HHS Public Access

Author manuscript

Eur J Pharm Biopharm. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Eur J Pharm Biopharm. 2018 January ; 122: 167–175. doi:10.1016/j.ejpb.2017.11.001.

Intranasal Immunization with Dry Powder Vaccines

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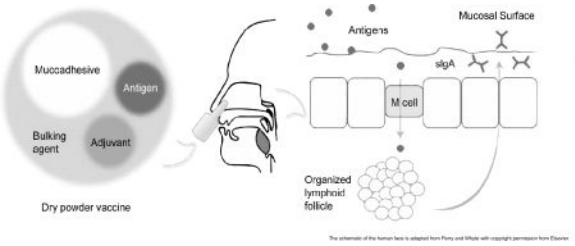
Abstract

Vaccination represents a cost-effective weapon for disease prevention and has proven to dramatically reduce the incidences of several diseases that once were responsible for significant mortality and morbidity worldwide. The nasal cavity constitutes the initial stage of the respiratory system and the first contact with inhaled pathogens. The intranasal (IN) route for vaccine administration is an attractive alternative to injection, due to the ease of administration as well as better patient compliance. Many published studies have demonstrated the safety and effectiveness of IN immunization with liquid vaccines. Currently, two liquid IN vaccines are available and both contain live attenuated influenza viruses. FluMist® was approved in 2003 in the United States, and Nasovac® H1N1 vaccine was approved in India in 2010. Preclinical studies showed that IN immunization with dry powder vaccines (DPVs) is feasible. Although there is not a commercially available DPV yet, DPVs have the inherent advantage of being relatively more stable than liquid vaccines. This review focuses on recent developments of DPVs as next-generation IN vaccines.

Graphical abstract



Keywords

Mucosal immune responses; NALT; Dry powder; Devices; Mucoadhesives

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Declaration of interest

The authors report no conflict of interest.

1. Nasal immune systems and its role in intranasal immunization

The nose is responsible for conditioning the inspired air and protecting the airways from large and potentially harmful particles, as well as functions as a sensory organ responsible for olfaction [1]. The air inhaled through the nose moves from the nasopharynx to the oropharynx, and then larynx and trachea until it reaches the deep lung [2].

The nasal cavity is divided by the nasal septum into two paired compartments, further separated by the folds of the superior, middle, and inferior concha that form three ducts called superior, middle, and inferior meatus [3] (Fig. 1). Based on the increased turbulence and air flow resistance, two major deposition areas of inhaled particles can be identified in the nasal cavity, the middle concha and the internal ostium [4].

The mucous membrane lining the nasal cavity secretes mucus that works as an innate immunity mechanism by washing away or entrapping potential pathogens [5]. Nasal secretions also contain protective elements such as immunoglobulins [6], lactoferrin [7, 8], and immune cells [9, 10]. The mucosal surfaces present inductive sites that can initiate an immune response to specific antigens independent from the systemic immune system. These inductive sites are collectively described as mucosa-associated lymphoid tissue (MALT) [11]. The nose-associated lymphoid tissue (NALT) is a subdivision of the MALT [12]. The term NALT in rodents refers to a pair of aggregated lymphoid tissue localized in the nose (i.e., the bottom of the nasal ducts) [13]. The Waldeyer's ring, a well-known group of tonsils that includes the adenoid, tubal, palatine, and lingual tonsils, is the key lymphoid tissue in human nose [14]. A post-mortem study by Debertin et al. provided the first evidence of the existence of a NALT, in addition to the Walderyer's ring, in young children [15]. This study in young children found disseminated aggregates of lymphoid tissue in the nasal cavity in 38% of the cases, mainly located in the superior meatus (30.1%), the middle concha (26.4%), the inferior concha (13.5%), and the superior concha (10.4%). In a recent review, Pabst stated that there is not reported data on the frequency of NALT in adolescents and adults [16].

Upon nasal vaccination, antigens can follow different routes that were reviewed by Kuper et al. [17]. While soluble antigens can potentially penetrate the nasal mucosa and contact antigen-presenting cells (APCs) such as dendritic cells and macrophages in the mucosa, particulate antigens can be either cleared by the mucociliary system or taken up by microfold (M) cells in the NALT. The route followed by the antigens in DPVs should mainly resemble the fate of particulate antigens. The uptake of antigens by APCs leads to the activation of T and B cells. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the immunoglobulin A (IgA) class of antibodies into the lumen, where they can interact and neutralize the specific antigens. This interaction leads to the formation of IgA-antigen complexes, which are easily entrapped in the mucus and then eliminated by the ciliated epithelial cells [18]. Also, lymphocytes activated in a mucosal surface can reach remote mucosal sites through the lymphatic system and transfer the immunity in a response called "the common mucosal immune system" [19]. The studies by

Johansson et al. demonstrated that in humans an IN vaccine could provide immunity to the cervicovaginal mucosa [20], but not to the gut mucosa [21].

