., 2014). This organization is thought to be essential for normal GI barrier homeostasis because it can separate most microorganisms in the GI lumen from the mucosal epithelia and immune system cells (Johansson et al., 2011, Faderl et al., 2015, Taherali et al., 2018).

Enterocytes can produce transmembrane mucins with various structures, which help create the glycocalyx on their apical side, a valuable resource for pathogen attachment, interaction, and interspecies communication (Pelaseyed et al., 2014). The ability of mucosal GI pathogens to pass through the mucus crossing the thick mucus layer is one of the significant characteristics that distinguish them from most commensals. The human GIT, particularly the colon, is considered the home for a diverse and complex microbiota containing approximately 1000 bacterial species (Corfield 2018). Fungi and phages/viruses are also thought to be residents of the intestinal microbiota (Hooper et al., 2002; Johansson and Hansson, 2016). Local pathogens compete for space in the mucosa with these resident microorganisms.

The innate immune system of the intestinal mucosa can sense infections, transmit signals to the immune system, inhibit invaded microbes, and renew injured or aged epithelial cells. The mucosal pathogens have versatile mechanisms that could modulate the host inflammatory and immune responses to manipulate host cell death and survival signal pathways, which would allow such pathogens to adapt to the intestinal mucosal environment, thus, facilitating infections. Nevertheless, vaccination can be used as part of a comprehensive control and prevention program, as a complete immune response is a complementary tool for controlling mucosal pathogens.

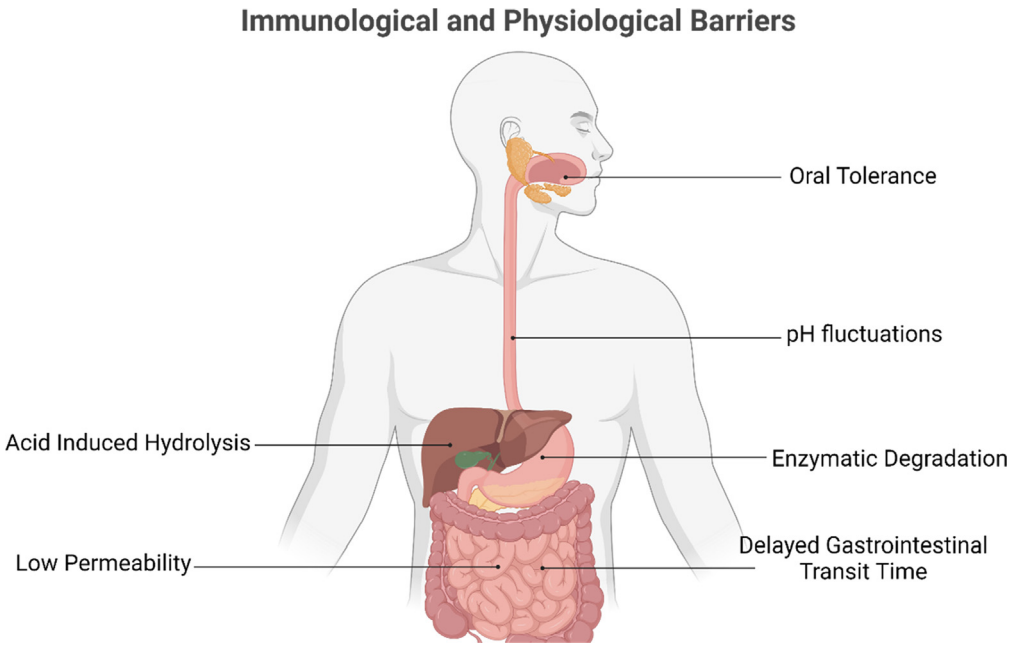

Fig. 2. Immunological and physiological challenges associated with the oral administration of vaccines. Created with Biorender.com.

