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# Nanoparticle-Based Mucosal Vaccine Delivery: Intranasal, Oral, and Pulmonary Formulation Strategies for Next-Generation Immunization

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**ABSTRACT**

The mucosal surfaces are considered to be an important entry point of pathogens into the human body; thus, they represent unique targets for vaccines. A nanoparticle-based drug delivery system is a modern technique used to deliver and enhance the effectiveness of mucosal vaccines. Modern studies give a better understanding of the formulation and delivery issues that need to be taken into consideration when developing effective and safe mucosal subunit vaccines via nanoparticles. They include novel nanomaterials, their physicochemical characteristics and formulation issues, interaction of nanoparticles with mucosal immune cells, as well as mucosal immunisation and delivery strategies. In this review, we will discuss modern achievements in the application of nanoparticle-based techniques in mucosal vaccine delivery and future research challenges in this area.

**Keywords:** Nanoparticles, Mucosal immunisation, Vaccines, Drug delivery, Adjuvants

## 1. INTRODUCTION

Vaccines are considered among the greatest achievements in medicine and have so far saved people from different kinds of disabilities in more than 750,000 children and prevented 3 million deaths per year [1]. With increased understanding of the mechanism of action of the mucosal immune system, mucosal vaccination is considered an important route for vaccine administration because it induces both mucosal and systemic immune responses [3,4]. Mucosal vaccination will create the first line of defence against infection and spread of pathogens at the site of entry of the pathogen [5,6]. Moreover, it has been observed from various studies that the maximum immune response is seen in the mucosal area exposed to antigen and in adjacent mucosa having specific connections [2,7].

The development of mucosal vaccines involves the proper choice of antigens, delivery, and adjuvant systems to enhance the effectiveness of the vaccine
[6,7]. Adjuvants are substances that can be incorporated into a vaccine formulation to enhance the immune stimulation of the immune system through antigen
presentation or co-stimulation. Adjuvants are also used to introduce antigens to the immune system in order to induce a specific immune response [6,7]. Nevertheless, there are only a few adjuvants that have been approved for human use and are mostly used in parenterally delivered vaccines.

## 2. INDUCTION OF MUCOSAL VACCINE DELIVERY

### 2.1. Induction of immune response

Numerous studies have provided ample evidence regarding the important role played by organised MALTs in the sampling of antigen and production of different kinds of lymphocytes, including specific IgA effectors, memory B cells, and T cells. This process includes lymphocyte proliferation, secretion of some cytokines and cellular traffic. Antigen sampling and immune response induction is a well-controlled process since an
effective immune response needs access to the antigen first. The MALT does not have a supply of antigen via the lymphatics but samples foreign materials from the epithelial surfaces.

### 2.2. First exposure of the mucosal immune system to antigens / Antigen uptake

The stratified squamous epithelium is not keratinised and therefore does not have any tight junctions. Antigen-presenting dendritic cells enter these epithelial layers, extract samples of the antigens present in the lumina, and then migrate out back into the organised lymphoid tissues. In the case of the single-layer intestinal and respiratory tract epithelial tissues that have tight junctions between their cells, antigens are specifically absorbed through the special regions of the follicle-associated epithelium. Specialised epithelial cells, M cells, absorb antigens from the lumen into organised lymphoid tissue in the mucosa through transepithelial transport of samples of foreign material. M cells, through the limited microvilli border, reduced glycocalyx and transcytosis, play an important role in delivering certain types of antigens to Peyer 5 patches for initiation of immune response involving active IgA secretion and induction of oral tolerance [8].

## 3. USE OF NANOPARTICLES FOR VACCINE DELIVERY SYSTEMS

Vaccines are made up of either living attenuated organisms, dead organisms, or parts of organisms. Though these have contributed significantly to the management of infections, some are not effective enough in protecting against infections. In addition, some of the live vaccines are not safe enough to be given to the large number of immunocompromised individuals in the world today. There are also a variety of infectious diseases for which there is no licensed vaccine yet. To solve these problems,
a new range of vaccines is being developed which will use either isolated proteins or polysaccharides, or naked DNA which encodes a protective antigen. Though these are safer, well-defined and less reactogenic than the traditional vaccines, they are poor
immunogens which require adjuvants to make them effective. The aluminium-based adjuvants are the most widely used ones. However, these can cause local reaction and cannot stimulate cell-mediated immunity [10]. Consequently, novel adjuvants and delivery systems are needed for the new generation of vaccines.