M cells are specialized cells that have been extensively studied in the gut-associated lymphoid tissue (GALT) for its role in the transport of antigens across the mucous membrane to underlying lymphoid cells [22]. Fujimura found M cells in excised human adenoid tissue [23]. These cells could take up influenza A virus, suggesting that M cells are sites of antigen uptake for induction of mucosal immunity after IN immunization [24]. Finally, antigen particles taken up by NALT are drained into lymph nodes. These antigens may induce the production of serum IgA and IgG in systemic lymphoid organs [17, 25]. However, the systemic immune response elicited by mucosal vaccination is usually weaker than that induced by parenteral immunization [26]. Therefore, the immune response provoked by DPV formulations is generally characterized by measuring specific sIgA and serum IgG titers as markers of local and systemic immune responses, respectively [27].

2. Advantages and disadvantages of intranasal immunization

2.1. Intranasal immunization versus parenteral injection

Most of the pathogens enter the body by penetrating mucosal surfaces, making the nasal mucosa one of the most promising routes for vaccinations due to the accessibility of the mucosal tissue, the improved patient compliance, and the potential for self-administration [28]. Moreover, in light of the continuous search for novel approaches for heat-stable and needle-free vaccines, the IN route of immunization denotes a great versatile strategy. Specifically, needle-free vaccines can potentially decrease the costs of vaccination, as they do not require trained professionals for administration and waste disposal, and represent a viable approach for mass vaccination campaigns. In addition, IN route allows for the administration of both liquid and dry vaccine formulations.

A hurdle in the development of nasal vaccine formulations is the limited time available for antigen absorption, due to the rapid mucociliary clearance of foreign particles [29]. This obstacle has been addressed by using mucoadhesives, which can increase the immunogenicity of vaccines by prolonging the residence of the antigens at the immune effector sites [30]. Chitosan is one of the most extensively studied mucoadhesives in IN vaccine formulations [31–33]. Its mechanism of action is based on its capacity to increase vaccine residence time as well as open tight junctions transiently in mucosal membranes, which improve antigen penetration [34]. Chitosan also enhanced the response to a parenteral immunization [33, 35]. It has been proposed that the observed increase in immune response induced by vaccine formulations that contain chitosan could be due to the presence of impurities in the chitosan [27]. Glycol chitosan has also been tested as a mucoadhesive in liquid IN vaccines. In preclinical studies, vaccine formulations containing glycol chitosan have shown better mucosal uptake and reduced nasal clearance [36], as well as stronger immunogenicity [37], as compared to those containing chitosan.

2.2. Intranasal versus pulmonary immunization

Currently, there are seven non-parenteral vaccines approved by the US Food and Drug Administration (FDA) [38]. Five of these products are for oral administration (i.e., adenovirus, cholera, rotavirus and typhoid vaccines) and two are liquid vaccines for intranasal administration (influenza vaccines). As compared to the nasal route, the pulmonary route offers a much larger mucosal surface area that has been extensively explored for local as well as systemic delivery of both liquid and powder drug formulations [39]. For vaccines, the high vascularization of the alveolar tissue may facilitate the systemic delivery of antigens with a greater potential of achieving both respiratory and systemic immunization [40]. The delivery of DPVs by inhalation has been explored *in vi*vo for tuberculosis, hepatitis, influenza, and measles with promising results [41–46]. The safety and tolerability of a dry powder measles vaccine administered by inhalation were also confirmed in a clinical trial [47].

The main challenge of this route of administration is the low efficiency of the drug delivery achieved by current products. Both the formulation and the device used to administer the formulation determine the critical particle size for lung deposition [48]. To reach the deep lung, the aerosolized particles should have an ideal aerodynamic size between 1 and 5 μm. Particles above this size tend to impact on the surface of the upper airways [49]. For vaccine delivery, the later may not be completely disadvantageous because these particles still can reach the lymphoid tissue in the oropharyngeal region. However, if aiming for pulmonary and systemic immunization, advanced processing is required to obtain a high fine particle fraction (FPF) as well as delivery devices that can prevent or reduce the loss of product in the mouth and throat [50]. Saluja et al. developed two influenza DPVs with FPF values of 37% and 23% that were able to trigger significantly higher serum IgG titers than IM immunization in a mouse model [42]. The animals were immunized through an oropharyngeal tube.