4. Oral vaccines delivery criteria

The oral delivery of therapeutics, in general, has high patient compliance, yet, it suffers from low systemic bioavailability for several medicines through GIT degradation (Brown et al., 2020). In addition, other pharmacokinetics and pharmacological drawbacks, such as the inability to cross biological membranes, short half-lives, low stability, immunogenicity and the clearance susceptibility by immune cells, the liver or kidneys, have hindered the delivery of many therapeutics, in particular biopharmaceuticals, i.e. proteins, peptides and vaccines (Sharma 2007, Patel et al., 2014). These limitations have forced patients to receive higher or more frequent doses, which reduced patient compliance and enhanced the possibility of side effects (Schiffter 2011). Consequently, there is a clear need for developing such products into delivery systems that could protect them from the abovementioned challenges, preserving their bioactivity and controlling their release. Hence, an overall reduction in the dose and the frequency of administration will be achieved.

Various digestive enzymes in the GI environment are crucial for absorbing nutrients; nonetheless, they possess a major difficulty in oral vaccines administration. The main enzyme responsible for protein degradation is pepsin, followed by subsequent involvements of other peptidases that break down proteins into amino acids to facilitate their absorption in the small intestine (Frizzell 2019). The pH alteration in the GI is another factor that needs to be considered. Owing to the conformational changes in the antigenic epitopes of vaccines at specific pH levels, their oral administration would be difficult, as their stability will deteriorate. GI-transit time is another variable that depends on changes in the individual's ingestion behavior. GI-transit time can range from 2 h in the fasting state to 16 h under the fed-state. The longer the vaccine remains in the GIT, the more enzyme-induced proteolysis can occur, particularly under the acidic pH of the stomach (Kang et al., 2018). The mucus layer of the goblet cells is a significant physical barrier to the successful delivery of drugs in general. Hence, targeting drug molecules to M cells may facilitate the translocation of such molecules from the intestinal lumen to the subepithelial layer, as M cells serve as the leading portal of entry (Kang et al., 2018). Fig. 2 summarize the challenges associated with oral vaccine administration.

5. Conventional methods in developing oral vaccines

Conventional approaches to manufacture oral vaccines tend to alter the structure of proteins during the production stages due to the high temperature and the use of harsh solvents. Complex coacervation is an associative liquid-liquid phase separation caused by electrostatic and entropic interactions between oppositely charged macro-ion and is often applied in the pharmaceutical and food industries (Timilsena et al., 2019, Mi et al., 2020). During this process, the coacervated polymer is deposited around the active compound, causing the settling of the internal core. Complex coacervation has been widely used for the micro/nanoencapsulation of flavors and thermally sensitive compounds to improve the product stability against light, humidity and temperature (Timilsena et al., 2019). Using a complex of polypeptides, such as poly-lysine and poly-glutamate, to thermos-stabilized porcine parvovirus through complex coacervation strategy has improved the stability of this non-enveloped virus for two weeks at 60 °C. However, applying complex coacervation to enveloped viruses, such as the bovine viral diarrhea virus, against high temperature did not have any significant stabilizing effect compared to the non-enveloped virus at a similar temperature (Mi et al., 2020). The pH sensitivity and ionic strength are significant barriers of complex coacervation technology, as the coacervation occurs within a very narrow pH window (Augustin et al., 2006, Timilsena et al., 2019). The presence of trace amounts of salt can also cause complex coacervates to dissociate (Timilsena et al., 2019).

Spray drying is a continuous process used to produce a dehydrated powder from a liquid feed, resulting in a bulk product ready to be packaged in variable quantities (Fleming 1921). This process consists of the following steps: liquid feed atomization, droplet drying, powder collection, and subsequent processing. The mixing of the liquid feed and the pressurized nebulizing fluid will be forced to leave a nozzle, where the quick decompression of the liquid will be converted into a spray of droplets (McAdams et al.,

2012). The fluid droplets could be furtherly adjusted to specific characters by manipulating the liquid feed properties, such as solvent choice, surface tension or feed concentration, the size of spray nozzle, and the nature of nebulizing fluid (liquid, suspension, or emulsion). After spraying, the droplets of the fluid are then dispersed into a heated chamber filled with dry inert gas for their drying (McAdams et al., 2012). In the case of vaccines development, the drying gas temperature should be controlled to avoid deteriorating the proteins by the high-temperature exposure. Additionally, many variables of the formulation, for example, buffer and salts, should also be considered to prevent changes in the pH and ionic strength during the drying process and reconstitution (McAdams et al., 2012). Currently, no marketed vaccine is fabricated by spray drying (Kanojia et al., 2017). However, various studies have been conducted for evaluation, such as a live attenuated MMR vaccine formulated using trehalose, sucrose, glycerol and L-arginine as protecting agents, followed by spray drying. This technique has extended the stability of the vaccine for almost two months at 37 °C (Ohtake et al., 2010).