### 3.1. Strengths and weaknesses of nanoparticles Nanoparticle-Based Therapeutic Vaccines: Recent Advances and Future Challenges

| Strengths | Weaknesses |
|---|---|
| 1. The nanoparticles can easily be manipulated for rapid and non-resistant targeting. | 1. Drug release from nanoparticle-coated cells causes cell death. |
| 2. Prolongation of exposure of the drug at the targeted site helps in having a good immune | 2. Due to the smaller size of nanoparticles and larger surface area, they are hard to manipulate and coat drugs, especially when in liquid form |

| | |
|---|---|
| response and also increases the bioavailability of the drug. | |
| 3. They are capable of containing high amounts of drug without undergoing physical or chemical changes and also have better biocompatibility. | 3. Nanoparticle-based drugs are costlier than normal drugs. |
| 4. Particular ligands can be attached to help nanoparticles target particular sites for treatment. | 4. They are toxic to the body. |
| 5. Nanoparticles can be administered orally, through the nose, and through the optic nerve [10]. | 5. They lack of scientific knowledge regarding their interaction with the body system [12]. |

## 4. INTRANASAL NANOPARTICLE VACCINE FORMULATION STRATEGIES

### 4.1. Live-attenuated intranasal vaccines

Live attenuated vaccines delivered through the nose as a means of protecting from influenza viruses during regular and pandemic seasons are being researched at present. The benefit that lies in the use of live-attenuated vaccines consists in their ability to mimic the natural infection process and expose the influenza antigens in the natural form to the nasal mucosa without causing any symptoms of the disease [19]. Flumist by AstraZeneca is one such vaccines; it is the first nasal trivalent vaccine for seasonal influenza [19,20]. When compared to the injected trivalent influenza vaccine, the Flumist vaccine demonstrated a greater period of protection, cross-protection, efficacy and immunogenicity [19].

### 4.2. Mucoadhesion improvement

Antigens generally lack affinity for the nasal epithelial cells and are rapidly cleared through the mucociliary clearance mechanism [15,16]. The administration of mucoadhesive agents together with the antigen in an attempt to prolong nasal stay and hence allow interaction with the immunological systems becomes inevitable [16, 18]. Promising results using biodegradable mucoadhesive polymer carriers in mucosal vaccine delivery have made them important vehicles used in nasal vaccine delivery. Polymers including polylactide-co-glycolide (PLGA), chitosan, alginate, and carbopol are examples of the polymers used in antigen delivery via the nasal route [15]. Hydrophilic polymers such as sodium alginate and carbopol bind with the mucin layer by hydrogen bonding, thus increasing the nasal retention period [16,17].

### 4.3. Particle-based delivery systems

Particles employed as vaccine delivery vehicles in the nasal route include liposomes, ISCOMS, and polymeric particles, including virosomes [19,20]. The particulate antigen is transported using the transcellular pathway to deliver the antigen to the lymphoid tissues, where it will target the M cell. The M cell, a component of the NALT, provides the portals of entry that allow the antigen to get into the areas containing professional APCs (dendritic cells), B cells, and T cells, leading to an enhancement in both the humoral and cellular immunity [15,17,18,19]. Particles can deliver multiple antigen molecules and share the same dimensions as the pathogen.

## 5. ORAL NANOPARTICLE VACCINE FORMULATION STRATEGIES

### 5.1. Delivery method for nanoparticle-based oral vaccines

For several decades now, the field of nanotechnology has grown immensely with no end in sight, transforming an extensive range of disciplines including medicine, the food industry, agriculture, and environmental sciences, to name just a few; it has become an indispensable part of our lives [21]. Within

the field of pharmacy, the technology has transformed the field of drug delivery by improving the intracellular delivery of hydrophobic drugs and their solubility.

### 5.2. Nanoparticles Commonly Used for Oral Vaccine Delivery

The common nanoparticles used for oral vaccine drug delivery include polymeric nanoparticles such as chitosan and PLGA, lipid nanoparticles such as liposomes and solid lipid nanoparticles, protein nanoparticles, inorganic nanoparticles such as silica and calcium phosphate, and virus-like nanoparticles [21,22].

