Expert Opinion

Design of nanoparticle-based dry powder pulmonary vaccines

Brian Pulliam, Jean C Sung & David A Edwards†
†*Harvard School of Engineering and Applied Sciences, 322 Pierce Hall, 29 Oxford Street, Cambridge, MA 02138, USA*

The development of needle-less vaccination for pulmonary delivery may require dry forms of vaccines whose powder properties allow for a low cost, heat and freeze tolerance, efficient aerosolization, and the ability to target cells of the immune system. For each of these reasons, nanoparticles can play a critical role in the formulation, development and delivery of needle-less vaccination. This review aims to communicate present biomaterial design issues surrounding the incorporation of nanoparticles into pulmonary vaccines.

Keywords: adjuvants, aerosols, biocompatible polymers, microparticles, nanoparticles, poly(lactic-co-glycolic acid), pulmonary delivery, vaccines

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1. Introduction

Pulmonary delivery presents an attractive alternative to injection for the vaccination of children and adults. Elimination of the syringe and needle can simplify administration, ameliorate the pain and suffering associated with injections, improve regimen compliance, reduce the risk of transferring blood-borne infections, and mitigate a serious waste disposal problem [1,2]. As nearly all respiratory pathogens gain entry through mucosal membranes, establishing immunity at or near the site of infection might result in better first-line defenses against infectious agents [3,4]. Furthermore, generating mucosal immunity in the lungs can provide a broader base of protection, as the respiratory tract encompasses a large interrelated network of associated lymphoid tissues – the larynx, nasopharynx, and bronchoepithelium – that readily share antigenic information [5] and are known to be capable of inducing systemic immunity [6]. The favorable physiological environment of the deep lungs relative to other mucosal tissues such as the nose and gut (e.g., physiological pH and reduced mucociliary action) may circumvent many problems that exist with other non-invasive vaccine targets, namely rapid clearance, poor absorption, exposure to digestive enzymes, and the antigenic tolerance developed in tissues frequently exposed to common environmental substances [7]. Given the extensive mucosal surface area (equivalent to the surface of a single's tennis court) available to inhaled particles on deposition, an efficient system for delivering vaccines in aerosol particle form might permit delivery of vaccines at lower doses than via injection and allow for greater diversity in the types of antigens that could be administered, including those based on nucleic acids [8]. Aerosol-based vaccines have the additional benefit of being easily formulated as dry powders, using material science strategies developed over the last decade for aerosolized bio-therapeutics, such as insulin and growth hormone [9,10]. These can potentially reduce or eliminate cold-chain requirements, promote sterility, and increase the overall stability of antigens [11,12]. For reasons such as these, pulmonary vaccination strategies have recently generated a great deal of research interest and shown potential notably in the successful demonstration of dry powder vaccines against influenza [13] and measles [14].

Many challenges remain before achieving safe and effective vaccination via the lungs. For whole-cell vaccines, like attenuated *Mycobacterium bovis* in Bacille Calmette-Guérin (BCG) vaccination against tuberculosis, present vaccines cannot be effectively delivered by aerosol in their traditional dry powder (lyophilized) state. In liquid form these vaccines require large volumes of clean water and long delivery times (i.e., via nebulization) relative to traditional injection delivery. Delivery via nebulization is moreover generally inefficient and variable. For antigen-based vaccines, the delivery of successful adjuvants that marshal an appropriate immune response to the lungs [15] can pose a safety risk, notably given that the same conditions that make the pulmonary delivery of vaccines attractive (i.e., the inefficient clearance of very small particles) can lead to a significant and long-lasting presence of highly inflammatory material in the lungs. Additionally, as many of the infectious diseases requiring vaccination disproportionably afflict the poor, aerosol vaccines must be cheap, easy to produce, and stable for distribution.