Freeze-drying, or lyophilization, is another technique used to stabilize vaccines, which is used for approximately 25% of the vaccines approved by the FDA (Preston and Randolph 2021). The advantages of using freeze-drying are minimizing the damage to vaccines via the application of very low temperature during the processing and the ease of reconstituting the dry product. Nevertheless, the stress applied to the vaccine's components such as proteins, destabilization, nucleic acid degradation through internal ice formation can affect the vaccine stability (Hansen et al., 2015). The vacuum foam drying technique is an alternative to lyophilization that could be used for biological products and vaccines. A liquid solution will convert to a dry foam-like structure under moderated temperature and vacuum. This method has a major advantage of overcoming the potential stress on the vaccine components by avoiding the use of extremely high or low temperatures and pressure (Lovalenti et al., 2016). However, vacuum foam drying is still immature, and more investigation is required to evaluate the development of vaccines using this technique.

Emulsions are often used in manufacturing pharmaceuticals, cosmetics, and food. Membrane emulsification is one of several methods that are used for making emulsions. The concept of this method is to force a dispersed phase to permeate through a uniformed pore-size membrane, using a low-pressure pump, into a continuous phase that flows along the membrane surface (Joscelyne and Trägårdh 2000). The system consists of a tubular microfiltration membrane, a pump, a feed vessel, and a pressurized container used to disperse the oil phase (dispersed phase). Before initiating the process, it is crucial to control several parameters and conditions, such as membrane's pore size and porosity, membrane type, continuous phase velocity, dispersed phase flux, transmembrane pressure, and emulsifier concentration and type (Joscelyne and Trägårdh 2000). Polysorbate 80 is an example of an emulsifying agent that could be used as a vaccine component to protect the protein from any mechanical damage (Agarkhed et al., 2013). Polysorbate 80 has been used in various vaccines, such as the quadrivalent influenza vaccine (Krantz et al., 2021) and the AstraZeneca COVID-19 vaccine (Sellaturay et al., 2022).

All the abovementioned techniques are known for their ability to manufacture vaccines; however, avoiding high temperature, harsh solvents, buffer, and salts are currently attracting great attention in biological product development.

6. Promising methods in developing oral vaccines

An ideal oral delivery system of vaccines should protect the loaded active compound, either by complexation or encapsulation within the carrier matrix and control the payload release through a sustained release fashion. This delivery approach should also have a high therapeutic load and cellular uptake profile. Different drug delivery strategies have been devised to maximize the efficacy of oral vaccines, namely liposomal, polymeric and adenoviral, and oil-based formulation.

Lipid-based nanoparticles are a desirable delivery approach due to their amphipathicity and high biocompatibility. Since liposomes mimic the natural structure of the cell membranes, phospholipid bilayers with internal aqueous cavities have been used widely as a biocompatible drug delivery system for many decades now (Bulbake et al., 2017, He et al., 2019). Previous studies have investigated cationic liposomes as effective oral vaccine carriers owing to their ability to encapsulate water-soluble molecules, such as proteins and nucleic acids, and protect these payloads from being degraded in the GI environment (Schwendener 2014, Vela Ramirez et al., 2017). Wang *et al.* reported an oral liposomal DNA vaccine via the encapsulation of Mycobacterium antigen Ag85A. The results demonstrated an immune antigen-specific response after three oral doses in mice with tuberculosis (Wang et al., 2010). Another study by Liu *et al.* has reported an oral DNA vaccine using cationic liposomes encoding the M1 gene of influenza A virus, and the findings showed stimulated immune responses and protection against influenza virus after three oral doses, at weekly intervals, in a mouse model (Liu et al., 2014).

