BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e21769

Article

Oral and nasal vaccination: current prospects, challenges, and impact of nanotechnology-based delivery systems

Natália Floriano Paiva, Fabiana Testa Moura de Carvalho Vicentini

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil

Currently, mucosal vaccine administration has stood out as an easier and non-invasive application method. It can also be used to induce local and systemic immune responses. In the COVID-19 pandemic context, nasal and oral vaccines have been developed based on different technological platforms. This review addressed relevant aspects of mucosal vaccine administration, with emphasis on oral and nasal vaccinations, in addition to the importance of using nanotechnology-based delivery systems to enable these strategies.

Keywords: Mucosal vaccination. Oral vaccine. Nasal vaccine. Nanocarriers. Nano-vaccinology.

INTRODUCTION

Vaccines have contributed hugely to the maintenance of global health (Zhang, Billingsley, Mitchell, 2018a). Large-scale immunization is crucial to reducing the risk of infection in individuals who are unvaccinated or cannot be vaccinated. It acts by interrupting disease transmission, whether direct and restricted to the human population such as measles and rubella, or indirectly such as yellow fever (Fernandes *et al*., 2021). Advances in vaccinology have provided significant benefits to public health, eradicating diseases, preventing epidemics, and reducing deaths from infectious diseases. In this context, advances in biotechnology have enabled the development of increasingly safe and effective vaccines (Braz *et al*., 2014). The main objective of a vaccine is to present a specific antigen or set of antigens to the host's immune system, as a way to fight against a pathogen (Negahdaripour *et al*., 2017) or a developed pathology, such as cancer, for example.

According to the literature, vaccines are classified according to methods used in antigen preparation into three groups or generations. First-generation vaccines are traditional or classic vaccines that use intact (entire) pathogens but are subjected to treatments for their inactivation or attenuation. They include for example those vaccines against pertussis, smallpox, polio, rubella, measles, and adenovirus (Diniz, Ferreira, 2010).

Live-attenuated vaccines are those composed of pathogens unable to infect or replicate in humans, but still can evoke the immune response (Rockwell, 2017). Attenuated suspensions of pathogenic microorganisms are obtained by serial passage method or chemical mutagenesis, both techniques produce mutant strains with reduced virulence and toxicity (Jiskoot, Kersten, Mastrobattista, 2013). Among the advantages, these vaccines have high immunogenicity and can stimulate Toll-Like Receptors (TLR) to the same extent as in live viral pathogen infection. They can also offer longer-lasting protection against viruses, for example (Han *et al*., 2021). However, despite being an efficient strategy, the following disadvantages should be highlighted: integration of the pathogen's nucleic acid sequence into the host's genome, somewhat unlikely reversal to a more virulent strain or pathogen reactivation, and potentially serious complications in children and immunodeficient individuals. Thus, their use becomes restricted, not being an efficient and safe method for the prevention and/or

***Correspondence:** F. T. M. de C. Vicentini. Faculdade de Ciências Farmacêuticas de Ribeirão Preto. Universidade de São Paulo. Av. Café S/N, Monte Alegre, Ribeirão Preto, São Paulo, Brasil. 14040-903, Ribeirão Preto, SP. Phone/Fax: 00 55 16 33154257 / 33154232. E-mail: fabtesta@fcfrp.usp.br. ORCID: https://orcid.org/0000-0002-0842-9130

treatment of several diseases such as viral hepatitis, acquired immunodeficiency syndrome (AIDS), and cancer (Jiskoot, Kersten, Mastrobattista, 2013).

Another important approach is vaccine production from inactivated pathogens through heat, radiation, or chemical products (e.g., formalin and formaldehyde) (Rockwell, 2017). These processes reduce the ability of microorganisms to multiply, consequently causing disease, without losing their immunogenic potential. The inactivation technique promotes changes in the envelope permeability and protein conformation of pathogens, besides damaging their genetic material (Fernandes *et al.*, 2021). Inactivated vaccines display a complete repertoire of immunogenic components of intact pathogens and, compared to live-attenuated vaccines, they pose no risk of pathogen reactivation (Li *et al*., 2020). As inactivated vaccines are non-live vaccines, they cannot infect the host and cause disease for not containing any living or infectious particles; therefore, they have a good safety profile even in immunocompromised individuals (Vetter *et al.*, 2018). However, like the attenuated ones, they also have disadvantages, such as little or no activation of cell-mediated immunity; and more adverse effects compared to the attenuated and second- and third-generation vaccines, which will be discussed below (Jiskoot, Kersten, Mastrobattista, 2013). During the inactivation process, structural deformation of pathogen immunogenic epitopes may also occur, leading to a decline in immune protection (Li *et al*., 2020). Therefore, these vaccines are considered less immunogenic, not being able to provide long-term protection, thus requiring adjuvant addition and/ or multiple doses (Han *et al*., 2021).

Second-generation vaccines are based on the fact that, for some pathogens, protection can be given by inducing antibodies directed at specific targets, such as the toxin responsible for disease symptoms or sugar present on the surface, allowing the host's immune system to neutralize and eliminate pathogen (Diniz, Ferreira, 2010). Thus, this class includes subunit vaccines, which use pathogen-specific fragments and components, such as proteins, carbohydrates, and capsids, to stimulate the activation of a strong immune response (Han *et al*., 2021). These purified antigens may be extracted from natural or synthetic sources, or even derived from recombinant DNA technology (Braz *et al*., 2014).

In this sense, subunit vaccines include only pathogen fragments as antigens, e.g., proteins, polysaccharides, or viral parts capable of producing virus-like particles (VLPs). The former can be produced by purified antigenic proteins from the entire pathogen, or by recombinant genetic engineering. This technique includes inserting a gene that encodes an antigenic protein into an expression system capable of producing large amounts of the antigen of interest in cell cultures. First-generation polysaccharide vaccines are based on capsular polysaccharides purified from whole pathogens; however, these vaccines have poor immunogenicity, short-term protection, and reduced immune response after some administrations. Accordingly, conjugate vaccines have emerged, resulting from polysaccharides chemically bonding to carrier proteins to induce a strong immune response (Vetter *et al*., 2018). Ultimately, virus-like particle vaccines are based on ordered viral self-assembly from protein subunits that are noted in many different virus families. Such vaccines may present structures that are highly antigenic to the immune system but are safer due to the absence of the viral genome, which can revert to virulence (Afrough, Dowall, Hewson, 2019).

As for its positive aspects, the subunit vaccine stands out for its absence of risks associated with handling live pathogens in the laboratory (Han *et al*., 2021). Besides being safer and easier to produce, other relevant advantages can be highlighted, such as the use of a greater variety of pathogenic microorganisms, increased stability, and fewer allergic and autoimmune responses (Negahdaripour *et al*., 2017). However, when it comes to disadvantages, these vaccines contain fewer antigens than those previously described, in addition to their purification processes often eliminating innate immunity-triggering components, which may be less immunogenic (Vetter *et al*., 2018). Therefore, this problem can be minimized by the use of adjuvants and fusion with immunostimulant molecules (Li *et al*., 2020). Vaccines against tetanus, diphtheria, and hepatitis B are examples of subunit vaccines, while vaccines designed to control meningococcal meningitis and pneumonia can

be mentioned as an example of polysaccharide subunit vaccines (Diniz, Ferreira, 2010).