3. Advantages and disadvantages of dry powder vaccines

FluMist® (MedImmune, LLC) is the first live attenuated influenza virus IN vaccine that was successfully approved and commercialized in the US and Europe (as Fluenz®) [51]. Nasovac® (Serum Institute of India, Ltd.) is an IN flu vaccine approved in India [52]. The inactivated IN influenza virus vaccine Nasalflu® (Berna Biotech AG, Switzerland) was available for a short period of time before it was discontinued in 2001 due to a potential association with partial facial paralysis [53]. Other vaccines for IN administration have been extensively studied against infectious diseases (e.g., measles, meningitis, tuberculosis, and pneumonia) [33, 54–60], or autoimmune diseases (e.g., arthritis) [61]. However, most of these formulations are liquids such as drops, sprays, and even emulgels.

Liquid vaccine, similar to other biologic products, are susceptible to physical, chemical, and thermal instability that can affect their potency and efficacy by undergoing degradation, aggregation, and/or hydrolysis [62]. Therefore, to stabilize them, liquid vaccines need preservatives, buffers, and cold-chain for storage and transportation [54]. Dry powder vaccine formulations can potentially eliminate these requirements, leading to more stable, more efficacious, and less expensive vaccines, which would be highly advantageous in

particular for remote areas in tropical developing countries because these vaccines would become available for the population in the areas [63]. Data from many studies evidenced the superior storage stability of DPVs at different temperatures and humidities, as compared to liquid vaccines [27, 64–67]. Most of these studies evaluated the stability of the stored vaccines after reconstitution using *in vitro* assays, but Wang et al. instead demonstrated that an IN anthrax DPV stored for 2 years at room temperature was as immunogenic as the fresh DPV [68].

DPVs, as other dry powder formulations for inhalation, need to be protected from humidity [69]. The composition and quality of the packaging system are key elements for the chemical and physical stability of dry powder formulations [70]. Suitable containers for DPVs may well follow the packaging used in the recently available drug powders for nasal delivery [71]. In these intranasal products, the drugs are protected from humidity either in sealed pouches with desiccant (e.g., Onzetra Xsail®, sumatriptan nasal powder) or within the device with a built-in desiccant chamber (e.g., Rhinocort Turbuhaler®, budesonide nasal powder).

4. Dry powder vaccines tested *in vivo*

DPV formulations usually contain the same components of traditional vaccines (i.e., antigen and adjuvant), in addition to bulking agents, stabilizers, and mucoadhesives. Spray drying (SD) and freeze-drying (FD) are the most commonly used pharmaceutical processes to produce dry powders of biologic compounds. Spray freeze-drying (SFD) uses the same principle of liquid atomization of SD, but the atomized liquid is instantly frozen in liquid nitrogen and then lyophilized. SD was compared to SFD in the preparation of DPV formulations for pulmonary delivery, and it was noted that both methods produce stable vaccines with good inhalation properties [42]. Thin-film freeze-drying was also reported as a method to successfully convert liquid vaccines containing aluminum salts to DPVs [72]. A more recent approach instead is based on the encapsulation of the vaccine components in mucoadhesive particles, and then the particles are converted into a dry powder [73–76].

Finally, carrier-based formulations have been proposed as an alternative method to improve the nasal deposition of DPVs and consequently reduce the lung deposition of vaccine particles. Again, the carrier-based vaccines are converted into a dry powder for IN immunization. Sugar alcohols [76], polylactic acid-polyethylene glycol nanoparticles [77], and poly (lactic-co-glycolic) acid microspheres [78] are some examples of carriers tested for IN vaccines. Table 1 summarizes the different DPVs investigated for IN administration in preclinical and clinical studies. Below is described in detail each type of vaccine.

4.1. Influenza

Influenza represents the leading cause of respiratory diseases in humans [79]. The first reported influenza DPV formulation was developed using whole, inactivated influenza viruses [64]. This IN vaccine generated a potent nasal mucosal immune response in rats, in addition to a systemic immune response that was comparable to that induced through intramuscular (IM) injection. Data from that study also demonstrated that chitosan increased the immune response induced by the DPV [64]. Garmise et al. prepared and characterized

influenza DPVs by testing the polydispersity of particles and the flow properties of DPV formulations containing whole, inactivated influenza viruses, lactose or trehalose, and chitosan [80]. In another study, the research group focused on identifying the effect of different mucoadhesive compounds on the DPV formulations and found a consistent trend between the nasal residence time of the formulations and the strength of the resultant immune response. Maximal mucosal and systemic responses were observed when using sodium alginate and carboxymethylcellulose-high molecular weight [27].

Different ratios of the bioadhesives, starch, and polyacrylic acid, were evaluated as carriers of the viral influenza antigen by Coucke et al. [81]. These powder formulations were able to induce systemic immune responses. It was observed that the levels of IgG titers induced by the IN vaccine had a dose-dependent correlation with the polyacrylic acid content.