Coupling liposomes with additional polymers/ligands can solve the stability problems of liposomes. A study by Ma *et al.* has successfully conjugated liposomes with polylactide-co-glycolide acid (PLGA) to protect the antigenic epitopes from exposure to the GI, yet, this system suffered from a modest GALT transportation rate and low immunological response compared to the IM formulation (Ma et al., 2014). Another study by Wang *et al.* increased the specificity of liposomes by attaching mannose moieties to bind to mannose receptors presented on the surface of antigen-presenting cells such as macrophages and dendritic cells. This approach has led to a more robust immune response with elevated levels of serum IgG and mucosal IgA antibodies compared to IM administration (Wang et al., 2014).

Bilosomes are non-ionic lipid-based nanoparticles that contain bile salts, i.e. sodium deoxycholate, and have been used for oral vaccination due to their rapid uptake by M cells. Several previous studies have reported efficient mucosal immune response after oral administration of bilosomes encapsulating proteins. A study by Shukla *et al.* demonstrated triggered mucosal and systemic immune responses after orally administrating recombinant hepatitis B surface antigen-loaded bilosomes by the enhanced uptake of this delivery system in gut-associated lymphoid tissues (Shukla et al., 2008). The oral vaccination of ferrets with recombinant hemagglutinin-loaded bilosomes was investigated by Wilkhu *et al.* The results showed this vaccine's ability to reduce fever, lung inflammation, and the overall viral load in an influenza model (Wilkhu et al., 2013). Immune-stimulating complexes (ISCOMs), considered second-generation liposomes, are cage-like structures of glycosides presented in cholesterol, phospholipids and antigens that can be used as carriers and immunostimulants for vaccine delivery (Fleck et al., 2019). The use of ISCOMs is due to their high immunogenicity and balanced stimulations of humoral and cellular immune responses (Vela Ramirez et al., 2017). Mohamedi *et al.* have reported an oral immunization and efficient protection of ISCOMs loaded with Herpes simplex virus type 2 (HSV-2) antigens against a heterologous lethal dose of HSV-2 in mice (Mohamedi et al., 2001).

Polymeric nanoparticulate systems use natural or synthetic polymers, such as chitosan or PLGA, respectively, as candidates to implement oral vaccine delivery owing to their biocompatibility, biodegradability and stability. However, the susceptibility to

enzyme-induced degradation and poor absorption in the GIT can hinder the oral application of such polymers (Kang et al., 2018). The disintegration kinetics of polymeric particles also need to be considered, as these particles should be resistant enough to prevent premature unravelling before reaching the PPs. Some polymers' mucoadhesiveness and immunostimulant properties, particularly chitosan, might be an additional advantage owing to their adhesive tendencies to mucosal layers and strong activation of antigen-presenting cells and lymphocytes, respectively (Kriegel et al., 2013, New 2019). This mucoadhesion is due to the electrostatic interaction between the positively charged chitosan and the negatively charged mucous layer (Deacon et al., 2000). Chitosan has been used as a polymer of choice in numerous nanodrug and gene delivery studies. It is naturally occurring, biocompatibility, biodegradability, and its degradation products are nontoxic, non-immunogenic non-carcinogenic, making this polymer a strong candidate for oral vaccination delivery (Jazayeri et al., 2021).

Metallic nanoparticles, mainly gold and silver nanoparticles, have been previously investigated as oral vaccine systems owing to their inertness, rigidity, low toxicity, high immunogenicity, and simplicity to synthesize with low costs (Carabineiro 2017). A previous study by *Barhate et al.* has reported that chitosan functionalized gold nanoparticles carrying tetanus toxoid and an immunostimulant agent Quillaja Saponaria extract can induce immune responses by 28-fold compared to the controls in mice (Barhate et al., 2013). Another study by Jazayeri *et al.* has shown a high antibody production and cellular immune response of silver nanoparticles H5 DNA vaccine against avian Influenza virus H5 in young chicks after a single oral dose (Jazayeri et al., 2012).