For oral vaccine drug delivery, the polymeric nanoparticles facilitate sustained release of the antigen and promote an effective immune response.

## 6. PULMONARY NANOPARTICLE VACCINE FORMULATION STRATEGIES

### 6.1. Research on Inhalable Nano-Formulations is Thoroughly Performed

Methods of synthesis. Many methods of synthesis are known for inhalable nano-formulations, which can be classified into top–down and bottom–up methods. The former include the breakdown of large solid particles into nanoparticles under the influence of external forces like high-pressure homogenization and wet milling. The latter include the synthesis of nanoparticles from molecules via precipitation, crystallisation, and solvent removal, including extrusion, solvent evaporation, and antisolvent methods [23,24].

#### 6.1.2. Structural analysis.

In the case of synthesised nano-formulations, structural analyses must be done by identifying the nanoarchitectonics in terms of their atomistic, molecular, nanoscale, and mesoscale structure. This may be achieved through techniques like ultraviolet spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, X-ray diffraction, and X-ray photoelectron spectroscopy [25,26].

### 6.2. Research Status Regarding the Delivery of Inhalable Nano-Formulations via DPIs

The principle of drug delivery using DPIs is that the microscale powder of the drug and the carrier (lactose), respectively, is aerosolised into the lungs due to the turbulence created by the breathing of the patient and the internal resistance of the instrument [27]. Thus, from this point of view, one can state that the research questions regarding the DPIs of the nano-formulations should be as follows:

a) whether there is any influence of the drying process on the nano-formulation;
b) how the nano-formulation is transformed into solid microscale powder and whether it is possible to deposit it into the lungs; and
c) whether it is possible to aerosolise the solid microscale powder deposited in the lungs in its original nano-formulation form. Research Status Regarding the Delivery of Inhalable Nano-Formulations via DPIs

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### 6.3. Aerosol-Based Delivery Technologies

#### 6.3.1. New Approaches to Inhalation Medicine

While inhalation medicine has been practised since ancient times, the scientific research about the effectiveness of such methods was started only in the 1950s [29]. Modern development in the area includes various designs of inhalers and more than 230 types of devices and medicines that are used. Inhalation devices that deliver inhalation medicine in practice can be divided into two general categories: inhalers and nebulisers. Among inhalers, there are dry powder inhalers (DPIs), metered dose inhalers (MDIs), and soft mist inhalers (SMIs). Nebuliser aerosol is achieved through ultrasonic, vibrating mesh, or fluid jet atomization. The difference between device categories lies in the state of aerosol that is produced by them; DPIs produce solid aerosol, while MDIs, SMIs, and nebulizers liquid aerosol. All inhalers can work with different types of chemicals and produce aerosol that has a healing effect. Yet, depending on the patient and dosage required, some devices would be preferable for certain purposes [30,31].

In the present review, our objective is to provide an overview of the emerging paradigms in pulmonary drug delivery, along with the current opportunities in this field. Firstly, we are going to consider the technologies and developments in the field of pulmonary drug delivery, customised inhalation medicine, in which the innovations aim at the optimisation of the deposition efficiency of the drug in areas of lung lesions of different patient populations. Then, we are going to discuss the shifts in cargo complexity, which took place in the field of pulmonary drug delivery, resulting in the expansion of the scope of the APIs that can be delivered through inhalation. As new technologies and particles enable the delivery not only of small molecule APIs but also macromolecule APIs, namely the nucleic acids, proteins, antibodies, and enzymes, we are going to discuss the advances in inhaled biologics (i.e., macromolecules such as protein, enzyme, and antibody APIs) and the emerging opportunity for delivering inhaled nucleic acids for gene therapy. Finally, we are going to address the diversified targets of pulmonary drug delivery, including oncolytics, vaccines, and antiviral drugs.

### 6.4. Fundamentals of inhalation delivery

#### 6.4.1. Engineering Marvel of the Lung:

Physical Barriers That Prevent Aerosols From Penetrating

The main purpose of the lungs is to facilitate the interchange of oxygen and carbon dioxide as necessary for cellular respiration. Therefore, the lungs need to effectively take up oxygen from the environment and deliver it to different parts of the body rapidly, and at the same time get rid of the waste carbon dioxide. Consequently, the lungs are directly involved with the circulation as a means of delivering gases throughout the body and constitute the cardiopulmonary system. But due to this close relationship of the lungs with the environment and internal organs, the lungs also have the important role of screening, cleaning, and preparing the environmental air to enter into direct contact with cells and tissues.

