Nanoparticles development for pulmonary vaccination: Challenges and opportunities

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ABSTRACT

Pulmonary vaccination is unique immune system protection treatment for the respiratory tract. Lungs contain large surface area for interaction with antigens. Nanoparticles as efficient drug carriers have been used for pulmonary vaccination. These structures contribute to the process either by encapsulating, dissolving, surface adsorbing or chemically attaching the active ingredients. Development of pulmonary vaccines via sub-micron particles has been investigated in this study. The nanoparticles deposited on the respiratory mucus, based on their size and charge, are either locally trapped or diffuse freely. Therefore, different mechanisms of particle deposition are defined based on the particle size and surface charges. Advantages and disadvantages of nanoparticles preparation methods as they pertain to pulmonary vaccine applications are comprehensively depicted. The adverse side effects of nanoparticles encountering immune cells is also discussed. Finally, the side effects and challenges of nano-pulmonary vaccines are discussed, offering a series practical suggestion for further industrial development and manufacturing of nanoparticle-empowered pulmonary vaccines.

Keywords: Challenges, Development, Nano particle, Pulmonary vaccination

Introduction on the mechanism of pulmonary vaccination

Pulmonary vaccination is a unique vaccine application strategy to boost the immune system for the respiratory tract. The large surface area in the lungs is crucial for effective interaction with antigens [1]. Additionally, the lungs possess characteristics which are advantageous for pulmonary vaccine applications such as a dense vasculature, rapid absorption, a thin alveolar epithelium, lower enzyme activity and a high solute exchange capacity [2].

The lung is classified into two physiological sections: the conducting transitional airways and the lung parenchyma. A thickheaded mucus layer with dendritic cells (DCs) was existed in conducting part that facilitates trapping of vaccines. The cover of lung parenchyma are included from epithelial cells and prepared from antigen-presenting cells (APCs). As antigens input the lung, they are absorbed either by the macrophages or DCs [3].

Inhaled particles with AD (aerodynamic diameters) more than 5 mm deposit in the upper parts of the conducting airway. The diameter of particles put down in lower parts of respiratory ways are in the range of 1-5 mm. Using PRINT (Particle Replication in Non-wetting Template) technology [4], the design of particles with aerodynamic characteristics relevant to the desired deposition features have become feasible. Deposition profiles in the lung could be one of the effects of Non-spherical structures existence. In a reported study, PRINT enables a high weight percent loading of bioactive molecules on a 50 mg of inert particles and thus the respective local and stable pulmonary delivery showed at low particle concentration, therapeutic efficacy could be seen [5]. Vaccines with pulmonary delivery specification is induced sectional immunity more effectively than typical vaccination [1,6,7]. Due to the importance of pulmonary vaccines,

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several vaccines in clinical trial was listed in Table 1 [8]. One of the advantages of this vaccination approach is the possibility of NP (nanoparticle) loading to enhance the vaccination. The long residence time of NPs in the lungs due to their ability to escape from the clearance mechanisms such as macrophage uptake, and translocation to the systemic circulation is amongst the key advantages of NPs [3]. For example, using PEGylated phospholipid nanocarriers as inhalation aerosols can significantly enhance the respiratory delivery through the nasal and pulmonary routes (Table 2) [9].

Several factors such as antigen dose, adjuvant type, deposition site, antigen release, and exposure frequency determine the outcome of pulmonary vaccination [15]. DCs, macrophages, monocytes, and B cells provide perfect physiological preconditions. In Mexico a randomized clinical trial in school children was done. In this research the diameter of aerosols of a measles vaccine were lower than 5 µm. Results of this experiment showed aerosolized measles vaccine induced a better antibody response than injected vaccine [15]. However, vaccine stability remains a concern when a liquid vaccine is utilized for pulmonary delivery.

Pulmonary vaccination using dry powder is more efficient and repeatable than that of the liquid aerosols. Particle properties can better control when making a dry powder vaccine, hence ensuring optimum inhalation and efficient delivery to the target site in the lungs. Recently, the efficacy of liquid and powder pulmonary influenza vaccines were evaluated in many preclinical studies involving mice and sheep [16,17]. Adjuvants were used to reduce the amount of antigen and induce CTL responses upon pulmonary vaccination. The advantages of adjuvant utilization for this application extends beyond these effects.

Adjutants can be used to manipulate the ratio of different antibody subtypes prepared in response to the vaccine [15]. NPs are generally referred to particles with 1-100 nm however, NPs larger than 100 nm are required for well-organized drug loading [2,15]. Important physicochemical parameters influence the interactivity between antigen-loaded nanoparticles, immune cells and the immunological outcome include shape, size, hydrophobicity, surface charge, colloidal stability, solid-state characteristics, and bio-adhesive properties. Nanoparticles with 20-100 in diameter exhibit prolonged circulation time, as compared with smaller or larger particles. Late studies shows that 50-nm particles are more fruitful taken up by DCs in the pulmonary mucosa and induce costimulatory signals, as compared with 500-nm particles [18]. Following interaction with the biological milieu, hydrophobic NPs are coated by adsorption of plasma proteins, which prime the nanoparticles for approval by the reticuloendothelial systems [19]. In contrast, hydrophilic particles display extends circulation half-life in vivo [20]. The colloidal stability also affects the cellular interaction, as nanoparticle suspensions can aggregate over time and become internalized by mononuclear cells [21].

Table 1. Examples of Pulmonary vaccines in clinical trials [8]

Vaccine	Pathogen/condition	Administration route	Clinical trials (government identifier)	Status/phase
Neisseria lactamica	Meningitis	Nasal	NCT03549325	Recruiting/Not applicable
RSV ΔNS2/Δ1313/ I1314L and RSV 276	RSV infection	Nasal	NCT03227029	Recruiting/I
RSV 6120/ΔNS2/1030s	RSV infection	Nasal	NCT03387137	Recruiting/I
Ad5Ag85A	Tuberculosis	Intrapulmonary	NCT02337270	Recruiting/I

Table 2. Examples of aerosolized PEGylated nanocarrier delivery to the lungs [9]

Aerosol type	PEG length	Therapeutic	Carrier	Disease	Reference
DPI	2,3, and 5 kDa	Paclitaxel	PEG	Lung cancer	[10]
DPI	5 kDa	Carcumin	PLGA-PEG chitosan	Ashtna, COPD, Cystic Fibrosis	[11]
DPI	8 kDa	Ciprofloxacin	PEG	Respiratory infections	[12]
Colloidal dispersion	2 kDa	Ciprofloxacin	PEGylated liposome	Respiratory infections	[13]
DPI	-	insulin	PEG	Diabetes mellitus	[14]

Additional factors are considered during utilizing polymeric NPs in formulation of vaccines, include glass transition temperature, crystallinity, and bioadhesiveness[22]. The antigen release correlates with amorphous parts of polymers. It is noted that the crystalline state results in decreased release rate of the loaded antigen [22,23]. Inclusion of bio adhesive polymers, improves the exchange and lengthens the contact time with the mucosal surfaces. Due to this fact, these drug delivery mechanisms are efficient for targeting drugs to specific cells [24].