The use of adenoviruses, the double-stranded DNA viruses with about 40 kb genome, has become a qualitative leap in the world of vaccines development. Actually, some of the most virulent viral diseases, such as Ebola, dengue, rabies and influenza, have been targeted directly by adenoviral vaccine carriers due to their superior ability to generate strong cellular and humoral immune responses (Vela Ramirez et al., 2017). Nevertheless, adenoviruses are susceptible to degradation by the gastric acidic environment; hence, it is recommended to encapsulate the recombinant adenoviruses into enteric-coated tablets (Kang et al., 2018). Potent immune responses were demonstrated by Deal *et al*., who used live adenovirus serotypes 4 and 7 to trigger an immune response that showed more than 95% effectiveness in protecting against acute respiratory diseases (Deal et al., 2013). However, the use of specific adenovirus serotypes could potentially induce host cytotoxicity and an over-inflammatory response due to pre-existing systemic immunity. The clinical efficacy of adenoviral vaccines is limited, as the T-memory cells and the attachment of neutralizing antibodies to the adenoviruses would swiftly clear when re-exposed to the same antigens adenoviruses before they undergo transgene expression (Zaiss et al., 2009). This could explain the reduction in the strength of the immune response in individuals with pre-existing immunity when a low dose of the virus is administered. Still, to overcome this, different vectors for the prime and booster vaccines should be used. A previous study has proven this method successfully on rodent models, where significant T- and B-cell responses against HIV antigens were induced by primarily using an attenuated version of *Listeria Monocytogenes*, i.e. a bacterial vector then subsequently using Adenovirus serotype 4 as the booster vaccine. Safety remains a concern, as live-attenuated vectors might transfer into a pathogenic form once ingested in the GIT via a mutation or a transfer of genes from the surrounding microbes (New 2019).

M cells have a propensity for translocating hydrophobic substances hence the potential of using oil droplets may be a suitable delivery system, given its hydrophobic nature. However, most of the former oil-based formulations tend to hold the antigen at the outside rather than within the oil compartment itself, making the antigen susceptible to enzyme-induced proteolysis in the GI environment. Another concern of this delivery approach is the incompatibility between the oil's hydrophobic nature and the antigens' hydrophilicity. However, double-emulsions could solve this issue, yet, stability remains a burden (New 2019). Incorporating an additional component, such as polymeric nanoparticles, can help improve this formulation's stability and inhibit the premature release of the encapsulated drug/antigen (Liau et al., 2015).

Microfluidic technology has emerged as a novel approach for gene delivery to overcome limitations associated with gene-based vaccines manufacturing, such as reproducibility (i.e. size-to-size distribution). Liposomal and other polymers, such as chitosan, can be formulated using a microenvironment system under controllable hydrodynamic flow through a nano-scale channel (Kastner et al., 2015). The assembled MNPs by microfluidics can be further optimized to achieve a uniform size in a repeatable manner which is vital to maximizing the delivery efficiency (Carugo et al., 2016). Promising results on delivering nucleic acid materials, such as pDNA, siRNA and mRNA, were reported that unlocked a new technology of fabricating vaccines, particularly gene-based vaccines (Walsh et al., 2014, Zukancic et al., 2020, Loo et al., 2021). Despite the advantages of microfluidic technology for vaccine development, including cost-effectiveness, low energy and volume use, increased sensitivity, and reduced human error, no approved vaccine has been manufactured using this technology (Shirzadfar and Khanahmadi 2018). One of the main challenges of microfluidic is the industrial scale-up production (Lemoine et al., 2020). The next generation of microfluidic devices should incorporate parallel nano mixing channels in a single microfluidic device for rapid and efficient production (Shepherd et al., 2021).

All delivery systems mentioned above hold high promises in oral vaccine administration; nonetheless, they suffer from low stability, low immunological response induction, early antigen release, scale-up difficulties or safety concerns that hinder their development. Therefore, the search for a novel approach for manufacturing oral vaccines is still on.