Finally, third-generation and newer vaccines are characterized by making use of the pathogen genome. They are nucleic acid vaccines obtained by genetic engineering methods, which encode relevant antigens to induce host immunity (Diniz, Ferreira, 2010). Immunization with nucleic acid vaccines involves the administration of genetic materials such as plasmid DNA or messenger RNA (mRNA), which encode antigens of interest (Jiskoot, Kersten, Mastrobattista, 2013). In short, a specific nucleic acid is introduced into the host cells to initiate pathogen-protein synthesis; then, the host immune system recognizes it as a foreign agent and triggers an immune response against it, as in a live pathogen infection (Han *et al*., 2021). In general, nucleic acids allow the encoding of a wide variety of antigens and can induce long-term humoral and cellular immunity (Jiskoot, Kersten, Mastrobattista, 2013). Thus, different vaccines can be developed without the need for new production, purification, and validation methods, leading to fast, flexible, and cost-effective development and production since the characteristics of these vaccines are independent of the encoded proteins (Fernandes *et al*., 2021).

Plasmid DNA is produced by replicating bacterial cells, such as *E. coli*, which is further purified using established methods. Its administration route is intramuscular due to its low turnover rate, preventing DNA from being rapidly dispersed to cells in the division process. After intracellular DNA uptake, the encoded protein is expressed on the surface of host cells (Jiskoot, Kersten, Mastrobattista, 2013). Compared to other vaccine technologies, DNA vaccines offer a platform for rapid and flexible development and production, making it an attractive strategy for combating emerging epidemics, such as the COVID-19 pandemic. Furthermore, these vaccines produce antigens in target cells, promoting the return of native conformation and post-translational modification of pathogen antigens. However, DNA vaccines pose some challenges that must be considered. One is the need for adjuvants and delivery systems due to their limited immunogenicity, as they cannot be propagated and amplified *in vivo*. Another factor is potential mutagenesis or oncogenesis due to their integration with the host chromosome, requiring integration studies on DNA vaccine safety (Li *et al*., 2020). Its relatively low immunogenicity is probably due to a low transfection rate, as a non-condensed plasmid has a highly distended and negatively charged structure, making it difficult to enter the cell. Even if cell entry does occur, entry into the nucleus, which is required to achieve transcription, is extremely inefficient. Therefore, to increase DNA vaccine immunogenicity, plasmids may be produced to encode antigens, as well as encode together immunostimulatory molecules to induce a strong immune response (Wallis, Shenton, Carlisle, 2019).

Among the third-generation vaccines, RNA vaccines consist of linear RNA molecules transcribed *in vitro* and then purified before administration. These vaccines have genetic information with appropriate translational elements, which provide efficient production of the encoded antigen (Sasso *et al*., 2020). They are currently a promising alternative to conventional vaccines, as they have high potency, rapid development, and low production costs. Besides that, they present a greater safety profile, high efficacy, and fast production. First, mRNA is not infectious and its platform is unable to integrate into the human genome, with no risk of infection or insertional mutagenesis. Second, its high efficiency is due to potential structural changes that make mRNA more stable and highly translatable using delivery systems, allowing a rapid uptake and expression within the cytoplasm. Lastly, they display a high yield of *in vitro* transcription reactions, resulting in fast, low-cost, and scalable production. There are two main types of RNA used in vaccines: non-replicating (conventional) and virus-derived self-replicating. Conventional RNA vaccines encode the antigen of interest and contain untranslated regions (UTRs), while self-amplifying RNA ones encode not only the antigen of interest but also the machinery needed for viral replication, allowing intracellular RNA amplification and abundant protein expression (Pardi *et al*., 2018).

In this context, viral vector vaccines are a versatile platform for delivering the genetic code through viruses to produce antigens in the cells of vaccinated individuals. These vaccines can make use of attenuated

and non-replicating viral vectors to deliver antigens in the form of genetic information directly to cells, improving its generation, targeting, and processing. This way, the antigen will undergo cellular synthesis and processing similar to those of a natural infection. Furthermore, viral vector vaccines can be administered without additional adjuvants and accept variations as a function of the vector used. They can induce a robust specific cellular and humoral immune response against the target antigen, with a better safety profile and high-yield production processes, which are essential in high-demand situations such as the covid-19 pandemic. However, some negative points should be highlighted, such as the fact that viral vectors are genetically modified organisms that may show potential health risks when exposed to the environment. Some safety issues are of concern such as a potential integration into the host genome or very high or persistent replication of vaccines. Finally, each viral vector employed requires different cellular systems for production, which makes the process complex and more expensive (Fernandes *et al*., 2021).

Vaccines under development, or in use, in clinical trials against COVID-19 may fall within the different technological platforms described above (Table I). Additionally, according to World Health Organization (WHO) guidelines, most of the ongoing projects for vaccination against COVID-19 have parenteral administration through intramuscular injection, intending to induce the production of high-titer neutralizing systemic antibodies that can control the infection. However, one of the most negative points of such a vaccination strategy is the induction of a mucosal immune response, whose efficacy and durability are low, allowing the virus to enter the body through the oral-respiratory tract (Ashraf *et al*., 2021).

TABLE I - Vaccines under development or in use against COVID-19 fall within the different technological platforms (adapted from https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines - last update: 03/11/2022)

Vaccine platform	Type of vaccine	Number of doses	Route of administration	Phase	Developers
Live attenuated virus	MV-014-212 A live attenuated vaccine that expresses the spike protein of SARS-CoV-2	1	Intranasal	Phase 1	Meissa Vaccines, Inc.
Live attenuated virus	COVI-VAC	1-2	Intranasal	Phase 3	Codagenix and Serum Institute of India
Inactivated virus	CoronaVac Inactivated SARS-CoV-2 vaccine	2	Intramuscular	Phase 4	Sinovac Research and Development Co., Ltd
Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	2	Intramuscular	Phase 4	Sinopharm, China National Biotec Group Co and Wuhan Institute of Biological Products
Protein subunit	CoV2-OGEN1, protein based vaccine	1-2	Oral	Phase 1	USSF/Vaxform
Protein subunit	RBD + AgnHB	3	Intranasal	Phase 1/2	Center for Genetic Engineering and Biotechnology (CIGB)

TABLE I - Vaccines under development or in use against COVID-19 fall within the different technological platforms (adapted from https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines - last update: 03/11/2022)