Dehghan et al. observed the highest increase in IgG titers after the administration of chitosan nanospheres loaded with whole UV-inactivated influenza virus A together with synthetic CpG oligodeoxynucleotides (CpG ODNs) or *Quillaja* saponin as adjuvants in a rabbit model, demonstrating the feasibility of including vaccine adjuvants in IN DPV formulations [73, 82].

4.2. Diphtheria and Meningitis

Diphtheria is a disease caused by a toxin produced by *Corynebacterium diphtheria*. The cross-reacting material (CRM_{197}) of the diphtheria toxin, not toxic but still antigenic, is used extensively as a licensed polysaccharide antigen carrier in human vaccines [83]. CRM_{197} can be further treated with formaldehyde to increase resistance to proteolytic degradation [84]. McNeela et al. found that the addition of chitosan to a liquid formulation of CRM_{197} treated with 0.18% formaldehyde resulted in significantly enhanced immunogenicity measured by specific IgG titers in guinea pigs [33]. In another study, the group demonstrated that the vaccine formulated as a DPV was well tolerated in humans [85]. This IN vaccine induced significantly higher secretory IgA levels and similar protective levels of serum IgA and IgG, compared to those induced by IM immunization, and the same DPV caused a strong Th2-biased response in humans after IN immunization [86].

Menjugate-C® is a meningitis C vaccine developed and commercialized in Europe by Chiron Corp. (now Novartis Vaccines and Diagnostics, Inc.). It contains meningococcal C oligosaccharide conjugated to CRM_{197} in a powder form to be resuspended before IM administration [87]. Huo et al. used this vaccine powder mixed with chitosan for nasal immunization in a clinical study [88]. IN administration of the powder was well tolerated by the subjects, and the meningococcal-specific serum IgG and IgA levels observed 28 days after the administration were comparable to those seen after IM administration.

4.3. Viral gastroenteritis

Noroviruses are recognized as the main cause of epidemics of gastroenteritis in children and adults. The genetically variable *Norovirus* genus present three genogroups (GI, GII, and GIV) that can infect humans [89]. The expression of Norwalk virus (GI.1) capsid proteins without the viral RNA allows the development of immunogenic but noninfectious virus-like particles for vaccine formulations [90]. The immunogenicity of the norovirus virus-like

particles (NV VLPs) was later confirmed by intranasal administration using a liquid formulation in mice[91].

El-Kamary et al. conducted a phase 1 clinical study to evaluate the safety and immunogenicity of a DPV containing NV VLPs, monophosphoryl lipid A (MPL) as adjuvant and chitosan as a mucoadhesive [92]. This DPV was highly immunogenic and well tolerated with only mild side effects. Peripheral blood mononuclear cells (PBMCs) from subjects in these clinical studies were collected to analyze B memory cell responses [93]. The B memory cell frequencies determined by flow cytometry were correlated with the serum levels of NV VLP-specific serum antibodies [93]. A significant NV-specific IgA and IgG responses were obtained in all subjects immunized with 100 μg/dose. In a different clinical study, the same DPV containing NV VLP, MPL, and chitosan provided significant protection against gastroenteritis after exposure to a homologous virus [94].

Velasquez et al. formulated a vaccine powder containing these NV VLPs in combination with a gelling agent derived from *Aloe vera* (GelSite®) and gardiquimod as an adjuvant. *In situ* gelation as well as mucosal and systemic immunity were shown in guinea pigs when this formulation was given IN [29]. Two novel adjuvant-free norovirus vaccines have been developed containing NV VLPs from genogroups I and II.4 with GelSite® as a mucoadhesive. The preclinical study conducted by Springer and colleagues reported that these DPVs showed maximal immunogenicity with smaller doses (greater than 15 μg) in guinea pigs [95]. Recently, the same group reported a bivalent DPV containing the antigens GI and GII.4 [96].

4.4. Anthrax

This lethal disease is caused by an exotoxin produced by *Bacillus anthracis*. Investigated IN vaccine formulations include those containing the recombinant protective antigen (rPA) protein and synthetic CpG oligonucleotides (as an adjuvant) [97, 98]. Mikszta et al. evaluated the protective effect of this vaccine in rabbits [99]. IN administration of the DPV showed better protection against anthrax (100% of survival) than IN liquid vaccine (67% survival). In a following study, the same complete protection was observed when a lower dose of this DPV was used to immunize rabbits [97]. Finally, this DPV formulation allowed the maintenance of protein integrity for one month at ambient temperature and in accelerated stability studies [65].