7. Electrospraying in vaccine development, clinical translation and scale-up

As previously mentioned, there is vast attention toward the clinical application of vaccines and other biological products using micro/nanotechnology to overcome the drawbacks associated with their formulation and administration (Cao et al., 2019). Micro/Nanoparticles (MNPs) come in different sizes and shapes. They can be synthesized using numerous materials such as metals, lipids, sugars, viruses, inorganic materials, and natural- or synthetic-occurring polymers; hence, they will have various physicochemical and biological properties (Cho et al., 2008). There are different methods to fabricate MNPs; however, electrospraying, in particular, has been utilized enormously in the past decade to overcome the drawbacks associated with conventional forms of their preparation, such as low drug loading capacity, particle aggregation, scale-up difficulty, batch-to-batch variation and high cost of production (Chakraborty et al., 2009).

Electrospraying technique involves the atomization of a liquid solution or suspension to form droplets through the application of an electrical force (voltage), which will permit the dispersion of the liquid that flows out of a capillary nozzle into fine droplets (i.e., spraying) (Wu and Clark 2008). The remaining charges on the particles and the evaporation of the solvent during the process can avoid the aggregation of the produced particles (Jaworek and Sobczyk 2008). Owing to the use of low concentrations of liquid

solutions or suspensions, electrospraying can produce particles that are uniform with particle sizes that may reach down to less than 10 nm (Chakraborty et al., 2009).

The principles of the electrospraying technique involve the flow of a polymer liquid out of a needle connecting to a syringe at a constant rate through a pump, and a high electrostatic force is attached and applied to that needle. This electric force will overcome the liquid's surface tension, changing the liquid droplet's sphere-like shape into a cone-like form known as Taylor cone (Doshi and Reneker 1995, Sill and von Recum 2008, Williams et al., 2018). The liquid will then be ejected from the tip of that cone to form a jet in a collector ground, through which it will be interrupted to yield highly fine charged particles that repel each other, creating a spray. In addition, the evaporation of the solvent will take place during the liquid ejection process, which will control the particles' size and shape along with the liquid concentration (Doshi and Reneker 1995, Sill and von Recum 2008, Williams et al., 2018).

Among all materials that can synthesize MNPs, natural and synthetic polymeric MNPs are considered the ideal delivery systems, also called vectors, due to their biodegradability, biocompatibility, high stability, and mucoadhesiveness benefit their clinical application. The choice of a suitable polymer for preparing electrosprayed MNPs may influence the encapsulated therapeutic agent's size, shape, and drug release rate (Zamani et al., 2013). The reason behind selecting a particular polymer specification (molecular weight and concentration) along with a specific solvent that dissolves it is to avoid electrospraying process complications, such as the solidification of liquid droplets on the tip of the needle (Nguyen et al., 2016).

The applied voltage, flow rate and tip-to-collector distance are vital process parameters to initiate the formation of electrospray jet via overcoming the surface tension of a polymer solution, and thus, synthesizing stable and uniform MNPs (Doshi and Reneker 1995, Sill and von Recum 2008, Williams et al., 2018). Additionally, both the voltage and flow rate can directly relate to the size of the yielded particles, in which reducing their levels will generate smaller particles. In contrast, the effect of the distance on the size is inversely related. The impact of these processing parameters affects the polymer solution flight time. An adequate flight time is necessary to allow the polymer molecular chain entanglement, hence, overcoming the surface tension of the polymer solution (Chakraborty et al., 2009). Polymer solution parameters, such as viscosity, conductivity, and surface tension, are essential for the quality of the resulting particles, as well as the electrospraying process (Doshi and Reneker 1995, Sill and von Recum 2008, Williams et al., 2018). Decreasing the viscosity of the solution by reducing the polymer concentration or molecular weight can lead to smaller particle size while reducing the conductivity and surface tension will produce a larger size particle (Bhattarai et al., 2018). Increasing the solution viscosity will start to produce elongated particles (or beads), then beaded fibers, and eventually fibers, in which the process will be named 'electrospinning' (Pillay et al., 2013). The solvent type could also affect the quality of the particles, as high solvent volatility will accelerate the solvent evaporation during the process, thus, producing a smaller particle size (Doshi and Reneker 1995, Sill and von Recum 2008, Williams et al., 2018). Temperature and humidity are two essential environmental factors that could control the reproducibility of the resulting particles. Increasing the temperature may reduce the polymer solution viscosity and surface tension, resulting in smaller particles, while at higher temperatures, i.e. above 40 °C, could form larger particles due to increasing the solvent evaporation rate (Chakraborty et al., 2009). Humidity above 60% can enhance water absorption, leading to wet particles. All these parameters are related to each other and should be considered to produce high quality and reproducible MNPs.