Drug carriers like NPs function either by encapsulating, dissolving, surface adsorbing or chemical attachment for active component. Typical presentation of the lung structure focusing on the size of particles depositing is shown in Fig 1.
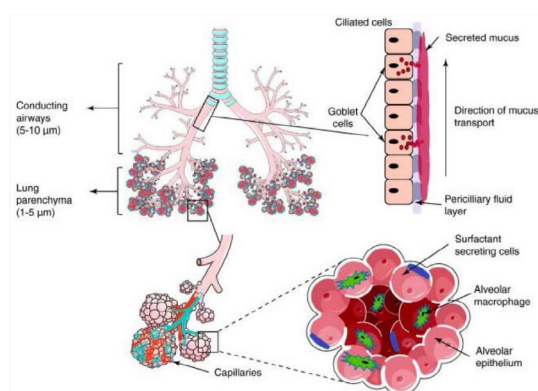

Fig 1. Schematic representation of the lung structure with indication of the size of particles depositing in the depicted areas [15]

In this study, development of pulmonary vaccines concerning on the sub-micron particle loading is investigated.

The therapeutic targets selected for vaccines include various regions of lung. The pulmonary vaccines for pathogen colonies that form on the upper parts of the lung (e.g. upper bronchi region) such as chlamydia pneumonia utilize carrier nanoparticles with particles larger than 5 micrometer average sizes [25]. In order to combat infections deep in the bulk of the lung tissue (e.g. streptococcus pneumonia and bacillus anthracis) particles with diameters smaller than 3 micrometers are used to produce the respective pulmonary vaccines [26]. There are various design strategies based on the need for specific target for the vaccines in development. Nano-in-Micro (NiM) formulation, multi core-shell structure design,

utilization of excipient particulates along with the vaccine particles, and dispersion manipulation are among some of these strategies [27].

Which nanoparticles could be used in pulmonary vaccines?

Although studies have shown direct pulmonary administration of NPs is not feasible, NPs could be converted to dry powder inhaler (DPI) formulations by using safe excipients [9]. This technique enabled improved deposition and stability of NPs compared to direct administration of NPs.

Both natural (albumin, gelatin, alginate, collagen, cyclodextrin, and chitosan) [28] and synthetic (poly (lactide-co-glycolide) (PLGA) [28], polyacrylates and polyanhydrides) polymers were used in formulation of pulmonary nanovectors [29]. Due to the mucoadhesive characteristics of chitosan, it could be favorable for mucosal vaccine delivery. It was reported that Chitosan has been broadly studied for delivery of vaccine antigens delivery in the mucosal surfaces during preclinical animal infection models [30-32]. However, chitosan presents low solubility under physiological conditions. Due to this issue its use for biomedical applications has been limited [33]. To miss this downside a number of fuctionalizes chitosan have been improved. Numbers of chitosan derivatives are contained trimethyl chitosan, hydroxyethyl chitosan, chitosan ester, phosphorylated chitosan, and sulfated chitosan [34]. Trimethyl chitosan is the most studied chitosan derivative for mucosal vaccine utilizations. Trimethylated chitosan NPs induce strong mucosal immunity against hepatitis B virus following nasal administration [35]. Trimethyl chitosan-coated liposomes were exhibited to persuade high mucosal and systemic antibody titers upon nasal administration [36] and had a protection against infection of Group A *Streptococcus* [37].

Gamma polyglutamic acid reveals high water solubility and biodegradability and was used for mucosal vaccinnation. Gamma polyglutamic acid-based NPs have been presented to induce protective immunity against influenza virus infection following nasal immunization [80]. Poly-gamma glutamate/chitosan NPs induced protective mucosal immunity against influenza virus infection [38]. Acceptable mucosal immune responses in the nasal mucosa against Group A *Streptococcus* infection using these NPs were reported [37].

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Hyaluronic acid has a high biocompatibility, biodegradability, and mucoadhesiveness, which are desired properties for mucosal vaccination. It represents a multifunctional carbohydrate mediator of immune process [40]. Delivery mechanisms using hyaluronic acid-based have been tested for mucosal vaccination. Hyaluronic acid linked with heat labile toxin-based mucosal adjuvant (LTK63) was shown to induce systemic and mucosal antibody responses following nasal administration with the influenza hemagglutinin antigen [41]. In another study, nasal delivery of F1-V, which is a candidate recombinant antigen from *Yersinia pestis*, using NPs composed of hyaluronic acid and cationic liposomes induced robust humoral and a balanced Th1/Th2 immune responses [42].

The other major water-soluble linear polysaccharide is pullulan, which has been used for NP-based vaccine delivery systems designed to deliver antigens across the mucosa. A nanogel consisting of a cationic type of cholesteryl group-bearing pullulan formulated with *Clostridium botulinum* type-A neurotoxin induced strong tetanus toxoid- specific systemic and mucosal immune responses after nasal immunization [43]. Besides, the pullulan-based nanogels induced Th2 and Th17 cytokine responses in the serum and respiratory tract tissues of macaques after nasal coadministration with pneumococcal surface protein A [44].

Nasal administration of cholesteryl pullulan-encapsulated tumor necrosis factor-α NPs exhibits strong immunity against influenza [45].

High surface area of Nanovectors, could be resulted in developed drug loading, and these particles are small enough to cross biological barriers and transfer their cargo into cells [28]. Particles were entered in lung sections based on the particle size. The huge particles (1-10 μm) are located in the trachea and bronchi, whereas smaller particles (such as NPs) could be seen in the deeper parts of the lung. Inhaled particles may be deposited in the lung by the following

Table 3. NPs used for pulmonary vaccine delivery

Nano particle	Vaccine type	Effect	Reference
DNA-lipid nanocomplexes	HIV	Targeting the large population of DCs and lining the alveoli	[53]
lipid-chitosan hybrid NP	Hepatitis B	Generated long-lived antigen-specific antibody	[53]
PGA-co-PDL polymeric (NPs)	NPs are suitable for targeting lung DCs. NCMPs are promising carrier for pulmonary vaccine delivery.	Bovine serum albumin adsorbed poly PGA-co-PDL polymeric NPs within L-leucine microcarriers for dry powder inhalation. Showing a low toxicity profile	[54]
Micelles (Peptide Cross-linked micelles-PCMs), 50nm	HIV	PCMs encapsulate immuno stimulatory DNA in the core and bind peptide antigens through disulphide linkages. In the presence of a high concentration of glutathione they deliver antigenic peptides and immuno stimulatory DNA to APCs	[2]
Liposomes	-	Dimyristoyl phosphatyl-choline (DMPC):cholesterol(CH)-(7:3) liposomes were prepared by dehydration-rehydration followed by freezing-thawing method. The enzyme, GUS, was successfully encapsulated and showed encouraging activity following decreolization	[2]
Polymersomes, 250 nm	influenza hemagglutinin (HA)	Polymersomes acted as an immune adjuvant and showed an improved immunogenicity.	[2]
Poly(lactic-co-glycolic acid) (PLGA), 900nm	Hepatitis B	PLA and poly lactic-co-glycolic-acid were tested for pulmonary delivery in rat spleen. Enhanced immune responses was reported	[2,55]
polyanhydride nanovaccines encapsulating F1-V	H5N1 avian influenza	Amphiphilic CPTEG: CPH nanovaccine enhanced early CD8+ T cell expansion and differentiation into effector memory phenotypes.	[56]
PGA-co-PDL NPs in Pulmonary dry powder vaccine	Streptococcus pneumoniae	Antigen PspA adsorbed onto the surface of polymeric NPs encapsulated in L-leucine microparticles. PspA released from the dry powders-maintained antigen stability, integrity and activity.	[57]
Dried bacterial rod-like structures (1-4 mm in length and 200-400 nm in diameter) coupled with small particles of leucine (1 mm)	Pulmonary vaccination	Showed drug efficacy and reduced the bacterial burden in guinea pigs.	[58]
DNA bind with the PLGA polyethyleneimine	DNA vaccine encoding the bacterial latency antigen Rv1733c	Binding showed enhanced T-cell immune response	[58]
Alginate NPs	Diphtheria	Toxoid-loaded alginate NPs showed highest humoral immune response	[59]
NP and CpG	-	Better protection against challenge	[1]
Chitosan-DNA	Mycobacterium tuberculosis	Enhances the immunogenicity of a DNA vaccine encoding HLA A*0201-restricted T-cell epitopes of tuberculosis	[60]