Another antigen used for anthrax vaccines is a peptide from the capsule of *B. anthracis*. DPVs were developed by lyophilization with mannitol and MPL using rPA in combination with either the free peptide or the peptide conjugated to rPA (rPA-conjugated). The powder was then resuspended to obtain liquid vaccines or mixed with chitosan to prepare DPVs for IN administration [100]. These vaccines were used to immunize rabbits exposed later to a lethal aerosol spore challenge. All DPVs demonstrated 100% protection, but only the animals immunized with rPA plus rPA-conjugated capsular peptide did not show signs of illness after 14 days. Also, the DPVs induced serum IgG titers that were higher than those induced by IN liquid vaccination and similar to that induced by IM vaccination. Klas et al. tested a different formulation containing rPA and the capsule peptide mentioned above in rabbits [101]. The peptide was conjugated to bovine serum albumin (BSA-conjugated),

lyophilized with mannitol and MPL, and then mixed with chitosan. Results after an aerosol spore challenge showed that a single IN immunization could protect the rabbits against the lethal spore challenge, up to 9 weeks post-treatment. Wang et al. used a different adjuvant, the mast cell activator C48/80, to prepare an rPA DPV without mucoadhesives [68]. IN administration of a freshly prepared DPV or a DPV stored for 2 years at room temperature induced immune responses comparable to those induced by IM administration of a liquid vaccine.

4.5. Tetanus

Tetanus toxoid (TT) has been used as a model antigen for IN vaccine development [74, 102]. Tafaghodi and Rastegar used TT and *Quillaja* saponin encapsulated in alginate microspheres with and without cross-linked dextran mucoadhesive microspheres for nasal immunization in rabbits [103]. These microspheres were tested for morphology, particle size, and *in vitro* release of TT and *Quillaja* saponin. They observed the highest mucosal and systemic immune responses when dextran microspheres were added to the formulation, which was converted into a dry powder and filled into a polyethylene tube (2 mm in diameter) connected to a syringe for IN administration.

5. Devices used for intranasal delivery of dry powder vaccines

The IN FluMist® Quadrivalent vaccine is a liquid formulation in a prefilled intranasal sprayer. However, dry powders for IN immunization require a different type of device to aerosolize the product. Dry powder inhalers (DPIs) are examples of commercially available inhalers developed for pulmonary drug delivery and have been on the market for decades. Depending on the aerosolization mechanism, DPIs are broadly classified as active or passive devices. Active devices have an aerosolization mechanism, while in passive devices the aerosolization is driven by patient's inhalation [104]. Following this classification criterion, most of the devices tested for IN delivery of DPVs can be classified as active devices. In fact, during the administration of IN vaccines inhalation should be prevented to keep the formulation in the nasal cavity to target the lymphoid tissues in the nasal cavity and prevent lung deposition.

In general, these devices for DPV delivery use a pneumatic force to expel the powder into the nasal cavity, which resembles the mechanism of action of a syringe. Indeed, most of the lab-made devices used for preclinical studies are based on syringes [64]. Some examples of commercially available devices for IN delivery of powders are the Monopowder single-dose disposable device (Valois Pharmaceutical Division), the Unit-dose system (AptarGroup, Inc.), and the Powder-Jet® device (RPC Bramlage GmbH). The Unit-dose system has been used to intranasally dose DPVs in preclinical studies in rabbits and guinea pigs [68, 95]. The Monopowder single-dose disposable device has been used for intranasal DPV administration in preclinical and clinical studies [85, 86, 100, 101]. The Powder-Jet®, different from the other devices, is a multidose system and was used by Trows and Scherließ to characterize their carrier-based DPV formulations [76]. A detailed review of devices for nasal drug delivery is available [105]. Since vaccines are aimed for long-term immunization, there is no clear need for multi-dose chambers nor reusability, which are important aspects of devices

used for chronic diseases. An ideal IN DPV product should be pre-loaded in a ready-to-use, disposable device to prevent manipulation and loss of the product. The packaging of the device should be moisture-sealed to ensure protection from humidity until use. Onzetra Xsail® (Avanir Pharmaceuticals, Inc.), approved by the FDA in 2016, is a sumatriptan powder for nasal delivery with the breath-powered device OptiNose® (OptiNose US, Inc.) [106]. This passive device is actuated when the patient blows air in the mouthpiece that releases the drug into the nasal cavity through the nosepiece. Even though OptiNose® has not been tested for DPVs delivery, it sets the precedent of an approved device that can be used for the study of future DPVs. Also, blowing through the mouth causes the closure of the soft palate between the nasal and the oral cavity, which prevents powder from flowing into the oropharynx and lungs [107, 108]. Therefore, this characteristic could be helpful to evaluate in clinical studies the immune responses triggered at the nasal mucosa without the contribution of additional responses from the airway mucosa outside the nasal cavity.

A list of the main findings, key challenges, and potential future directions of IN DPVs research can be found in Table 2.