Vaccine platform	Type of vaccine	Number of doses	Route of administration	Phase	Developers
Protein subunit	Full length recombinant SARS-CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	2	Intramuscular	Phase 3	Novavax
Protein subunit	MVC-COV1901 (Spike-2P protein + adjuvant CpG 1018) MVC-COV1901 (Beta)	2	Intramuscular	Phase 4	Medigen Vaccine Biologics, Dynavax and National Institute of Allergy and Infectious Diseases (NIAID)
Virus like particle	Receptor Binding Domain (RBD) SARS-CoV-2 HBsAg VLP vaccine	2	Intramuscular	Phase 1/2	Serum Institute of India, Accelagen Pty and SpyBiotech
Virus like particle	Coronavirus-like particle COVID-19 (CoVLP)	2	Intramuscular	Phase 3	Medicago Inc.
RNA based vaccine	Comirnaty BNT162b2 (3 LNP – mRNAs)	2	Intramuscular	Phase 4	Pfizer/BioNTech and Fosun Pharma
RNA based vaccine	mRNA-1273 Spikevax	2	Intramuscular	Phase 4	Moderna and National Institute of Allergy and Infectious Diseases (NIAID)
DNA based vaccine	BacTLR-Spike oral DNA vaccine	1	Oral	Phase 1	Symvivo Corporation
DNA based vaccine	AG0301-COVID19	2	Intramuscular	Phase 2/3	AnGes, Takara Bio and Osaka University
DNA based vaccine	INO-4800 + eletroporation	2	Intradermal	Phase 3	Inovio Pharmaceuticals, International Vaccine Institute and Advaccine (Suzhou)
Viral vector (replicating)	rVSV-SARS-CoV-2-S vaccine	1	Intramuscular	Phase 2/3	Israel Institute for Biological Research
Viral vector (replicating)	Intranasal flu-based-RBD	2	Intranasal	Phase 3	University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy

TABLE I - Vaccines under development or in use against COVID-19 fall within the different technological platforms (adapted from https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines - last update: 03/11/2022)

Vaccine platform	Type of vaccine	Number of doses	Route of administration	Phase	Developers
Viral vector (non-replicating)	PIV5 vector that encodes the SARS-CoV-2 spike protein	1	Intranasal	Phase 1	CyanVac LLC
Viral vector (non-replicating)	VXA-CoV2-1 Ad5 adjuvanted oral vaccine platform	2	Oral	Phase 2	Vaxart
Viral vector (non-replicating)	BBV154, Adenoviral vector COVID-19 vaccine	1	Intranasal	Phase 3	Bharat Biotech International Limited
Viral vector (non-replicating)	Covishield ChAdOx1-S (AZD1222)	1-2	Intramuscular	Phase 4	AstraZeneca and University of Oxford
Viral vector (non-replicating)	Ad26.COV2.S	1-2	Intramuscular	Phase 4	Janssen Pharmaceutical Johnson & Johnson
Viral vector (non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector)	1	Intramuscular	Phase 4	CanSino Biological Inc./Beijing Institute of Biotechnology

In this way, mucosal vaccines stand out in the production of local and systemic immune responses, having been shown to trigger a systemic response analogous to that of parenteral administration. Therefore, it is extremely important to evaluate mucosal vaccination strategies that can effectively trigger systemic and mucosal immunity (Shahiwala, Vyas, Amiji, 2007). Thus, this review will address aspects relevant to mucosa administration of vaccines, with emphasis on oral and nasal strategies, in addition to the importance of nanotechnology-based administration systems to make them feasible.

MUCOSAL VACCINATION

Parenteral administration of vaccines by needle and syringe has low acceptability by children and infants, in addition to a high risk of infection due to the inappropriate reuse of these materials worldwide (Kalam, Khan, Alshamsan, 2017). Within this framework, in addition to the current occurrence of pandemics and bioterrorism, studies have been dedicated to developing systems that allow vaccine administration in a non-invasive way (without needles), as they are safer methods (Zheng *et al.*, 2018).

Parenteral vaccination has been linked to several successful cases of individual protection against major pathologies. However, in addition to its safety and low-acceptability issues, another limitation of this strategy, which is extremely relevant, might be its failure to induce immunity when the pathogen enters the site, that is, mucosal surfaces (Corthésy, Bioley; 2018). Therefore, mucosal delivery of vaccines stands out as a practical, non-invasive, and effective alternative to induce local and systemic responses (De Magistris, 2006). When compared to parenteral vaccines, mucosal administration can promote more effective protective immune responses due to the induction of secretory immunoglobulin A (IgA), cell-mediated immunity in mucosal tissues, and immunity at mucosal sites where pathogens enter the body (Rhee, 2020).

Mucosal vaccines can stimulate all immune system components, protecting against pathogens infecting the host through both mucosal surfaces and other routes. In more detail, mucosal vaccination stimulates the production of IgA antibodies against specific antigens at infection sites, as well as systemic IgG antibodies. Induction of secretory IgA production is essential because, in the intestinal region, for example, they have greater stability due to their secretory component, in addition to helping to prevent the development of pathogenic colonies in intestinal tissues through pathogen agglutination, entrapment, and elimination (Van der Weken, Cox, Devriendt, 2020). In addition, mucosal vaccination can also induce immune responses at more distant sites due to the expression of specific receptors by lymphocytes, besides activating cellular responses mediated by CD4+ T helper lymphocytes and CD8+ T cytotoxic lymphocytes, which are of great importance in combating intracellular pathogens (De Magistris, 2006).

Thus, mucosal immunization stands out in its importance due to several reasons, including: (1) greater adherence of individuals and greater capacity for mass immunization; (2) the potential of self-administration as it does not need specialized professionals, decreasing vaccination costs significantly; (3) simpler methods of production and storage as it does not need to follow strict sterilization protocols and have a high purity level; (4) no interference with cultural or religious issues; (5) induction of mucosal and systemic humoral immunity by antigen-specific IgA and IgG antibody responses; and (6) elimination of potential disease asymptomatic carriers, thus interrupting transmission of infections among individuals (Skwarczynski, Toth, 2020).

However, despite its many advantages, the strategy still has challenges concerning inefficient immunogenicity, uptake, and presentation of antigens; and the occurrence of enzymatic degradation and immune tolerance (Srivastava *et al*., 2015). Overall, effective strategies for mucosal immunization involve three important characteristics: (a) overcoming physiological barriers of mucosa; (b) efficient specific targeting of antigen-presenting cells (APCs) in the mucosa for proper processing of antigens that activate specific T and B cells, and (c) control of antigen and adjuvant delivery kinetics to promote a long-term protective adaptive immune memory (Woodrow, Bennett, Lo, 2012).

Vaccine mucosal administration can be done via oral, nasal, pulmonary, rectal, vaginal, ocular, sublingual, or transcutaneous routes (Thakur, Foged, 2020). However, current studies have focused in particular on oral and nasal vaccinations, which can induce stronger mucosal immune responses (Kang, Cho, Yoo, 2009).

Oral delivery

Oral vaccines have several advantages over conventional parenteral vaccines: large-scale low-cost production, ease of administration, and induction of immunity in the intestinal mucosa blocking disease transmission and increasing herd immunity (Parker *et al*., 2018). Oral vaccination is associated with increased adherence, lower occurrence of adverse reactions, and potential for self-administration, which make them attractive for mass vaccination campaigns (Rhee, 2020).