Another benefit of using electrospraying for MNPs fabrication is its improved scale-up capacity compared to other techniques (Parhizkar et al., 2017). This is due to its continuous processing, low shear force, and rapid solvent evaporation rates that make it a suitable technology for nucleic acid- and protein-based therapeutics delivery, which avoids the denaturation of such materials (Steipel et al., 2019). Only solvents listed as Class 3 in the FDA classification for residual solvents in therapeutics, such as acetone and ethanol, are two preferable solvents to be used when electrospraying, other than water (Genito et al., 2021). The aptitude of using a multi-axial needle (co–, tri and quad-axial needle that produce two, three and four layered-particles, respectively) can also be another advantage, as by applying an additional layer, it may either control the release of the drug that is located at the core or add one more therapeutic agent for a dual-action (Davoodi et al., 2015, Williams et al., 2018).

Therefore, electrospraying can be used to encapsulate and release broad-spectrum therapeutic agents, including proteins and nucleic acids, which offers an elegant and scalable technique to develop vaccines. Several previous attempts utilized electrospraying for delivering vaccines, which are shown in Table 2. The choice of the polymer, along with the application and study outcome, were also highlighted.

These previous studies have demonstrated the efficient delivery of electrosprayed MNPs protein- and peptide-based vaccines that resulted in robust cellular and humoral protective responses. Among these studies, an oral vaccine formulation developed using ovalbumin loaded alginate coated chitosan MPs showed an elevated level of IgG and IgA, suggesting a promising oral vaccination system (Suksamran et al., 2013). As the application of electrospraying in vaccine development is growing, only a few attempts have focused on developing oral vaccines, which could hold many advantages such as vaccine self-administration, enhanced vaccine accessibility and rapid epidemic containment (Moreira et al., 2021). Therefore, more attention on developing oral vaccines using electrospraying technology should be clinically translated soon.

8. Promises, challenges and future remarks of oral vaccination

Infectious diseases have been a major cause of human mortality over the years, requiring a constant development of vaccines and antimicrobial agents as two promising therapeutic approaches. To circumvent the spreading of viral infections, the urge of using vaccines has shown to be effective and economical (Wang and Coppel 2008, Rashmi and Madhavi 2021). However, many commercially available vaccines are administered parenterally, either IM or SC, which possesses specific challenges, including patient non-compliance and safety concerns (Wang and Coppel 2008). In contrast, oral delivery of vaccines offers a more convenient, pain-free and self-administering alternative option (Marasini et al., 2014, Kang et al., 2021). Additionally, it is cheaper to administer oral vaccines, as they do not require hospital/clinical admission or specialized trained health personnel. The production of oral vaccines will involve less stringent regulatory requirements due to the overall reduction of their purification processes than the parenteral vaccines (Wang and Coppel 2008).

The vaccine distribution process is one of the significant burdens that can affect the impact of vaccine programs, particularly in developing countries, where oral vaccines can improve such distribution more than injectable ones. This is mainly because training and mobilization of professional health workers account for about 25% of vaccine's cost, whereas in oral vaccination, the potential of self-administration could save such cost (Vela Ramirez et al.,

Table 2
Different examples of vaccine delivery using electrospraying technology. The bolded study represents an oral vaccine delivery system.