6. Conclusion

Vaccines are a primary weapon to safeguard public health, and now even more important, with the continuous development of antimicrobial-resistant microorganisms. Injectable vaccines present some disadvantages such as the requirement of professional assisted administration, cold chain during storage and transport, as well as the costs of waste disposal. Needle-free IN vaccination represents an attractive approach to address these issues, safely and efficiently. The success with the IN liquid FluMist® and Nasovac® vaccines demonstrated the viability of IN immunization from both marketing and vaccinology perspectives. It is true that there is not an FDA-approved DPV yet, but the increasing amount of preclinical and clinical studies, as well as the approval of IN dry powder products, have shown the interest of the pharmaceutical industry in this new type of vaccines and route of administration. Preclinical studies showed that IN immunization with DPVs is feasible. Finally, intranasal immunization with DPVs has many advantages over with liquid vaccines, but different devices that can actively deliver the powder into the nasal cavity are needed to efficiently administer DPVs intranasally.

Acknowledgments

Tania Bahamondez-Canas would like to thank the CONICYT (Becas Chile) for the scholarship to support her Ph.D. studies. Z.C.'s research is supported by grants from the U.S. National Institutes of Health (CA135274, CA179362), the National Natural Science Foundation of China (81460454), and the Inner Mongolia Natural Science Fund (2014ZD05). The authors would also like to thank Daniel Moraga-Espinoza for his help in drawing Figure 1 and the figure in the graphic abstract.

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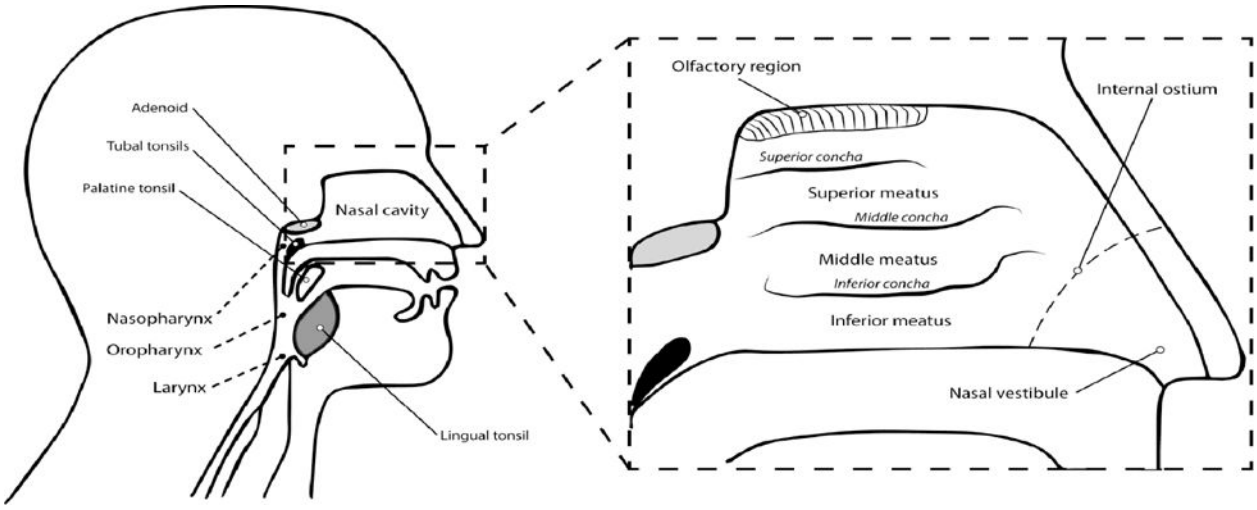

Figure 1.

Representation of the upper airways. Enlarged is a cross-section of the upper airway with details of the nasal cavity [2,3]. The adenoid, tubals, palatine tonsil and lingual tonsil are collectively called as the pharyngeal lymphoid ring or the Waldeyer's ring. Modified from Perry and Whyte with copyright permission [109].

Table 1

Intranasal dry powder vaccines tested *in vivo*.