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three mechanisms; impaction, sedimentation and diffusion. The particle size in impaction sedimentation and diffusion was more than 5 μm, between 1-5 μm in diameter and the sub-micron particles respectively [46]. Depending on size and charge, NPs deposited on the respiratory mucus were either locally trapped or diffuse freely.

Long NP persistence enables interaction with cells of interest for a longer time in order to become effective by standing in the lung or crossing the air-blood barrier. The interaction of NPs with pulmonary immune cells was of great interest because NPs can be easily impacted in the lungs and immediately interact with various cells in the immune system [46]. Several applications of NP in formulation of pulmonary vaccine were shown in Table 3.

Substance, size and surface functionality of NPs can be attentional to optimized the pulmonary vaccination. It was estimated that an average human lung includes of about 300 million alveoli providing an 80–90 m2 surface area of exchange. This surface area was enough to deliver a sufficient amount of antigen and NPs [2].

Immunostimulating complexes (ISCOM) are among the most efficient mucosal delivery systems. Particularly, they are effective in combating respiratory syncytial viral infections (RSV) [47]. These complexes include a matrix containing quillaja saponins, cholesterol, and phospholipids. The ISCOMs for pulmonary vaccination are geometrically hollow spherical structures including the mentioned molecules. They pose as multivalent antigens and promote phagocytosis by Antigen-possessing cells (APCs) [48]. Based on the size of ISCOMs they are considered as nanoparticles (50 nm) or microparticles (1000-50000 nm). The nanoparticle ISCOMS are among the most versatile nasal vaccine delivery systems [49]. Helgeby et. al. [50] showed that cholera toxin A1 (CTA1)-DD/Quil-A ISCOM could be prepared and showed that it yielded a high-efficiency and activity influenza virus vaccine to be administered nasally. In another report by Abdel Kader and coworkers [51], showed that via Span 60 assembly niosomes with ability to control successful ocular delivery and release of naltrexone hydrochloride (NTX). Although the size analysis of the ISCOM were in micrometer range, the Ex vivo transcorneal permeation study showed that the structure was able to deliver and release the active antigen with high efficiency.

Interbilayer-crosslinked multilamellar vesicles (ICMVs) are nanostructured vesicles in capsule-like shape with multilayer lipid bi-layer walls cross-linked together. These vesicles are the perfect carrier to deliver antigens used for pulmonary vaccination. These nanoparticles have been modified with monophosphoryl lipid A (MPLA) which then is readily used as HPV vaccines approved by FDA [52].

Importance of particle size classification and particle charge of NPs in pulmonary vaccination

Based on the equation (1), the particle standings inside the lungs is probed by the aerodynamic particle size (da), a sphere's diameter (density-1 g/cm3) in air with equal velocity to the particle in consideration [2].

$$d_a = d_g \sqrt{\rho/\rho_a}$$

Where ρ, ρa, and dg are the particle mass density (g/cm3), the unit density (1 g/cm3), and the geometric diameter (cm), respectively. Particles of 10 μm (da) and larger are usually impacted within the throat or sedimented in the bronchial region. However, being exhaled and often not being deposited in the alveolar region is the most common outcome of using particles smaller than 1 μm (da). It can be anticipated 1 to 5 μm (da) particles pass through the throat and reach the periphery of the lung. Since <1 μm particles main drive is diffusion and are often exhaled, they have to be delivered within microparticles. Microparticles prepared from NPs are about 1–5 μm (Optimal aerodynamic size for pulmonary delivery) and encompass inert pharmaceutical excipient acting as carriers [2]. In contrast, 10 μm< AD particles are put in the throat while particles with the diameter lower than 1 μm most likely enter the alveolar areas or are exhaled. Due to this reason, they are not appropriate for pulmonary delivery [58]. Spray freeze drying (SFD) produces porous spherical powders, with more than 90% of particles with 1-5 μm aerodynamic size. Size determines the antigen delivery through governing the direction, speed, and translocation efficacy into the lung parenchyma, lymphatic system, specific pulmonary APC populations, as well as the blood circulation system [58].

Considering the delivery to peripheral lung parenchyma, it is not advised to utilize too small, or too large particles. The former type of particles

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lead to increased subsequent exhalation and the latter causes the particle deposition in upper respiratory airway as well as mouth and throat. The 1 -2 μm AD aerosol particles can be deposited in the lungs with up to 90% success rate should they be inhaled slowly and deeply. This method enables most of the aerosol to reach peripheral alveolar areas. Vaccine antigens are commonly categorized in three-dimensional types. The smallest antigens (< 10 nm) are comprised of proteins or viral subunit antigens that are often combined with adjuvants to form larger particles or aggregates.

The latter vaccine antigen preferable deposition occurs in the peripheral lung parenchyma. However, the former type (5- 10 μm) mainly reaches the upper regions of the airways. NPs of ADs of <100 nm can travel to the alveolar region. Nonetheless, their deposition is disrupted by exhalation. The antigen and/or its respective carrier geometrical size also determines the interaction with particular APCs and pharmacokinetics and pharmacodynamics of transferring the antigen to the draining lymph nodes (immune response generation area in the body). On the one hand, in studies with nanoparticles (< 30 nm), these nanoparticles rapidly enter the lymphatic system and drain to regional lymph nodes passively. On the other hand, larger particles (from ~100 nm to a few μm) efficiently transfer into the lymphatic vessel APCs. These observations indicate the importance of particle size for local delivery of vaccine (Fig 2 and 3) [61].
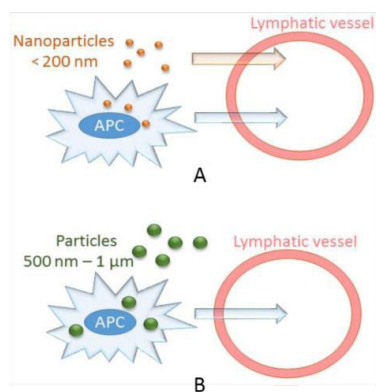