The airways of the lungs begin with the trachea, which splits into the right and left main bronchi at the anatomical structure called the carina, halfway down the chest cavity. This marks the first branching point of separation into the right and left lungs' macrostructural hierarchy. The main bronchus then continues to split in a symmetrical manner into the lobar bronchi and subsequently into the segmental bronchi. These branching points of the upper airway system are used to divide the major airway systems of each lung into

what is known as the lobes. The right lung contains three lobes - upper, middle, and lower - while the left lung has two - upper and lower. However, despite the major regional differentiation of the airway system of the lungs, the microstructure of the airways within the same regions remains similar, with each passage leading to two secondary passages which are shorter and narrower than the previous passage. Therefore, airway structure and size are normally described using bifurcation tracing and the increasing generation number of each branch point [32,33,34,35].

### 6.5. Distributing Aerosols to the Lung: The Engineering Phenomena Related to Contemporary Applications

The sizes of these particles, along with the dynamics of breathing, result in several complicated phenomena, which makes the transport of inhaled drugs quite hard to predict. Thus, the inhalation aerosols are usually described in terms of a particle size distribution in accordance with the aerodynamic diameter (dae), which is defined as the diameter of the unit density sphere having the same settling speed as an aerosol [36]. The deposition of aerosols will depend both on dae and on the characteristics of local airflow, because even though turbulence is common at the first generations, the subsequent generations have low-Reynolds-number developing flows due to numerous bifurcations [36,37].

Based on the above factors, it becomes evident that, according to common understanding, aerosols larger than 5 μm have the tendency to deposit mainly by means of impaction in the bronchi and extrathoracic airways, which comprise the mouth, pharynx, and larynx. Deposition via sedimentation or gravitational settling is the predominant method of deposition of aerosols around 0.5-8 μm in diameter and takes place due to gravitational effects; deposition takes place mainly in the range of bronchioles to alveoli and in the extrathoracic airways. Finally, aerosols smaller than 0.1 μm deposit mainly by diffusion through Brownian motion, enabling their departure from streamlines and deposition against the wall, unless they are exhaled beforehand [38,39]. Considering the above dominating methods of deposition, aerosols in the range of 1-5 μm can deposit in the lungs efficiently and are considered the optimal range for inhalation therapy [40,41].

### 6.6. Biological Barriers and Target

In addition to the geometry of the lungs, the composition of the environmental conditions conditions the air that is inhaled into the airways and delivered to the alveoli. Where symptoms of disease present themselves as macroscopic alterations of the airway leading to airway constriction, obstruction, or deterioration (e.g. airway remodelling), there are microscopic changes in the airway lining fluid that can be attributed to the effect of disease as well. Airways from the trachea down to the terminal bronchioles are lined with mucus. The conducting airways turn into the respiratory airways through multiple branch points where increasingly more alveoli appear in the airway wall. Alveoli are made up of alveolar type I (ATI) and alveolar type II (ATII) epithelial cells; ATI cells are long, thin cells facilitating gas exchange, whereas ATII cells produce pulmonary surfactant. The pulmonary surfactant is a distinctive entity compared to the airway mucus in that it is designed specifically to help balance the alveoli. Breathing involves the expansion and contraction of the lungs, leading to changes in volume of the alveoli; pulmonary surfactant helps to keep surface tension enough to keep the alveoli open [42,43]. Therefore, inhaled drugs have to get past.