Oral administration is the most used route since the intestinal epithelium has a high absorption capacity due to its extensive surface area (around 300 to 400 m^2). In addition, another important characteristic is the presence of lymphoid tissue, also known as gut-associated lymphoid tissue (GALT). The tissue contains inductive sites where immune responses begin, in addition to effector sites where adaptive immune responses are made (Davitt, Lavelle, 2015). Studies have indicated that antibody-secreting cells in the intestinal mucosa can persist for decades, then plasma cells provide long-lasting mucosal immunity. In some reports, oral immunization could more easily activate cytotoxic T lymphocytes and long-acting mucosal memory immunoglobulins A when compared to systemic immunization (New, 2019).

The development of oral vaccine should include an understanding of the physical-chemical aspects of the barriers that constitute the host's first defense against pathogens (Davitt, Lavelle, 2015). In general, gastrointestinal mucosa has a protective mucous membrane and a layer of epithelial cells, which

provide the host's protection against the unregulated entry of pathogens and harmful substances. A good mucosal coating is a physical barrier that limits molecular diffusion and penetration of microorganisms into the epithelium. Despite variable structures and functions according to its location (oral, gastric, and intestinal), the main function of the epithelial layer is to prevent the absorption of undesirable pathogens and macromolecules. In this context, additional forms of protection can be cited, such as secretion of antimicrobial compounds within the mucous membrane, low pH of the stomach region, presence of bile salts and digestive enzymes in the intestinal portion, and intestinal motility leading to gastric emptying (Coffey, Gaitha, Traverso; 2021). Thus, vaccine oral delivery has faced several challenges since vaccines need formulations able to maintain their stability in the gastrointestinal environment and avoid tolerance induction, resulting in the need for higher doses of antigens to perform effective protection (Vela Ramirez, Sharpe, Peppas, 2017). Although intestinal microbiota interferes with oral vaccination efficacy, this aspect is not very well established (Van der Weken, Cox, Devriendt, 2020).

The development of suitable oral vaccines must meet important criteria such as sufficient protection against antigens in the gastrointestinal environment, use of high loads of antigens or other vehicles with encapsulation capacity, prolonged exposure of antigens to antigen-presenting cells, ability to reach intestinal cells, long-term systemic and mucosal memory, and high safety. To overcome these challenges, one important strategy has been the study and application of delivery system technologies for oral vaccination (Kang *et al*., 2018). In addition to protecting antigens against the gastrointestinal environment, different delivery systems have also been developed for targeted delivery to immunological induction sites that allow their absorption by antigen-presenting cells, enhancing immune response robustness (Van der Weken, Cox, Devriendt, 2020).

Currently, given the COVID-19 pandemic, two oral vaccines are in progress to protect against the coronavirus. One of them is produced by the American company Vaxart and consists of a recombinant oral vaccine in a pill form. This vaccine is composed of an enteric coating with an adenoviral vector capable of coding genes for the production of S and N nucleocapsid proteins of SARS-CoV-2. The enteric coating is essential for protecting vaccine formulation from the acidic environment of the stomach, as the tablet dissolves in the digestive tract, and hence provides induction of protective mucosal immunity against viral infection. The other proposal, called OraPro-COVID-19TM, includes the use of a non-replicating viral vector that expresses the S protein in an encapsulated form, providing thermal stability for the formulation. Like the previous one, this proposal also has an enteric coating and, when orally administered, directly reaches the intestinal lymphoid tissues. As a result, there is an induction of cellular (CD4+ and CD8+ T-cell mediated) and humoral (antibody-mediated) immune responses (Ashraf *et al*., 2021).

Intranasal delivery

As mentioned for the oral route, intranasal administration for local and systemic delivery of therapeutic compounds is non-invasive and painless, requires no sterilization, and can be self-administered. Besides these advantages, the nasal pathway has a large mucosal surface area due to the presence of numerous microvilli, porous endothelial membranes, and highly vascularized epithelium (Kang, Cho, Yoo, 2009).

In terms of anatomy, the nasal cavity has a surface area of 150 cm^2 and a volume of 15 to 20 mL, which is divided into five regions according to anatomical and functional characteristics: nasal vestibule, atrium, breathing zone, olfactory region, and nasopharynx. Drugs or vaccine antigens can be delivered in the respiratory region due to their high permeability, large surface area, and rich vascularization. The region is composed of a pseudostratified epithelium, in which columnar and goblet-shaped cells are connected by tight junctions, preventing paracellular passage of particulate materials in inhaled air (Rhee, 2020). Thus, the epithelial cell layer represents a barrier to penetration of pathogens, with the help of mucus production and the presence of ciliary structures, as well as its ability to detect and capture these organisms by endocytosis

or recognition of patterns such as Toll-Like Receptors (TLRs) (Yusuf, Kett, 2017).

Another important characteristic of the nasal cavity is nasopharyngeal-associated lymphoid tissue (NALT) (Rhee, 2020). The nasal mucosa immune system is composed of lymphoid tissue, B cells, T cells, and APCs, which are covered by an epithelial layer containing memory (M) cells specialized in transporting antigens through the epithelium. The action of epithelial cells, together with lymphocytes and underlying antigen-presenting cells, induces an innate immune response to the invasion of foreign agents into the body (Yusuf, Kett, 2017). The associated lymphoid tissue, composed of immunocompetent cells and present in this nasal epithelium, is an ideal target for intranasal administration of vaccines capable of inducing an appropriate immune response (Islam *et al*., 2012). In addition to inducing a local immune response, it also induces an immune response in mucous tissues distant from the nasal cavity, such as the vagina. This is because mucosal lymphocytes are functionally connected, which can be advantageous for developing vaccines against sexually transmitted pathogens such as human immunodeficiency virus - HIV (Li *et al*., 2016).

One of the advantages of intranasal vaccination is the induction of protection against pathogens reaching the respiratory tract, with responses generated in lymphoid tissue associated with the nasal cavity providing long-term protection. Several diseases, such as influenza, respiratory infections, measles, and meningitis, are developed by the entry of microorganisms via the respiratory mucosa. In this context, a vaccination that allows defense through this route is highly desirable, as it prevents infection of both the individual and the environment. In addition, reduced activity of degradation enzymes, better patient compliance, and cost reduction should also be considered, as it is a simpler method of application (Pan *et al*., 2014; Köping-Höggård, Sánchez, Alonso, 2005).

In the context of the COVID-19 pandemic, studies conducted by Du Y and coworkers demonstrated that intranasal administration of a subunit vaccine containing a recombinant SARS-CoV-2 receptor binding domain generated an excellent humoral immunity induction profile and immunity on the mucosal surfaces of the nasal cavity, lung, genital tract, and intestine. They also reported that mucosal B cells secrete IgA into the nasal cavity and lung, which provides the first line of defense against viruses that enter the respiratory tract, thus preventing these microorganisms from invading the host's cells (Du *et al*., 2021).

However, the development of nasal vaccines has some challenges related to inefficient absorption of soluble antigens by the region's immune cells, rapid mucociliary clearance, short antigen residence time, permeation of restricted molecular size through epithelial barriers, absence of effectively safe adjuvants for human use, low stability of formulation against nasal enzymes, low pH, and reduced delivery volume due to low nasal cavity capacity (from 100 to 150 μL). Faced with these hindrances, the use of advanced delivery systems might help to increase the residence time of administered vaccines in the nasal cavity, formulation stability, and absorption of vaccine components (Marasini, Skwarczynski, Toth, 2017; Riese *et al*., 2014).