Fig 2. The size of the particle affects its entry into the initial lymphatic vessel. (A) Particles smaller than 200 nm in diameter can efficiently enter the lymphatic system; (B) Particles larger than 500 nm require active transport by APCs to the lymph nodes [53]
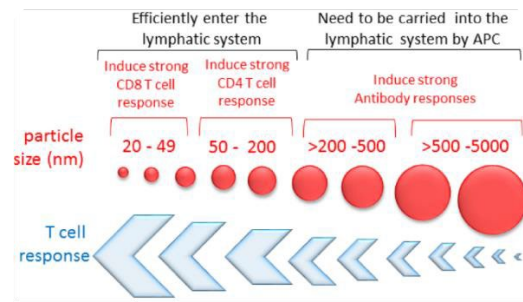

Fig 3. Dependency of efficiency of particle loading on size of particle [53]

It was reported that DC population of lung and the increase in production of lung chemo-attractants are related to cationic NPs. This effect can lead to utilization of NPs for pulmonary vaccine carrier applications [2]. Nonetheless, anionic NPs are internalized by AMs, which are considered as APCs. The main function of these APCs is clearing the lungs and sequestering foreign material and maintain homeostasis in the lungs. AMs are not the initial target of NP vaccines. Hence, rendering the design of DC-targeting particles more important. Anionic NPs secluded in AMs tend to be unsuccessful at evoking an adaptive immune response [2]. Cationic formulations offer superior responses specifically in the lung.

These cationic NPs lead to promoting an environment sufficient to express antigen-specific responses for vaccination, with minimal safety concerns and also modulated the local lung environment to improve the lung DCs recruitment and maturation. These milestones are accomplished while extensive AM clearance is avoided. In contrast, anionic NPs are proven to be inert in the lung from an immunological perspective. Hence, offering an alternative therapeutic potential towards tolerance promotion [2].

Effects of NPs loading on pulmonary vaccination

Nano- in micro-particle (NiM) was one of the effective structures for drug and vaccine delivery. It was presented that a dry nano-in-microparticle structure developed by a two-step method could be a promising route for vaccine applications. During that process, preparation of primary NPs via ionic gelation, was followed by dry powder vaccine fabrication via spray drying method. In a practical investigation, chitosan was found to be a reliable adjuvant and particle forming excipient as it possesses mucoadhesive and adjuvant properties

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[63-65]. It was shown that these powder vaccines can cause high capsule and device retention and consequently a failed vaccine delivery and ultimately injured the inhalator. The solution for this drawback was to add 1% magnesium stearate solution to the dry powder followed by blending for 3 hr.

A strong cellular immune response was elicited via treating NP-based vaccines attached to adjuvants as an immediate response to viral infections. Drug delivery approaches to the lungs for treating asthma would likely be accomplished by larger particles which release their active ingredients to extracellular spaces. Conversely, in the case of loading a respiratory vaccine, smaller particles with more potential of antigen cells uptake and delivery to lymph nodes are deemed more appropriate. Latest findings showed that regardless of their size, particles approximately remain in the lungs for up to 7 days after instillation. Hence, rendering them able to provide sustained localized delivery of therapeutic active ingredients via particulate formulations [61]. This observation was significantly different from the fast pace clearance shown for small particles (50 nm AD) and reflected the importance of design parameters for particle-driven therapeutic intervention applications [61].

Many identified DC subsets are categorically divided into cDCs and pDCs. Comprehensive investigations on murine and human lungs showed that two major subsets of cDCs were present in steady state conditions [65]. In murine lungs, these cDCs subsets expressed CD103 and CD11b, respectively [65,66]. While NPs of both positive and negative surface charges were able to transfer to the draining lymph nodes, both CD11b and CD103 lung DC subsets were exclusively associated with cationic NPs. Instillation of cationic NPs yielded the upregulation of Ccl2 and Cxc10. Therefore, the increased efficacy of pulmonary vaccine carriers with positively charged surfaces was explained through these cellular mechanisms [67].

An anatomic approach was to deliver NPs deep in the lung to target the large population of DCs lining the alveoli, which actively extend processes into the alveolar lumen to survey for microbes [53]. Vicente et al. produced lipid-chitosan composite NPs. This structure was comprised of an oily core with imiquimod and surrounded by a phospholipid layer and a chitosan coating. A hepatitis B protein antigen was adsorbed on the chitosan layer [53].

This design was in corroboration with the recent findings indicating that NPs captured by APCs are exclusively effective in the depth of respiratory tract. Sanders *et al.* [53] showed that when the antigen carried with ISCOMATRIX NPs delivered to the total respiratory tract rather than the upper respiratory airways led to stronger immune response induction.

Employing the mucoadhesive NPs could enhance the vaccine uptake by immune cells. This concept led to developing a process of co-conjugation of the danger signal flagellin to PPS-NPs. Through this approach an increased antibody titer to the vaccine antigen was achieved. Furthermore, when ovalbumin-containing 30-nm PPS-NPs were mixed with CpG, and delivered via pulmonary routes, induced enhanced cytotoxic CD8+ T-cell responses. Hence, enhancing the protection against an influenza virus in a more effective way compared to the soluble pulmonary vaccines.

NPs have also been designed to improve epithelial cell binding and transport across the epithelial barrier, further enhancing the pulmonary vaccine responses. hemagglutinin adhesion protein that binds to Heparin has been identified as an antibody able to bind to heparin-sulfate-containing targets on epithelial cells of lung [53].

The required particle dimensions to optimally deposit antigen onto a specific lung compartment can be different from the theoretically ideal particle dimensions to target a specific APC. This dilemma was observed for vaccines using nanocarriers as they can abruptly reach draining lymph nodes with insignificant deposition in the respiratory tract. Hence, limiting their interactions with APCs. A new approach utilized incorporation of NPs into micrometer-scale hybrids as a means to address this problem. Through this technique, NPs were incorporated to form hollow or porous microparticles. These "Trojan" particles are empowered via the virtues of both nano- and micro-scale particles as the micronized hybrids were deposited in the lung. Nevertheless, the size-dependent features of NPs were maintained post-release triggered by lung's humid environment and its lining fluid [61].

Effective method of nanoparticle processing in pulmonary vaccines

One of the major parameters affected on the

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price and quality of pulmonary vaccines was the method of NP loading or preparation. Therefore, optimized method of NP preparation in pulmonary vaccine have been investigated in this study.

Pulmonary vaccination can be attained using aerosolized liquid or dry powder vaccines [58]. It was shown dry powder-based influenza vaccines were specifically attractive as they offer higher stability in comparison with liquid vaccines that at extremely low and elevated temperatures are prone to degradation. Creating stable dry powder compounds before nebulization has been identified as a solution to this difficulty. This modified vaccine could improve local and systemic antibody production [1]. Dry pulmonary vaccine could be produced by several drying methods like jet-milling, spray-freeze drying, supercritical fluid drying, and spray-drying. The engineering of particles that are hard to be readily synthesized by common manufacturing processes was made possible via spray-drying. Additionally, nanotechnology can be a practical route to systematically design and manufacture high-performance dry powder formulations [68].