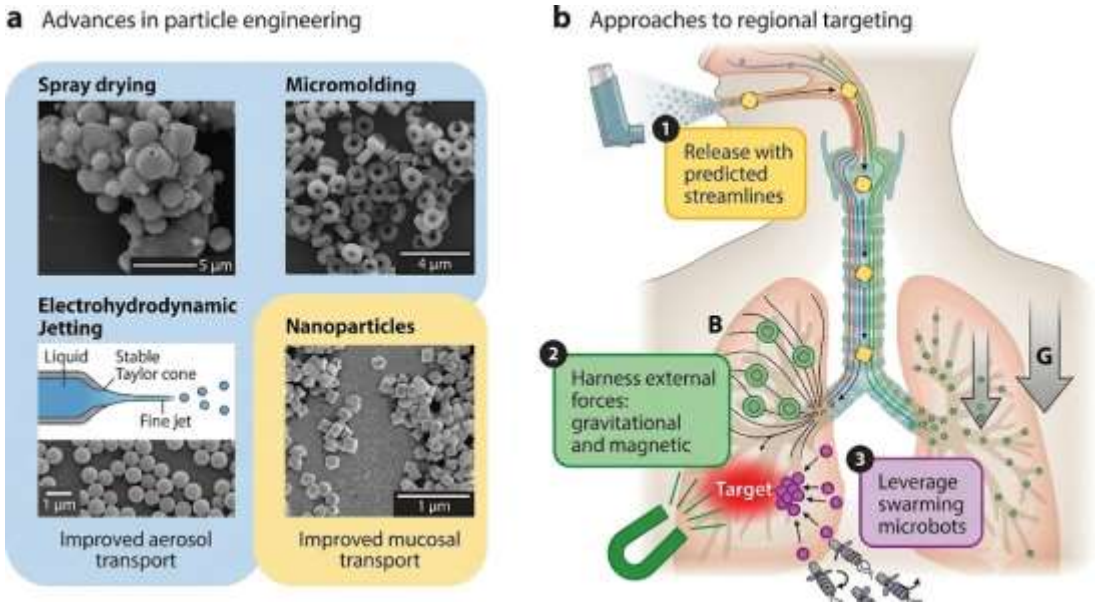
### 6.7. Biological Barriers and Target

The structure of the lungs and the composition of the environment affect the air that is inhaled through the airways and then reaches the alveoli. Whereas macroscopic changes in the airway resulting in airway

constriction, obstruction, or deterioration (airway remodelling) occur due to the presence of the symptoms of disease, the microscopic changes can be seen in the liquid lining fluid within the airway as a consequence of the disease process. The airways, which go from the trachea to the terminal bronchioles, are lined with mucus. The conducting airways become respiratory airways through many branch points as more alveoli are found in the walls of the airway. Alveoli consist of alveolar type I (ATI) and alveolar type II (ATII) epithelial cells, where ATI cells are thin cells for gas exchange, while ATII cells synthesise pulmonary surfactant. The pulmonary surfactant is a specific substance as opposed to the airway mucus because it is specially designed to keep the balance of the alveoli. Breathing consists of the lungs' expansion and contraction; this means that the volume of the alveoli changes; thus, the pulmonary surfactant maintains enough surface tension to keep the alveoli open [44,45].

### 6.8. Facilitating Innovations in Particle Engineering

The emergence of DPI technology has made possible several innovations in particle engineering that have helped change the entire paradigm of inhalation therapy, giving rise to more options to tailor delivery according to specific applications. Usually prepared using the technique of spray-drying, dry powders make it possible to gain patient-controlled aerodynamic characteristics of drug delivery because the patients themselves provide the energy necessary to aerosolise the drug by inspiring breaths. The process of spray drying allows efficient delivery that can be done inexpensively and that works well with various modes of treatment delivery with higher efficiency [46,47,48,49,50].




## CONCLUSIONS

The use of nanoparticles in the design of novel delivery systems for subunit vaccines and therapies presents a unique opportunity compared to classical vaccines, which use live or inactivated microorganisms. Some nanoparticle-based delivery systems for mucosal vaccination have been described; each system has its advantages and disadvantages. A novel generation of mucosal vaccines that rely on components of the microbes for eliciting an immune response certainly requires not only the development of novel classes of delivery systems for vaccines and adjuvants but also knowledge about the mechanisms of action of currently available delivery systems and adjuvants. Although some significant advancements have been made in the development of nanoparticles for delivery of antigens across the mucosal surfaces, there are

still some challenges remaining. Targeted delivery in mucosal vaccination today is only aimed at the mucosal epithelium and can benefit from the development of targeted delivery to M cells. Additionally, mucosal adjuvant and vaccine immune correlates of protection are not well established, knowledge of which will allow for developing novel mucosal vaccines. Lastly, the ultimate goal of most adjuvant studies is the development of new clinically applicable adjuvants.

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