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
Diphtheria	CRM_{197} formaldehyde treated (50 μg/dose)	N/S		Chitosan and mannitol	Monopowder (Valois)	Human	· IgA and IgG serum levels: Equivalent to IM immunization	(Mills *et al.*, 2003)
	CRM_{197} formaldehyde treated (50 μg/dose)	N/S		Chitosan, phosphate salts, and mannitol	Monopowder (Valois)	Human	· First study to demonstrate systemic T cell subtype response induced by a nasal vaccine	(McNeela *et al.*, 2004)
Influenza	Whole inactivated virus strain H1N1 (100 μg/dose)	FD		Chitosan and trehalose	Plastic housing unit attached to a syringe (WO 2002055133 A3)	Rat	· Stability: Higher stability than liquid vaccine after 12 weeks of storage at 25 °C. · IgA and IgG serum levels: Equivalent to IM and liquid IN immunization	(Huang *et al.*, 2004)
	Whole inactivated virus strain H1N1 (5 μg/dose)	SFD		Chitosan, trehalose, HPMC-HMW, CMC-HMW and SA.	Lab-made device	Rat	· Stability: Higher stability than liquid vaccine after 12 weeks of storage at 25 °C. · Mucoadhesives: SA and CMC-HMW increased the residence time · IgA and IgG serum levels: Equivalent to IM and liquid IN immunization	(Garmise *et al.*, 2007)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
	Whole heat-inactivated virus strain H3N2 (50 μg/dose)	FD	LTR192G	Starch and polyacrylic acid	Lab-made device	Rabbit	· Evaluation of different mucoadhesives ratios. · IgG serum levels: Correlated with the polyacrylic acid content. · sIgA nasal levels: Not detected.	(Coucke *et al.*, 2009)
	Whole UV-inactivated virus strain H1N1 (45 μg/dose)	Nanoencapsulation	CpG or *Quillaja* saponin	Chitosan	Lab-made device	Rabbit	· Evaluation of different adjuvants. · All DPV formulations were equal or better than IM vaccination. · IgG serum levels: Highest values obtained with CpG. · sIgA nasal levels: Highest values obtained with CpG.	(Dehghan *et al.*, 2014)
Meningitis	Meningococcal C oligosaccharide-CRM_{197} conjugated (10 μg/dose)	N/S		Chitosan	Combitips plus® (Eppendorf)	Human	· IgA and IgG serum levels: Equivalent to IM immunization. · sIgA nasal levels: Restricted to the immunized side.	(Huo *et al.*, 2005)
Anthrax	rPA (50 μg/dose)	FD or SFD	CpG	Chitosan and trehalose	Plastic housing unit attached to a syringe (WO 2002055133 A3)	Rabbit	· Evaluation of different administration routes (IM, ID, IN, and topical) and drying methods. · IgG serum levels: Highest with SFD	(Mikszta *et al.*, 2005)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
							• % survival after anthrax challenge: Equivalent to IM immunization (100%) and higher than liquid IN group (67%).	
	rPA (10 μg/dose)	SFD	CpG	Chitosan and trehalose	Plastic housing unit attached to a syringe (WO 2002055133 A3)	Rabbit	• Lower dose than in a previous study. • IgG serum levels: Lower than IM but equivalent to liquid IN immunization. • % survival after anthrax challenge: Higher than IM (86%) and liquid IN (63%) immunizations.	(Huang *et al.*, 2007)
	rPA (90 μg/dose) combined with free capsule peptide (18 μg/dose) or with rPA- conjugate (90 μg/dose)	FD	MPL	Chitosan and mannitol	Monopowder (Valois)	Rabbit	• Evaluation of different antigens and effect of chitosan. • Anti-rPA IgG serum levels: DPVs containing chitosan were equivalent to IM and higher than liquid IN immunization. • Anti-capsule IgG serum levels: Only observed when the peptide was conjugated to rPA and where equivalent with or without chitosan. • Eating behavior after anthrax challenge:	(Wimer-Mackin *et al.*, 2006)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
							Anorexic behavior in rPA + free peptide group compared to normal behavior in rPA + rPA− conjugate group.	
	rPA (50–150 μg/dose) alone or combined with BSA-conjugate (150 μg/dose)	FD	MPL	Chitosan and mannitol	Monopowder (Valois)	Rabbit	• Evaluation of different doses and antigens. • Anti-rPA IgG serum levels: All DPV were equivalent and lower than IM immunization after 3 weeks, but higher after 6 weeks. • % survival after anthrax challenge: Higher in the rPA + BSA-conjugate group (80%) than and rPA alone group (60%).	(Klas *et al.*, 2008)
	rPA (30 μg/dose)	SFD	C48/80	Trehalose	Unit-dose system (Aptar)	Rabbit	• Stability: Higher stability than liquid vaccine after 90 days at 25 °C. DPV maintained its activity after 2 years. • IgG serum levels: Equivalent to IM and liquid IN immunization.	(Wang *et al.*, 2012)
Tetanus	Tetanus toxoid (40 Lf/dose)	Microencapsulation	*Quillaja* saponin	Alginate and cross-linked dextran	Lab-made	Rabbit	• Evaluation of different excipients.	(Tafaghodi and Rastegar, 2010)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
							• IgG serum levels: Equivalent between DPVs but lower than IM immunization. • sIgA nasal levels: Equivalent between DPVs and higher than IM immunization. Highest level observed when cross-linked dextran was included.	
Gastroenteritis	Norovirus virus-like particles (GI.1) (10 μg/dose)	SD	Gardiquimod	GelSite®	Lab-made	Guinea pig	• Evaluation of the sIgA and sIgG levels in different mucosal surfaces after IN immunization: Significantly higher levels using GelSite®. • IgG serum levels: Highest values observed after 6 weeks with GelSite®.	(Velasquez *et al.*, 2011)
	Norovirus virus-like particles GI and GII.4 (0.1–100 μg/dose)	FD		GelSite®	Unit-dose system (Aptar)	Guinea pig	• Evaluation of different doses of two monovalent DPVs. • IgG serum and sIgG vaginal levels: Significantly higher values observed above 15 μg/dose in both DPVs. • IgA serum levels: Equivalent at all concentrations of both DPVs.	(Springer *et al.*, 2016)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
	Norovirus virus-like particles GI and GII.4 (5100 μg/dose)	FD		GelSite®	Unit-dose system (Aptar)	Guinea pig	· Evaluation of different doses of a bivalent DPV. · IgG serum and sIgG vaginal levels: Dose-dependent response for both antigens. Highest values observed 3 weeks after the second dose. · IgG intestinal leve ls: Dose-dependent response up to 50 ug/dose.	(Ball *et al*, 2017)
	Norovirus virus-like particles (GI.1) (5–100 μg/dose)	FD	MPL	Chitosan, sucrose, and mannitol	UniDose DP (Bespak)	Human	· Phase 1 clinical studies · Common adverse events: Nasal stuffiness and discharge. · IgA serum levels: Response observed above 50 μg/dose · IgG serum levels: Response observed above 15 μg/dose	(El-Kamary *et al.*, 2010)
	Norovirus virus-like particles (GI.1) (50–100 μg/dose)	FD	MPL	Chitosan, sucrose, and mannitol	UniDose DP (Bespak)	Human	· Phase 1 clinical studies · B memory cell population: above 4-fold increase in expression of CD27+ and CD38+ · IgA and IgG B memory response: In 100% of patients	(El-Kamary *et al.*, 2010, Ramirez *et al.*, 2012)