Smith et al., performed split influenza virus vaccine subunit encapsulation in spray-dried microparticles which contained distearoyl-phosphatidylcholine (DSPC) and DPPC. Following that process, freeze-drying of the product yielded inulin-stabilized influenza vaccine powder which was then successfully delivered to the lungs in mice subjects [69]. Advantages and disadvantages of NP preparation methods were shown in Table 4. It is crucial to investigate NP properties influence on modifying pulmonary immune responses [2].

Pulmonary vaccination device

There are two primary methods for introducing medicine into the lungs: inhalers and nebulizers. Both types of devices essentially perform the same function: produce small droplets of medicine (increased surface area) administered directly to the patient during inhalation events.

Inhaler – Aerosol

Inhalers are portable devices filled with medicine that can be administered on demand by the patient in single, discrete doses.

Table 4. Advantages and disadvantages of preparation methods of NPs used in pulmonary routs. Inhalers are currently used to treat a variety of lung conditions including asthma, bronchitis, pneumonia, and COPD. Inhalers are broken up into two categories: metered dose inhaler (MDI) and dry powder inhaler (DPI). MDIs, such as Aerospan, aerosolize the liquid medicine through a special nozzle to make administering the medicine more effective during normal inhalation. DPIs, such as Advair, aerosolize dry medicine during a forceful inhale to ensure the dry medicine breaks up into smaller powder and fly into the lungs. Both forms require special formulation considerations to ensure the delivery of medication is effective [70,71]. Both inhaler types will work for nanoparticle based pulmonary vaccination treatments. Nanoparticle vaccine can be specially formulated to maintain a consistent concentration in a liquid suspension or in a dry powder [70,72]. The challenge with nanoparticle-based vaccines lies in the formulation for these types of applications to maintain stability, proper aerosolization, and effective time-of-flight in the pulmonary tract to reach the target zone.

Nebulizer – Aerosol

Nebulizers are powered devices which

Table 4. Advantages and disadvantages of preparation methods of NPs used in pulmonary routs

Method	Advantages	Disadvantages
Emulsification/Solvent Evaporation	Hydrophilic and hydrophobic drugs can be encapsulated	Agglomeration of nanodroplets during evaporation, [2]
Emulsification and Solvent Displacement	Control over the size of NPs	Possibility of water-soluble drug leaking into the external aqueous phase, Large amounts of water to be removed, [2]
Salting Out	High loading efficiency, Easy scale-up	Removal of electrolytes, Incompatibility of salting-out agents with drugs, [2]
Nanoprecipitation	Simple, fast and reproducible, Easy scale-up, low surfactant concentrations required	Less polymer in the organic phase, [2]
Spray-dried NPs of Bacille Calmette Gue´rin (BCG) in	The effectiveness of aerosol delivery immunity against Mycobacterium tuberculosis	[68]
Spray nozzles (four-fluid spray nozzle and two solution mixing type nozzle)	Preparation of these nozzles can produce nanocomposite particles in one-step. Spray drying is suitable for scaling-up.	[68]

aerosolize liquid medicines into a mist that is easy to inhale. These are considered an active form of administration where the patient dons a mask covering the nose and mouth and can breathe normally while the nebulizer generates the mist for usually 5-10 minutes. Nebulizers are not as portable as inhalers and so are typically used for longer term treatments or for applications where inhalers would create more stress for the patient. There are three types of nebulizers: jet, which uses compressed gas to make aerosols by bubbling through a liquid reservoir and a nozzle; ultrasonic, which produces aerosols through ultrasonic vibrations (largest particles); and mesh, where the aerosol is made by pushing a liquid through a fine mesh (smallest particles). For all these types, the nanoparticle vaccine formulation would need to properly aerosolize with compressed gas, standup to ultrasonic vibrations and vaporize, and effectively form discrete droplets when pushed through a mechanical mesh [70,71]. Nanoparticles by their nature can be designed to effectively form discrete units, much like a colloid suspension, in the air during inhalation. The nanoparticle size can be tuned based on the vaccine type to properly dose the correct quantity of vaccine during a normal nebulizer session.

Side effect and challenges in nano-pulmonary vaccines

One of the main properties of aerosolized vaccines has not been clarified yet is safety considerations, especially in high-risk childs with asthma and HIV. Due to this fact, randomized trials contain disease-related end points on the clinical effectiveness are needed to assess utility and address safety of aerosolized vaccines [46].

A clear and final conclusion on pulmonary vaccines cannot be drawn, despite the various clinical tests. Most of obstacles and concerns during pulmonary vaccination was related to special groups such as children. A comprehended inhalation maneuver control is required to have effective drug delivery in the target aera, especially when the location of target is in the deep lung. Holding the breath for a minimum period of 5 seconds is desired for sedimentation time of particles. The older people may have larger residual lung volumes in comparison with their alveolar volumes; due to this issue finding a convective aerosol transport into this final airway generation is impossible. In addition, the total inhalation maneuver, taking more than 10 to 15 s including the breath-hold period, may be too long. Kids also have high breathing frequencies.

Under 6 to 12 months babies are only nose breathers. Only children 4-6 years old can comply with the inhalation instruction given [1]. It is evident that the side effects of pulmonary vaccination would be different due to the various age of customers.

Several trial researches on pulmonary vaccination, have paid attention to safety considering the side effects. Investigations indicates that fever was the only adverse reaction presented more frequently for the aerosol group. In the dose-escalating study on pulmonary HPV16 vaccination in 18-45 years old women, mild pharyngeal discomfort was the only recorded symptom. One of the volunteer found that dyspnea, chills and fever after a booster vaccination. In this study, on pulmonary vaccination study with the valent pneumococcal vaccine (32 – 12 years), dry mouth, headache, diarrhea, dizziness, and chills was reported as side effects. However, it should be noted almost no volunteers with the pulmonary

Table 5. Side effects of NPs for pulmonary immunological applications [62]

NP	Disadvantage
Liposomes, 30-200nm	• Poor stability and drug loading efficiency
Polymeric Micelles ,10-100nm	• Poor translation of micelle platform between protein antigens • Encapsulation lead to antigen degradation
Solid polymer particles	• Inflammatory properties of some depredated products
Dendrimers, 10-30nm	• Limited tissue permeability due to the high molecular weight • Cause Inflammation and complement activation
Carbon NPs, 50-400nm	• Need functionalization to ensure solubility and cytotoxity • Some functionalized products can active innate immune system and inflammation • Non-biodegradable

vaccination developed measurable antibody titers. The main side effects in the control group receiving the vaccine subcutaneous were pain at the injection site, general malaise, and headache. Consequently, the information on safety of pulmonary delivered vaccines is restricted. With regard to safety, a specific target group, like children and immune compromised populations, require extra attention [46]. In a vivo researches on pulmonary vaccines shows that particle size augments uptake into the immune cells of the lungs, with larger particles taken up less than smaller ones. This result suggests a duality in design considerations based on therapeutic features [73].