Figure 1 shows a schematic representation of immune response induction in nasal and stomach mucosa after nasal or oral vaccine administration, respectively. Table II displays a comparison between the main advantages and disadvantages of oral and nasal administration routes for vaccines.

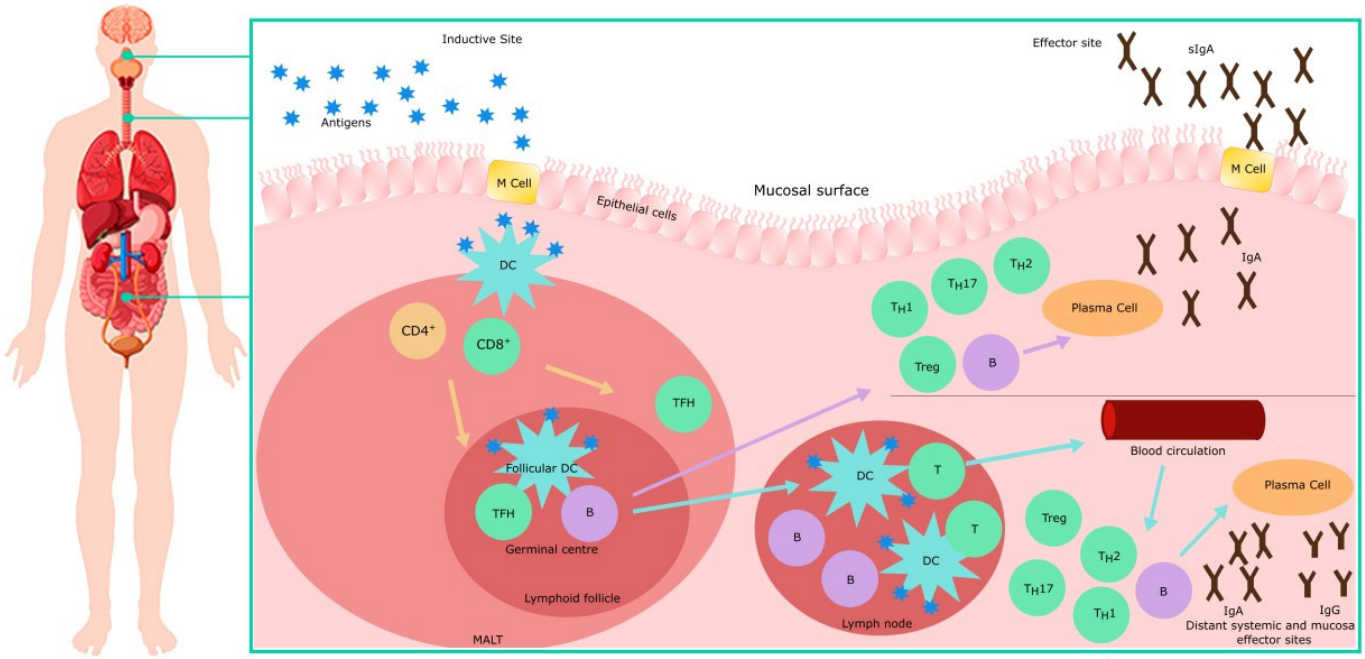

FIGURE 1 - Schematic representation of immune response induction in nasal and stomach mucosa after nasal or oral vaccine administration, respectively. Vaccine antigens found in the nasal cavity or gastrointestinal environment, depending on the administration route, are transported actively through microfold or epithelial cells to reach the lymphoid follicles. Then, on the inductive site, with the main one being mucosal-associated lymphoid tissues (MALT), there are organized lymphoid tissues where the antigen is taken up by dendritic cells (DCs) and other antigen-presenting cells (APCs). These antigens taken up by dendritic cells are presented to T cells, which, with the help of follicular dendritic cells (FDCs) and T follicular helper (TFH), contribute to the formation of germinal center in lymphoid follicles and activation of T and B cell responses in the inductive sites. Activated B cells differentiate into IgA-secreting plasma cells, which are responsible for IgA antibody secretion. DCs under maturation migrate to lymph nodes, but DCs already located in MALT can activate naïve CD4+ and CD8+ T cells, which results in the differentiation of activated CD4+ T cells into T helper 1 (Th1), Th2, or Th17 cells, regulatory T cells (Tregs) or follicular helper T cells (TFH). In the regional lymph nodes, DCs interact with T and B cells to produce IgG antibodies, which protect distant systemic or mucosal sites after reaching the bloodstream. In this context, the effector sites are responsible for protecting with the action of specific antibodies and CD4+ and CD8+ effectors, and where memory T cells reside.

TABLE II - Comparison between oral and nasal routes for vaccine administration

Route of administration	Advantages	Disadvantages
Oral and Intranasal	Possibility of self-administration Induction of mucosal immune response Non-invasive and painless method Applicable for mass vaccination	Requires higher doses than those used in the parenteral route
Oral	Ease of administration and increased adherence Large-scale production with low costs High absorptive capacity of the intestinal epithelium Presence of constituent lymphoid tissue	Mucosal coating is a barrier to absorption of macromolecules Secretion of antimicrobial compounds occurs within the mucous membrane Gastric degradation due to low stomach pH, presence of bile salts and enzymes First pass metabolism Intestinal motility and gastric emptying Inaccurate dosage and reduced bioavailability

TABLE II - Comparison between oral and nasal routes for vaccine administration

Route of administration	Advantages	Disadvantages
Intranasal	Large mucosal surface area, porous endothelial membrane and high vascularization of the epithelium Presence of lymphoid tissue associated with the nasopharynx Induction of the immune response in mucous membranes further away from the nasal cavity Protection against pathogens that enter the respiratory tract Prevents the spread of the pathogen through the air Reduced activity of degradation enzymes and avoids 1st pass metabolism	Epithelial cell layer forms a barrier to absorption Fast mucociliary clearance and short residence time Reduced delivery volume due to low nasal cavity capacity Nasal Obstruction is an important obstacle

NANOTECHNOLOGY APPLIED TO VACCINOLOGY

Notably, several vaccines have been successful in preventing major infectious diseases in the past. However, numerous vaccines still have not provided effective immunity to protect humans against some diseases, such as malaria, tuberculosis, and acquired immunodeficiency syndrome - AIDS (Fries *et al*., 2021). In this sense, nanotechnology studies have enabled the development of vaccine formulations with increased potency when compared to traditional formulations (Zhou *et al*., 2019).

Nanotechnology emerged in the 1980s through the development, synthesis, and manipulation of materials on a nanometric scale. It enabled visualizing biomolecular interactions, as well as obtaining nanostructures to eliminate disadvantages and obstacles related to the traditional pharmacological approaches (Contera, Bernardino, Tetley, 2020). According to Roco (2003), nanotechnology is defined as the ability to work at atomic, molecular, and supramolecular levels to understand, develop, and use materials, structures, devices, and systems with new properties and functions due to their small size. All biological systems have their first level of organization on a nanometric scale, within which fundamental properties and functions are clearly defined.