Vaccine	Polymer(s)	Application	Outcome(s)	Ref.
Ovalbumin MPs	**Calcium-alginate and calcium-yam-alginate coated with methylated *N*-(4-*N*,*N*-dimethylaminocinnamyl) chitosan**	**Immune response elevation in mice following oral vaccination**	**250 µg ovalbumin in the coated MPs showed the highest in vivo adjuvant activity in both IgG and IgA immunogenicity**	**(Suksamran et al., 2013)**
Recombinant EIT NPs	chitosan and trimethylated chitosan	Immunotherapy of Enterohemorrhagic Escherichia coli O157:H7 infected mice following intranasal administration or intraperitoneal injection	Only the nasal route of administration for both chitosan and trimethylated chitosan formulations produced significant secretion of IgA	(Doavi et al., 2016)
Cytomegalovirus peptides pp65 and IE-1 NPs	Polyvinyl alcohol (PVA)-coated Poly(lactide-coglycolide) (PLGA)	A peptide-based vaccine *in vitro* delivery	An increase in the proliferation and frequency of antigen-specific CD8(+) T cells	(Furtmann et al., 2017)
Resiquimod MPs	Acetalated dextran	Immune response elevation in mice following intravascular vaccination	Resiquimod MPs elevated the immune response in RAW macrophages and have reduced the Leishmania significantly in infected mice	(Duong et al., 2013)
Recombinant protective antigens (rPA) and resiquimod MPs	Acetalated dextran	Immunotherapy of Bacillus Anthracis infected mice following subcutaneous vaccination	Enhanced IgG antibody level and 50% survival of mice exposed to a lethal dose of Bacillus Anthracis by inhalation compared to zero survival for the FDA approved BioThrax vaccine	(Gallovic et al., 2016)
Cyclic dinucleotide 3′3′-cGAMP MPs	Acetalated dextran or PLGA	Immunotherapy against influenza-infected mouse following intramuscular injection	Both formulations had increased the IgG antibody level, interferon γ, IL-2, IL-6 and Th1-associated responses, with the acetalated dextran showed superior results	(Junkins et al., 2018)
3′3′-cyclic GMP-AMP (cGAMP) and resiquimod MPs	Acetalated dextran or PLGA	A potent cellular and humoral vaccine delivery	Both formulations had enhanced the antigen-specific cellular response and balanced the Th1/Th2 humoral response, with the acetalated dextran showed superior results	(Collier et al., 2018)
Murabutide or ovalbumin MPs	Acetalated dextran	An adjuvant or antigen vaccine delivery on endotoxin injected mice following intramuscular injection	Enhanced humoral and cellular responses for the Murabutide delivery, while the delivery of ovalbumin resulted in high antibody and cytokine production	(Chen et al., 2018)

2017). In addition to the improved accessibility and coverage, oral vaccination also has the advantage of stimulating strong mucosal immunity, which provides a primary barrier against infections initiating at the mucosal surface, as well as, produce systemic humoral and cellular immune responses (Wang et al., 2015, Vela Ramirez et al., 2017).

Despite the benefits oral vaccination might offer, there have been several challenges associated with this route of administration. Previous reports have shown that vaccines composed of proteins, DNA, and polysaccharides are usually more labile to degradation, resulting in loss of bioactivity (Sharma et al., 2015). The antigens in the vaccines can be diluted in the mucosal secretions, trapped in the thick mucosa, degraded by GI enzymes and acidic pH of the stomach, or stopped from being absorbed by the epithelial barriers (Neutra and Kozlowski 2006). These would lead to the use of relatively large doses of vaccines to ensure adequate quantities cross the mucosa and stimulate immune responses (Neutra and Kozlowski 2006). This, in turn, would increase the possibility of inducing tolerance instead of promoting a protective response (Hellfritzsch and Scherließ 2019). Furthermore, most available oral vaccines consist of live attenuated organisms, which can replicate in the mucosa, eliciting a sustained immune response. This has limited the use of oral vaccines for pathogens that cannot attenuate the production of such vaccines (Wang and Coppel 2008).

In this review, the barriers for oral vaccine development, including GIT limited absorption, bioavailability, and the harsh environment, were discussed, in addition to the humeral and cellular immunities involved in the intestinal mucosa. Several methods of developing oral vaccines were highlighted. In particular, as an advanced method of vaccines development, it holds a great potential to replace conventional approaches, such as coacervation, spray drying and membrane emulsification, owing to the lack of high temperature and toxic solvents use. Despite the more advance research exploring the application of electrospraying in protein, peptide, gene, and drug delivery, there is only a very limited studies in the field of oral vaccination. Therefore, more studies on the use of electrospraying in the development of oral vaccines are required in order to demonstrate their safety and efficacy profiles in clinical trials.

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