The immunological response to nanomaterials in human cells is one of the most important obstacles that should be attention in the next academic and industrial researches [46]. Moreover, the adverse side effects of NPs encountering immune cells also are important to be attention, such as immune-mediated destruction or rejection, which might cause the elimination of NPs and immune-toxicity, and damage to the immune system [46].

Important comments for scale up and industrial production of pulmonary vaccine

Some major instructions and parameters for understanding and solving the problem of pulmonary vaccine production are presented below:

Studies showed, with confirmation from academic reports, the use of pulmonary vaccine require more standardization of dosage, delivery device, and the aerosolized airway safety are needed for adoption confirmation of industrial products [15]. One of important factors for industries is sensitivity of nano-pulmonary vaccine to the temperature and maintenance of the cold-chain from the industry to the end customer which increased product price. Thermally staple NP carriers in pulmonary vaccines are suggested to overcome the temperature problem and decrease the production cost [69].

Dry powders may retain have a lower power over the same period in the absence of cold chain facilities in comparison with liquid phase. This is resulted in decreasing the safety risks from using contaminated materials. According to these considerations, dry powder improvement/ stabilization has been focused in both industrial and academic researches. Powders are generally

less complex and cheaper than that of the nebulizers and have the advantage of being more immunogenic in comparison with the liquids [1].

Pulmonary adoption for elders is another important focus of industries. Inhaler devices for low-age babies and children are too expensive to be disposable. Many inhalers, especially those having capsules, need the inhalation of 1.5 to 2.5 liters of air to exempt the entire dose in the inhaled air stream [1]. Prepare a stable adjuvanted influenza totally inactivated virus vaccine for pulmonary vaccination is one of the high-attentional academic approaches. Therefore, careful evaluation of antigen and adjuvant stability is required to determine storage temperatures and date of expiration [15].

In academic investigations, major fundamental questions have to be answered. For example, why does a particular vaccine induce a stronger immune response and protects against infection after pulmonary administration than that of the type of vaccine does not? This is because the vaccines are formulated differently can couse different aerosol properties, like particles size distribution. Additionally, optimal site of NP deposition is one of the important motivations in academic studies. Proper formulations and aerosols can be developed in combination with an inhalation equipment suitable for the target populations based on the deposition site [1].

Glass transition temperature (Tg) can have a major role in maintaining pulmonary powder properties at upper temperatures for a long period. This is because the residual moisture in the powder vaccine during storage. The presence of residual moisture in powder during packing can drastically reduce the Tg of the final product, compromising vaccine deposit at high temperature. Thus, evaluation of Tg after lyophilization is critical to define storage conditions for industries. Powder characteristics are the function of several complex phenomena such as diffusion velocities, distribution of components at the air-liquid phase, evaporation rate of droplets, and concentration gradients.

CONCLUSION

One of the important advantages of Pulmonary vaccines is the effective local immunity than that of the conventional vaccination. Other huge advantages of pulmonary vaccination is the possibility of utilizing NPs to enhance the

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vaccination. This study shows that based on size and charge, NPs deposited on the respiratory mucus are either locally trapped or diffuse freely. Depending on the particle size, various mechanisms of particle deposition in the lungs could be defined.

REFERENCES

1. Wouter FT, Kersten GF, Frijlink HW, Hinrichs WL, de Boer AH, Amorij JP. Pulmonary Vaccine Delivery: A Realistic Approach?. J Aerosol Med Pulm Drug Deliv. 2012; 25(5): 249-260.
2. Kunda NK, Somavarapu S, Gordon SB, Hutcheon GA, Saleem IY. Nanocarriers Targeting Dendritic Cells for Pulmonary Vaccine Delivery. Pharm Res. 2013; 30(2): 325-341.
3. Shiehzadeh F, Tafaghodi M. Dry Powder form of Polymeric Nanoparticles for Pulmonary Drug Delivery, Current Pharm Design. 2016; 22(17): 2549-2560.
4. Catherine A, Rahhal TB, Robbins GR, Kai MP, Shen TW, Luft JC, DeSimone JM. Nanoparticle surface charge impacts distribution, uptake and lymph node trafficking by pulmonary antigen-presenting cells. Nanomedicine: Nanotech Bio and Medicine. 2016; 12(3): 677-687.
5. Fytianos K, Drasler B, Blank F, von Garnier C, Seydoux E, Rodriguez-Lorenzo L, Petri-Fink A, Rothen-Rutishauser B. Current in vitro approaches to assess nanoparticle interactions with lung cells, Nanomedicine. 2016; 11(18): 2457-69.
6. Orga PL, Faden H, Welliver RC. Vaccination Strategies for Mucosal Immune Responses. Clin Microbiol Rev. 2001; 14(2): 430-45.
7. Shakya AK, Chowdhury MYE, Tao W, Gill HS. Mucosal Vaccine Delivery: Current State and a Pediatric Perspective. J Control Release. 2016; 240: 394-413.
8. Thakur A, Foged C, Nanoparticles for mucosal vaccine delivery. Nanoeng Biomat Adv Drug Delivery. 2020; 603-646.
9. Muralidharan P, Mallory E, Malapit M, Hayes DJ, Mansour HM. Inhalable PEGylated Phospholipid Nanocarriers and PEGylated Therapeutics for Respiratory Delivery as Aerosolized Colloidal Dispersions and Dry Powder Inhalers. Pharmaceutics. 2014; 6(2): 333-53.
10. Meenach SA, Anderson KW, Zach Hilt J, McGarry RC, Mansour HM. Characterization and aerosol dispersion performance of advanced spray-dried chemotherapeutic PEGylated phospholipid particles for dry powder inhalation delivery in lung cancer. Eur J Pharm Sci 2013: 49(4): 699-711.
11. Osman R, Kan PL, Awad G, Mortada N, El-Shamy AE, Alpar O. Spray dried inhalable ciprofloxacin powder with improved aerosolisation and antimicrobial activity. Int J Pharm. 2013; 449(1-2):44-58.
12. El-Sherbiny IM, Charles Smyth HD. Controlled release pulmonary administration of curcumin using swellable biocompatible microparticles. Mol Pharm. 2012; 9(2): 269-280.
13. Chono S, Suzuki H, Togami K, Morimoto K. Efficient drug delivery to lung epithelial lining fluid by aerosolization of ciprofloxacin incorporated into PEGylated liposomes for treatment of respiratory infections. Drug Dev Ind Pharm, 2011; 37(4): 367-372.
14. Insulin inhalation—Pfizer/Nektar therapeutics: Hmr 4006, inhaled peg-insulin—Nektar, PEGylated insulin—Nektar. Drugs R&D. 2004; 5(3): 166-170.
15. Padmanabh HP, Development of adjuvanted influenza vaccines for pulmonary delivery. University of Gronigen, PhD thesis. 2014.
16. Audouy SAL, van der Schaaf G, Hinrichs WL, Frijlink HW, Wilschut J, Huckriede A. Development of a dried influenza whole inactivated virus vaccine for pulmonary immunization. Vaccine. 2011; 29(26): 4345-52.
17. Wee JLK., Scheerlinck JP, Snibson KJ, Edwards S, Pearse M, Quinn C, Sutton P. Pulmonary delivery of ISCOMATRIX influenza vaccine induces both systemic and mucosal immunity with antigen dose sparing. Mucosal Immunol. 2008; 1(6): 489-96.
18. C.L. Hardy, J.S. Lemasurier, R. Mohamud, J. Yao, S.D. Xiang, J.M. Rolland, R.E. O'Hehir, M. Plebanski. Differential uptake of nanoparticles and microparticles by pulmonary APC subsets induces discrete immunological imprints. J. Immunol. 2013; 191: 5278–5290.
19. Li S.D, Huang L. Nanoparticles evading the reticuloendothelial system: role of the supported bilayer. Biochim. Biophys. Acta 1788. 2009: 2259–2266.
20. Petros R.A., DeSimone J.M., Strategies in the design of nanoparticles for therapeutic applications, Nat. Rev. Drug Discov. 2010: 9: 615–627.
21. Verhoef JJF, Groot AM, van Moorsel M, Ritsema J, Beztsinna N, Maas C, Schellekens H. Iron nanomedicines induce Toll-like receptor activation, cytokine production and complement activation. Biomat. 2017; 119 :68–77.
22. S.P. Vyas, P.N. Gupta. Implication of nanoparticles/microparticles in mucosal vaccine delivery. Expert Rev. Vaccines. 2007; 6: 401–418.
23. Makadia H.K, Siegel S.J. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Pol. (Basel). 2011;3: 1377–1397.
24. Jiao Y, Pang X, Liu M, Zhang B, Li L, Zhai G. Recent progresses in bioadhesive microspheres via transmucosal administration. Colloids Surf. B: Biointerfaces. 2016; 140: 361–372.
25. Bhide Y, Tomar J, Wei D, Vries-Idema J, W. Frijlink H, Huckriede A,Wouter L. J. H. Pulmonary delivery of influenza vaccine formulations in cotton rats: site of deposition plays a minor role in the protective efficacy against clinical isolate of H1N1pdm virus. Drug Delv. 2018; 25(1): 533–545.
26. Lu D, J Hickey A. Pulmonary vaccine delivery. Expert Rev Vaccines. 2007; 6(2):213-226.
27. Hellfritzsch M, Scherlie R. Mucosal Vaccination via the Respiratory Tract. Pharmaceutics. 2019; 11(8): 375.
28. Tynea AS, Chan JG, Shanahan ER, Atmosukarto I, Chan HK, Britton WJ, West NP. TLR2-targeted secreted proteins from Mycobacterium tuberculosis are protective as powdered pulmonary vaccines, Vaccine, 2013; 31(40):4322-4329.
29. Berlin JM, Tour JM. Development of novel drug delivery vehicles, Nanomedicine. 2010; 5(10):1487-1489.
30. Wu M, Zhao H, Li M, Yue Y, Xiong S, Xu W. Intranasal vaccination with mannosylated chitosan formulated DNA vaccine enables robust IgA and cellular response induction in the lungs of mice and improves protection against pulmonary mycobacterial challenge. Front Cell Infect Microbiol. 2017; 7: 445.
31. Dhaka S, Renu S, Ghimire S, Shaan Lakshmanapp Y,