Thus, nanotechnology studies have been carried out to develop new vaccines and collaborate with their worldwide implementation (Fries *et al*., 2021). Applications of nanotechnology in pharmacology (nanopharmacology) involve using small-scale particles to improve therapeutic performance. This improvement stems from the pharmacokinetic profile modulation of formulations, increasing bioavailability and/or half-life, as well as from changes in pharmacodynamics that lead to an increased efficacy (Apolinário *et al*., 2020).

The efficiency of a vaccine depends on its ability to promote immune responses different from those induced by natural infection. Accordingly, nanomaterials, or nanoscale materials, are advantageous due to their well-defined composition and length scale, and can safely engage major immune pathways (Fries *et al*., 2021). Nanoparticles may allow targeted delivery of antigens to immune system cells, especially those whose surface is modified through the addition of targeted ligands or antibodies. Such a delivery improves vaccine efficacy by facilitating absorption with a slow antigen release, inducing humoral and cellular immune responses (Rai *et al*., 2020). It is due to the ability of nanocarriers to deliver, together with active pharmaceutical ingredients, immunostimulating components and enable a synergistic effect on the immune system (Zhu, Wang, Nie, 2014). Furthermore, nanoparticles can activate APCs,

especially dendritic cells, thus increasing vaccine efficacy (Rai *et al*., 2020).

Therefore, the application of nanomaterials as vaccine delivery systems includes both antigen delivery and formulation adjuvant functions (Govindaraju *et al*., 2020) so that integration of delivery and immunomodulatory effects of nanomaterials promote highly relevant immunological outcomes for vaccination (Zhu, Wang, Nie, 2014). Finally, the unique size of nanoparticles, which enhances the delivery of vaccine components, is also highlighted as advantageous since there is more efficient drainage to lymphoid organs, wherein antigen uptake and processing occur (Zhou et al., 2019).

Overall, nanoparticulate systems used for vaccine delivery have three different components or parts: (1) nanoparticle composition material (natural or synthetic polymers, inorganic substances, among others); (2) immunogen or immunomodulating agent conjugated by covalent bond, adsorbed on the surface of nanoparticle or encapsulated within it; (3) targeting ligands and/or immunostimulators added to the surface of particles, including immune system-specific ligands, tissue-specific ligands, and pathogen-associated molecular patterns (PAMPs) (Rai *et al*., 2020).

In general, nanomaterials allow the investigation of structural characteristics and mechanisms of the immune system, which can be improved to increase immunogenicity, which is a result of the multivalence of nano-vaccines. Currently, several nanocarriers have been studied as delivery platforms, which, combined with advances in antigen design, allow us to obtain highly efficient vaccines that stimulate immune responses for still neglected diseases (Fries *et al*., 2021). The contribution of nanoparticles to vaccinology comprises their ability to load, protect, target, and deliver immunotherapeutic cargos to immune cells of interest, particularly to desired APCs (Boushehri, Dietrich, Lamprecht, 2020).

In the context of vaccination, free-form antigen delivery via the mucosa generally induces a weak immune response, which is related to the diffusion of antigens across mucosal barriers, mucociliary clearance, and the presence of degrading enzymes (Caetano, Almeida, Goncalves, 2014). Currently, several studies have contemplated the development of proper vaccine administration systems that contribute to improving aspects of the immunogenicity of antigens and induce a more efficient immune response, in addition to enabling reductions of applied dose and production costs (Jin *et al*., 2019).

Nanotechnological applications have enabled the targeted delivery of vaccine antigens across mucosal surfaces, improving the solubility, stability, and surface properties of antigens to achieve a better immune response (Thakur, Foged, 2020). Nanomaterials are advantageous carriers of vaccine formulations due to their biocompatibility, mucosal absorptivity, and biodegradability (mostly). They also have surface properties subject to modification and control, the ability to allow entry of molecules into cells, and the protection of formulations against degradation (Jiao *et al*., 2018).

Micro- and nanoparticulate delivery systems based on synthetic or natural polymers have been widely used for the development of vaccines delivered via mucosa. The use of nanoparticles, such as those mentioned above, helps to protect antigens from degradation, making formulations penetrate mucosal barriers and controlling the release of antigens and immunomodulators in cells and intracellular compartments of interest (Woodrow, Bennett, Lo, 2012). Thus, the application of nanoparticles in vaccine formulation helps increase antigen stability and immunogenicity, and also allows for its targeted delivery. Mehrabi and collaborators reported that chitosan nanoparticles have immunological activity and mucoadhesion properties, which are used as vaccine delivery systems for various antigens (Mehrabi *et al*., 2018). In addition, nanocarriers improve interaction with the epithelium, increasing absorption of antigens in mucosa-associated lymphoid tissues (MALT); therefore, this process favors the interaction of antigens with APCs, such as macrophages, and soon generates B and T lymphocytes, increasing the immune response at the site of interest (Caetano, Almeida, Goncalves; 2014).

Nanoparticles in oral vaccines

Currently, there are numerous alternatives to protect vaccines against the hostile gastrointestinal environment,

one of which includes transporting them inside or adsorbed to surfaces of nanoparticulate systems. Despite presenting less protection when antigens are adsorbed on the surface, they are more available for immune system recognition. Moreover, in both cases, additional protection can be obtained through an enteric surface coating on particles (Coffey, Gaitha, Traverso; 2021).

Among the strategies to improve oral vaccine immune response, efforts to overcome formulation instability in the gastrointestinal environment have excelled. In this context, acid-resistant biomaterials and coating techniques proved to be efficient for such a function (e.g., enteric coating of nanoparticles and packaging of antigen within the sturdy carrier). Enteric coating is based on polymers with different solubility at different pH, which allows the delivery of antigens to the GUT without being degraded in the acid environment (Zhang *et al.*, 2021).

Vehicles for oral vaccine delivery have been widely studied and employed, such as biodegradable and biocompatible polymers, for example, poly-D, L-lactide-coglycolide (PLGA), and polylactide (PLA), micelles, liposomes, solid lipid nanoparticles, dendrimers, and metallic nanoparticles. These polymers encapsulate antigens to protect them from metabolic degradation and also release them gradually. Furthermore, these formulations provide better stability and activity (Kour *et al.*, 2018).

In general, adjuvants for oral vaccination should be biocompatible, have a stable and controlled release, protect antigens from degradation in the gastrointestinal tract, have targeted delivery, and be capable of delivering antigens in a controlled manner to target immune cells to improve the presentation of antigens (Zhang *et al.*, 2018b).

Nanoparticles in nasal vaccines

Delivery systems have advantageous properties that allow protecting and transporting vaccine antigens to the desired location. Other important functions of these systems to improve the formulation of intranasal vaccines are: (1) increased mucoadhesion; (2) ability to overcome mucociliary clearance; (3) increased formulation permeation and penetration; (4) promotion of antigen retention; (5) more sustained antigen release; (6) antigen targeting ability; (7) delivery of immunogens to a preferential antigen processing pathway; (8) presence of adjuvant properties; and (9) foundation for binding immunomodulatory molecules (Riese *et al.*, 2014). In the nasal cavity, NALT is the most important region for vaccine delivery to induce a favorable immune response; therefore, formulations that allow increasing permanence time at this location may stand out (Tlaxca, Ellis, Remmele, 2015).