Disease	Antigen	Method of production	Adjuvant	Other excipients	Device	Model	Findings	References
							receiving 100 μg/dose and 90% of patients receiving 50 μg/dose.	
	Norovirus virus-like particles (GI.1) (100 μg/dose)	FD	MPL	Chitosan, sucrose, and mannitol	UniDose DP (Bespak)	Human	· Phase 1 and 2 clinical studies · Common adverse events: Nasal stuffiness and discharge. · Norwalk virus challenge: Significantly lower infection (61%) and illness (37%) compared to placebo (82% and 69%, respectively).	(Atmar *et al.*, 2011)

CRM197: cross-reacting material of the diphtheria toxin; FD: Freeze-drying; IN: intranasal; IM: intramuscular; SFD: Spray-freeze drying; HPMC-HMW: Hydroxypropyl methylcellulose, high molecular weight; CMC-HMW: Carboxymethylcellulose sodium, high molecular weight; SA: Sodium alginate; LTR192G: Heat-labile enterotoxin R192G mutant; rPA: recombinant Protective Antigen of *B. anthracis*; ID: intradermal; rPA conjugate; Capsule peptide conjugated to rPA; MPL: Monophosphoryl lipid A; BSA-conjugate: Capsule peptide conjugated to bovine serum albumin; GelSite®: *Aloe vera* derived polysaccharide polymer; Lf: Flocculation units; N/S: not specified

Table 2

Summary of key findings, main challenges, and future directions in intranasal DPV research.

Key findings in the literature about IN DPVs	Main challenges of IN DPVs	Prediction/future directions of IN DPVs
• Mucoadhesives such as chitosan and GelSite® improved the immunogenicity of DPVs by increasing the residence time of the formulation in the mucosal surface. • Mild formaldehyde treatment (0.18%) to stabilize antigens can enhance its immunogenicity. • The higher stability of DPVs compared to liquid vaccines was confirmed by the determination of its activity *in vitro* and *in vivo.* • Common mucosal response after IN vaccination can be tested to provide mucosal immunity in distant mucosal surfaces.	• Limited time for antigen absorption due to the rapid nasal mucociliary clearance. • Lack of guidelines for dry powder nasal products for *in vitro* performance and characterization. • Limited number of devices for dry powder nasal delivery for *in vivo* evaluation of DPVs. • Storage of the formulation protected from humidity to prevent loss of activity.	• Improvement of devices or development of new devices to efficiently deliver DPVs to nasal cavity only. • Novel formulations using particle carriers as bulking agents to modulate particle size and density to improve the deposition in the nasal cavity.