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Hogshead BT, Feliciano-Ruiz N, Lu F, HogenEsch H, Krakowka S, Lee CW, Renukaradhya G.J. Mucosal immunity and protective efficacy of intranasal inactivated influenza vaccine is improved by chitosan nanoparticle delivery in pigs. Front. Immunol. 2018 ;9: 934.

32. Rose F, Wern J E, Gavins F, Andersen P, Follmann F, Foged C, A strong adjuvant based on glycol-chitosan-coated lipid-polymer hybrid nanoparticles potentiates mucosal immune responses against the recombinant Chlamydia trachomatis fusion antigen CTH522, J. Control. Release 271 2018; 271: 88–97.

33. Cheung R.C, Ng T.B, Wong J.H, Chan W.Y. Chitosan: an update on potential biomedical and pharmaceutical applications. Mar. Drugs. 2015; 13: 5156–5186.

34. Riaz Rajoka MS, Zhao L, Mehwish HM, Wu Y, Mahmood S. Chitosan and its derivatives: synthesis, biotechnological applications, and future challenges. Appl. Microbiol. Biotechnol. 2019.

35. Tafaghodi M, Saluja V, Kersten G F, Kraan H, Slutter B, Amorij J P, Jiskoot W. Hepatitis B surface antigen nanoparticles coated with chitosan and trimethyl chitosan: impact of formulation on physicochemical and immunological characteristics. Vaccine. 2012; 30: 5341–5348.

36. Marasini N, Ghaffar K A, Giddam A K, Batzloff M R, Good M F, Skwarczynski M, Toth I. Highly immunogenic trimethyl chitosan-based delivery system for intranasal lipopeptide vaccines against group A streptococcus. Curr. Drug Deliv. 2017; 14:701–708.

37. Nevagi R J, Khalil Z J, Hussein W M, Powell J, M R , Capon R J, Good M F, Skwarczynski M, Toth I. Polyglutamic acid-trimethyl chitosan-based intranasal peptide nano-vaccine induces potent immune responses against group A streptococcus. Acta Biomater. 2018; 80: 278–287.

38. Okamoto S, Matsuura M, Akagi T, Akashi M, Tanimoto T, Ishikawa T, Takahashi M, Yamanishi K, Mori Y. Poly (gamma-glutamic acid) nano-particles combined with mucosal influenza virus hemagglutinin vaccine protects against influenza virus infection in mice. Vaccine. 2009; 27: 5896–5905.

39. Chowdhury M Y E, Kim T H, Uddin M B, Kim J H, Hewawaduge C Y, Ferdowshi Z, Sung M H, Kim C J, Lee J S. Mucosal vaccination of conserved sM2, HA2 and cholera toxin subunit A1 (CTA1) fusion protein with poly gamma-glutamate/chitosan nanoparticles (PC NPs) induces protection against divergent influenza subtypes. Vet. Microbiol. 2017; 201: 240–251.

40. Mummert M E, Immunologic roles of hyaluronan. Immunol Res. 2005; 31: 189–206.

41. Singh M., Briones M, O'Hagan D T. A novel bioadhesive intranasal delivery system for inactivated influenza vaccines. J. Control. Release. 2001; 70: 267–276.

42. Fan Y, Sahdev P, Ochy L J, Akerberg J, Moon J, Cationic liposome-hyaluronic acid hybrid nanoparticles for intranasal vaccination with subunit antigens. J. Control Release. 2015; 208: 121–129.

43. Nochi T, Yuki Y, Takahashi H, Sawada S, Mejima M, Kohda T, Harada N, Kong I G, Sato A, Kataoka N, Tokuhara D, Kurokawa S, Takahashi Y. Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat. Mater. 2010; 9: 572–578.