Therefore, nanoparticles are effective in improving nasal vaccine delivery by amplifying immune responses. These particles are also preferentially absorbed by the NALT system. In a study, Illum suggested that nanoparticles can be taken up by M cells in the NALT and transported to the lymphatic system, which then reaches the bloodstream (Illum, 2007). Positively charged particles can, for example, be cited as a nasal vaccine delivery system strategy, as they allow interaction between particles and mucus, which is negatively charged. This mucoadhesion reduces the vaccine clearance rate in the nasal cavity, which facilitates antigen uptake (Jia, Krishan, Omri, 2015).

Currently, the use of nasal administration of drugs and vaccines has grown significantly due to increasing studies in nanotechnology, imaging, and administration devices. In this sense, to overcome nasal route limitations, nanoparticulate carriers with the potential for nasal delivery of vaccines have been widely studied, which would allow for facilitating antigen absorption through nasal barriers, presenting antigens more efficiently to the immune system (Mato, 2019).

CONCLUSION

Vaccination is known to broadly contribute to the control of several diseases. However, new vaccine technologies must be developed to provide immune protection against various infectious diseases, including those transmitted through mucosal pathways. In this context, mucosal vaccination has the potential for mass immunization due to its several advantages. Two of them are ease of administration and induction of mucosal immunity. However, there is still a limited number

of licensed vaccines using this route. As previously mentioned, this is mainly due to a lack of efficient immunogenicity, which derives from difficulties of the epithelium in capturing antigens, as well as degradation and risk of immunological tolerance.

Vaccination via the nasal and oral mucosa has recently gained prominence due to nanoparticle-based delivery systems, which allow for overcoming delivery challenges in these routes. For oral vaccination, vaccine delivery systems must be capable of transporting antigens and adjuvants with high stability, as well as withstanding gastrointestinal conditions. As for intranasal vaccination, the delivery system must protect antigens against mucociliary clearance and be safe since the nasal cavity is close to the Central Nervous System. In general, nanoparticle-based delivery systems are crucial to promoting formulation stability under physicochemical conditions of the targeted environment, mucoadhesion to help antigen uptake through biological barriers, and targeted delivery to specific tissues and cells through the addition of targeting ligands.

To overcome the challenges above, nanotechnology, especially nano-carriers, has been widely used to develop vaccines for being non-invasive and painless administrated. Non-invasive vaccination, such as via oral or nasal mucosa, provides cost reduction for developing countries, reduces contamination by needle-borne diseases, and accelerates the vaccination process in situations such as the COVID-19 pandemic.

ACKNOWLEDGEMENTS

The authors would like to thank the São Paulo Research Foundation (FAPESP grant #2019/08891-8) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX 0846/2022) for financial support. NFP is the recipient of FAPESP-Brazil fellowship #2019/25548-5.

REFERENCES

Afrough B, Dowall S, Hewson R. Emerging viruses and current strategies for vaccine intervention. Clin Exp Immunol. 2019;196(2):157-166.

Apolinário AC, Salata GC, Bianco AFR, Fukumori C, Lopes LB. Abrindo a caixa de pandora dos nanomedicamentos: há realmente muito mais 'espaço lá embaixo'. Quím Nova. 2020;43(2): 212-225.

Ashraf MU, Kim Y, Kumar S, Seo D, Ashraf M, Bae YS. COVID-19 Vaccines (Revisited) and oral-mucosal vector system as a potential vaccine platform. Vaccines (Basel). 2021;9(2):171.

Boushehri MAS, Dietrich D, Lamprecht A. Nanotechnology as a platform for the development of injectable parenteral formulations: a comprehensive review of the know-hows and state of the art. Pharmaceutics. 2020;12(6):510.

Braz L, Guimarães DT, Vaz MRF, Nóbrega FF, de F. Contributions of biotechnology in the development and production of first, second and third generation vaccines. Rev Health Sci. 2014;3(30):189–206.

Caetano LA, Almeida AJ, Gonćalves LM. Approaches to tuberculosis mucosal vaccine development using nanoparticles and microparticles: a review. J Biomed Nanotechnol. 2014;10(9):2295-316.

Coffey JW, Gaiha GD, Traverso G. Oral biologic delivery: advances toward oral subunit, DNA, and mRNA vaccines and the potential for mass vaccination during pandemics. Annu Rev Pharmacol Toxicol. 2021;61:517-540.

Contera S, Bernardino JS, Tetley TD. Biotechnology, nanotechnology and medicine. Emerg Top Life Sci. 2020;4(6):551–554.

Corthésy B, Bioley G. Lipid-based particles: versatile delivery systems for mucosal vaccination against infection. Front Immunol. 2018;9:431.

Davitt CJ, Lavelle EC. Delivery strategies to enhance oral vaccination against enteric infections. Adv Drug Deliv Rev. 2015;91:52-69.

De Magistris MT. Mucosal delivery of vaccine antigens and its advantages in pediatrics. Adv Drug Deliv Rev. 2006;58(1):52-67.

Diniz MDO, Ferreira LCS. Biotechnology applied to the development of vaccines. Estudos Avançados. 2010;24(70):19-30.

Du Y, Xu Y, Feng J, Hu L, Zhang Y, Zhang B, et al. Intranasal administration of a recombinant RBD vaccine-induced protective immunity against SARS-CoV-2 in mouse. Vaccine. 2021;39(16):2280-2287.

Fernandes J, Lanzarini NM, Homma A, Lemos ERS. Vaccines. Rio de Janeiro: Editora Fiocruz, 2021.

Fries CN, Curvino EJ, Chen JL, Permar SR, Fouda GG, Collier JH. Advances in nanomaterial vaccine strategies

to address infectious diseases impacting global health. Nat Nanotechnol. 2021;16(4):1-14.

Govindaraju K, Dilip Itroutwar P, Veeramani V, Ashok Kumar T, Tamilselva S. Application of nanotechnology in diagnosis and disease management of White Spot Syndrome Virus (WSSV) in aquaculture. J Clust Sci. 2020;31:1163–1171.

Han HJ, Nwagwu C, Anyim O, Ekweremadu C, Kim S. COVID-19 and cancer: From basic mechanisms to vaccine development using nanotechnology. Int Immunopharmacol. 2021;90:107247.

Illum L. Nanoparticulate systems for nasal delivery of drugs: a real improvement over simple systems? J Pharm Sci. 2007;96(3):473-83.

Islam MA, Firdous J, Choi YJ, Yun CH, Cho CS. Design and application of chitosan microspheres as oral and nasal vaccine carriers: an updated review. Int J Nanomed. 2012;7:6077-93.

Jia Y, Krishnan L, Omri A. Nasal and pulmonary vaccine delivery using particulate carriers. Expert Opin Drug Deliv. 2015;12(6):993-1008.

Jiao H, Yang H, Zhao D, Chen J, Zhang Q, Liang J, et al. Design and immune characterization of a novel Neisseria gonorrhoeae DNA vaccine using bacterial ghosts as vector and adjuvant. Vaccine. 2018;36(30):4532-4539.