44. Fukuyama Y, Yuki Y, Katakai Y, Harada N, Takahashi H, Takeda S, Mejima M, Joo S, Kurokawa S, Sawada S, Shibata H, Park E J, Fujihashi K, Briles D E, Yasutomi Y, Tsukada

H, Akiyoshi K, Kiyono H. Nanogel-based pneumococcal surface protein A nasal vaccine induces microRNA-associated Th17 cell responses with neutralizing antibodies against Streptococcus pneumoniae in macaques Mucosal Immunol. 2015; 8: 1144–1153.

45. Nagatomo D, Taniai M, Ariyasu H, Taniguchi M, Aga M, Ariyasu T, Ohta T, Fukuda S, Cholesteryl pullulan encapsulated TNF-alpha nanoparticles are an effective mucosal vaccine adjuvant against influenza virus, Biomed Res Int, 2015: 2015: 471-468.

46. Blank F, Fytianos K, Seydoux E, Fink A, Von Garnier C, Lorenzo L, Fink A, Von Garnier C, Rothen-Rutishauser B. Interaction of biomedical nanoparticles with the pulmonary immune system. J Nanobiotechnol. 2017; 15(6).

47. Hu K, Elvander M, Merza M, Åkerblom L, Brandenburg A, Morein B. The immunostimulating complex (ISCOM) is an efficient mucosal delivery system for respiratory syncytial virus (RSV) envelope antigens inducing high local and systemic antibody responses. Clin Exp Immunol. 1998; 113(2): 235–243.

48. Tak W. Mak, Mary E. Saunders and Bradley D. Jett, Primer to the Immune Response. 2nd Ed. 2014.

49. Fujita Y, Taguchi H. Nanoparticle-Based Peptide Vaccines, Book Chap. 8, Micro and Nanotechnology in Vaccine Development, 2017:149-170.

50. Helgeby A, C Robson N, M. Donachie A, Beackock-Sharp H, K Lo¨vgren,K Scho¨n, Mowat A, Y. Lycke N. The Combined CTA1-DD/ISCOM Adjuvant Vector Promotes Priming of Mucosal and Systemic Immunity to Incorporated Antigens by Specific Targeting of B Cells. J Immonul. 2006: 176(6):3697-706.

51. Abdelkader H, Ismail S, Kamal A, G. Alany R, Design and Evaluation of Controlled-Release Niosomes and Discomes for Naltrexone Hydrochloride Ocular Delivery, J Pharm Sci, 2011: 100(5): 1833-1846.

52. V. Li A, J. Moon J, Abraham W, Suh H, Elkhader J, A. Seidman M. Generation of Effector Memory T Cell–Based Mucosal and Systemic Immunity with Pulmonary Nanoparticle Vaccination. Sci Trans Medicine. 2013; 204(5): 130-204.

53. Irvine DJ, Hanson MC, Rakhra K, Tokatlian T. Synthetic Nanoparticles for Vaccines and Immunotherapy. Chem Rev. 2015; 115(19):11109-11146.

54. Kunda N, M. Alfagih I, Rachel Dennison S, M. Tawfeek H, Somavarapu S, A. Hutcheon G, Y. Saleem I. Bovine Serum Albumin Adsorbed PGA-co-PDL Nanocarriers for Vaccine Delivery via Dry Powder Inhalation. Pharm Res. 2015; 32:1341–1353.

55. Muttil P, Prego C, Garcia-Contreras L, Pulliam B, Fallon JK, Wang C, Hickey AJ, Edwards D. Immunization of Guinea pigs with novel Hepatitis B antigen as nanoparticle aggregate powders administered by the pulmonary Route. The AAPS J. 2010; 12(3):330-337.

56. Ross KA. Synthetic nanoparticle-based vaccines against respiratory pathogens, Lova State University, PhD thesis, 2013.

57. Kunda N K, Alfagih IM, Miyaji EN, Figueiredo DB, Gonçalves VM, Ferreira DM, Dennison SR, Somavarapu S, Hutcheon GA, Saleem IY. Pulmonary dry powder vaccine of pneumococcal antigen loaded nanoparticles. Int J Pharm. 2015; 495(2):903-912.

58. Ozeki T, Tagami T, Drug/polymer nanoparticles prepared using unique spray nozzles and recent progress of inhaled formulation. Asian J of Pharm Sci. 2014; 9(5): 236-243.

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Nanomed. J. 8(1):1-13, Winter 2021

59. Sarei F, Dounighi NM, Zolfagharian H, Khaki P, Bidhendi SM. Alginate Nanoparticles as a Promising Adjuvant and Vaccine Delivery System. Indian J Pharm Sci. 2013; 75(4):442-9.

60. Bivas-Benita M, van Meijgaarden KE, Franken KL, Junginger HE, Borchard G, Ottenhoff TH, Geluk A. Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of Mycobacterium tuberculosis. Vaccine. 2004: 22(13-14):1609-15.

61. Blank F, Stumbles P, von Garnier C, Opportunities and challenges of the pulmonary route for vaccination, Expert Opin Drug Deliv, 2011: 8(5):547-63.

62. Jorquera PA, Tripp RA. Synthetic Biodegradable Microparticle and Nanoparticle Vaccines against the Respiratory Syncytial Virus. Vaccines. 2016; 4(4):45.

63. Lennart Buske SD. Chitosan as adjuvant and particle forming ex-cipient in a nano-in-microparticulate dry powder for nasal and pulmonary vaccine delivery. PhD thesis, Kiel University, 2014.

64. Silva A S, Tavares TM, Aguiar-Ricardo A. Sustainable strategies for nano-in-micro particle engineering for pulmonary delivery. J Nanopart Res. 2014; 16:2602.

65. Wang J, Huang X, Li FR. Impaired dendritic cell functions in lung cancer: a review of recent advances and future perspectives. Cancer Commun (Lond). 2019; 39(1):43.

66. Hammad H, Lambrecht BN. Lung dendritic cell migration. Adv Immunol. 2007; 93:256-278.

67. Von Garnier C. Nanoparticles in the Respiratory Tract: Modulation of Antigen-Presenting Cell Function, J Environ Immunol Toxicol. 2014.

68. Sou T. New developments in dry powder pulmonary vaccine delivery. Trends in Biotechnol. 2011.

69. Hugh DC, (Eds.). Pulmonary drug delivery, medicine for inhalation Chap. 2010; 1:181.

70. F Tonnis W, J Lexmond A, W Frijlink H, Boer A, LJ Hinrichs W. Devices and formulations for pulmonary vaccination, Expert Opinion on Drug Deliv. 2013; 10(10), 1383-1397.

71. Lu D, J Hickey A. Pulmonary vaccine delivery. Expert Rev. Vaccines. 2007; 6(2): 213-226.

72. Saleem I, Petkar K, Somavarapu S. Rationale for Pulmonary Vaccine Delivery: Formulation and Device Considerations, book Chap 19th, In Micro and Nano Technologies, Micro and Nanotechnology in Vaccine Development, William Andrew Publishing. 2017.

73. Roberts RA, Shen T, Allen IC, Hasan W, DeSimone JM, Ting JP. Analysis of the Murine Immune Response to Pulmonary Delivery of Precisely Fabricated Nano- and Microscale Particles. PLos one. 2013; 8(4): 1-13.

Nanomed. J. 8(1):1-13, Winter 2021

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