Jin Z, Gao S, Cui X, Sun D, Zhao K. Adjuvants and delivery systems based on polymeric nanoparticles for mucosal vaccines. Int J Pharm. 2019;572:118731.

Jiskoot W, Kersten GFA, Mastrobattista E. Vaccines. Pharm Biotechnol. 2013;27:439–457.

Kalam MA, Khan AA, Alshamsan A. Non-invasive administration of biodegradable nano-carrier vaccines. Am J Transl Res. 2017;9(1):15-35.

Kang SH, Hong SJ, Lee YK, Cho S. Oral vaccine delivery for intestinal immunity-biological basis, barriers, delivery system, and M cell targeting. Polymers (Basel). 2018;10(9):948.

Kang ML, Cho CS, Yoo HS. Application of chitosan microspheres for nasal delivery of vaccines. Biotechnol Adv. 2009;27(6):857-865.

Köping-Höggård M, Sánchez A, Alonso MJ. Nanoparticles as carriers for nasal vaccine delivery. Expert Rev Vaccines. 2005;4(2):185-96.

Kour P, Rath G, Sharma G, Goyal AK. Recent advancement in nanocarriers for oral vaccination. Artif Cells Nanomed Biotechnol. 2018;46(Sup3):S1102-S1114.

Li M, Zhao M, Fu Y, Li Y, Gong T, Zhang Z, et al. Enhanced intranasal delivery of mRNA vaccine by overcoming the nasal epithelial barrier via intra- and paracellular pathways. J Controlled Release. 2016;228:9-19.

Li YD, Chi WY, Su JH, Ferrall L, Hung CF, Wu TC. Coronavirus vaccine development: from SARS and MERS to COVID-19. J Biomed Sci. 2020;27(1):104.

Marasini N, Skwarczynski M, Toth I. Intranasal delivery of nanoparticle-based vaccines. Ther Deliv. 2017;8(3):151-167.

Mato LY. Nasal route for vaccine and drug delivery: Features and current opportunities. Int J Pharm. 2019;572:118813.

Mehrabi M, Montazeri H, Mohamadpour Dounighi N, Rashti A, Vakili-Ghartavol R. Chitosan-based nanoparticles in mucosal vaccine delivery. Arch Razi Inst. 2018;73(3):165-176.

Negahdaripour M, Golkar N, Hajighahramani N, Kianpour S, Nezafat N, Ghasemi Y. Harnessing self-assembled peptide nanoparticles in epitope vaccine design. Biotechnol Adv. 2017;35(5):575–596.

New RRC. Formulation technologies for oral vaccines. Clin Exp Immunol. 2019;198(2):153-169.

Pan L, Zhang Z, Lv J, Zhou P, Hu W, Fang Y, et al. Induction of mucosal immune responses and protection of cattle against direct-contact challenge by intranasal delivery with foot-and-mouth disease virus antigen mediated by nanoparticles. Int J Nanomed. 2014;9:5603-18.

Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines — a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261–279.

Parker EP, Ramani S, Lopman BA, Church JA, Iturriza-Gómara M, Prendergast AJ, et al. Causes of impaired oral vaccine efficacy in developing countries. Future Microbiol. 2018;13(1):97-118.

Rai M, Bonde S, Yadav A, Plekhanova Y, Reshetilov A, Gupta I, et al. Nanotechnology-based promising strategies for the management of COVID-19: current development and constraints. Expert Rev Anti-infect Ther. 2020;20(10):1299-1308.

Rhee JH. Current and new approaches for mucosal vaccine delivery. Mucosal Vaccines. 2020:325–56.

Riese P, Sakthivel P, Trittel S, Guzmán CA. Intranasal formulations: promising strategy to deliver vaccines. Expert Opin Drug Deliv. 2014;11(10):1619-34.

Rockwell PG. (Ed.). Vaccine science and immunization guideline: a practical guide for primary care. Cham: Springer International Publishing, 2017.

Roco MC. Nanotechnology: convergence with modern biology and medicine. Curr Opin Biotechnol. 2003;14(3):337-46.

Sasso E, D'Alise AM, Zambrano N, Scarselli E, Folgori A, Nicosia A. New viral vectors for infectious diseases and cancer. Semin Immunol. 2020;50:101430.

Shahiwala A, Vyas TK, Amiji MM. Nanocarriers for systemic and mucosal vaccine delivery. Recent Pat Drug Deliv Formul. 2007;1(1):1-9.

Skwarczynski M, Toth I. Non-invasive mucosal vaccine delivery: advantages, challenges and the future. Expert Opin Drug Deliv. 2020;17(4):435-437.

Srivastava A, Gowda DV, Madhunapantula SV, Shinde CG, Iyer M. Mucosal vaccines: a paradigm shift in the development of mucosal adjuvants and delivery vehicles. APMIS. 2015;123(4):275-88.

Thakur A, Foged C. Nanoparticles for mucosal vaccine delivery. Nanoeng Biomater Adv Drug Delivery. 2020:603–46.

Tlaxca JL, Ellis S, Remmele RL Jr. Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. Adv Drug Delivery Rev. 2015;93: 56-78.

Van der Weken H, Cox E, Devriendt B. Advances in oral subunit vaccine design. Vaccines (Basel). 2020;9(1):1.

Vela Ramirez JE, Sharpe LA, Peppas NA. Current state and challenges in developing oral vaccines. Adv Drug Delivery Rev. 2017;114:116-131.

Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. Ann Med. 2018;50(2):110-120.

Wallis J, Shenton DP, Carlisle RC. Novel approaches for the design, delivery and administration of vaccine technologies. Clin Exp Immunol. 2019;196(2):189-204.

Woodrow KA, Bennett KM, Lo DD. Mucosal vaccine design and delivery. Annu Rev Biomed Eng. 2012;14:17-46. Epub. 2012 Apr 18.

Yusuf H, Kett V. Current prospects and future challenges for nasal vaccine delivery. Hum Vaccin Immunother. 2017;13(1):34-45.

Zhang R, Billingsley MM, Mitchell MJ. Biomaterials for vaccine-based cancer immunotherapy. J Controlled Release. 2018a;292:256–276.

Zhang L, Yang W, Hu C, Wang Q, Wu Y. Properties and applications of nanoparticle/microparticle conveyors with adjuvant characteristics suitable for oral vaccination. Int J Nanomed. 2018b;13:2973-2987.

Zhang Y, Li M, Du G, Chen X, Sun X. Advancedoral vaccine delivery strategies for improving the immunity. Adv Drug Deliv Rev. 2021 Oct;177:113928.

Zheng Z, Diaz-Arévalo D, Guan H, Zeng M. Noninvasive vaccination against infectious diseases. Hum Vaccin Immunother. 2018;14(7):1717-1733.

Zhou J, Kroll AV, Holay W, Fang RH, Zhang L. Biomimetic nanotechnology toward personalized vaccines. Adv Mater. 2019;32(13):1901255.

Zhu M, Wang R, Nie G. Applications of nanomaterials as vaccine adjuvants. Hum Vaccin Immunother. 2014;10(9):2761–2774.

Received for publication on 03rd November 2021
Accepted for publication on 31st March 2022