At least some of the present challenges to pulmonary vaccination might be addressed through the development of nanoparticle vaccines. Biodegradable nanometer- and micron-sized particles appear to be well tolerated in the body and have gained acceptance as effective delivery systems for drugs and proteins [16]. Of particular interest for vaccination, these particles have demonstrated the capacity to act as immune system adjuvants if antigenic materials are encapsulated or adsorbed to the surface [17]. Methods for producing these particles that are amenable to industrial-scale production have gained prominence [18], and, if formulated appropriately, can provide an unparalleled opportunity to manufacture effective and safe aerosol vaccines at low cost. Recent advances in biomaterial and aerosol science that allow the low-cost formulation of particularly efficient nanoparticle aerosol delivery systems may help realize this opportunity, even as research and development remains at an early pre-clinical stage.

2. Nanoparticles

Biomedical nanoparticles generally refer to solid colloidal particles ranging in size from 10 to 1000 nm (1 μm) that consist of macromolecular materials in which active principle (drug or biologically active material) is dissolved, entrapped, encapsulated and/or to which the active principle is adsorbed or attached [19]. Many researchers refer to nanoparticles exclusively as particles of a size less than ~ 100 nm, hence the term 'microparticle' sometimes appears in the literature to describe particles of submicron size [20].

Nanoparticles can be engineered from many natural or synthetic polymers that degrade or are metabolized over time (**Box 1**). Polymers vary in their rate and mechanism of degradation, toxicity of byproducts, ease of antigen attachment or encapsulation, thermal stability, cost and availability. Each of these factors is an important criterion in the choice of the underlying substrate so as to ensure that nanoparticles are safe, capable of stimulating the immune system and suitable for formulation into aerosols.

A wide variety of polymers have been explored for use in drug delivery [21,22]. Of those demonstrated to be truly biocompatible, albumin provides the basis of the only commercially available nanoparticle therapy so far (Abraxane® for treatment of metastatic breast cancer) [23]; the natural polymer chitosan and the synthetic polymer poly(lactic-co-glycolic acid) (PLGA) have each been explored extensively in dry powder nanoparticle-based aerosol formulations.

Although chitosan is not yet approved for commercial use in medical applications, it has been widely studied and appears the most commonly used natural polymer in nanoparticle preparations [24,25]. Chitosan possesses several attractive features for use in particle-based pulmonary vaccines: strong mucoadhesive properties that increase particle retention time on mucus layers, a potentially high encapsulation efficiency, a polycationic nature suited for electrostatic adsorption, and high thermal stability [24,25].

PLGA continues to be the most extensively used polymer in biomedical applications and is well suited for pulmonary vaccine applications for a number of reasons. First, PLGA is a block copolymer composed of poly(lactic acid)(PLA) and poly(glycolic acid) that hydrolyzes completely to monomeric acids that are efficiently excreted from the body. Second, the PLA and poly(glycolic acid) subunits differ in their ability to recruit water; this permits control of nanoparticle degradation rates by varying their proportions in the polymer [26]. Such control allows for particle life-time to be designed to vary from several weeks to several years [27], the longer life-time having been argued to be critical to the development of single-dose vaccines [28]. Third, PLGA is very well characterized [29,30] and readily available in a variety of molecular weights and terminal group chemistries that allow researchers substantial flexibility in tuning nanoparticle properties. Finally, PLGA is FDA-approved for various commercial products and known to be safe, having been used extensively in medical devices, controlled drug delivery and tissue engineering applications [31,32].

Antigenic substances are associated with nanoparticles through encapsulation, surface adsorption or covalent attachment. Methods for creating nanoparticles are typically based on the mechanical homogenization of emulsions that contain antigens [33] or solvent displacement techniques where nanoparticles form spontaneously [34]. The former is used when encapsulation is desired, and often requires the presence of stabilizers, osmolytes or pH modifiers to offset the effects of organic solvents or the acidic internal nanoparticle environments caused by the hydrolysis of polymers [35,36]. Solvent displacement methods are more suited to the surface association of antigens (covalent or passive adsorption), as their encapsulation efficiency is low; these latter methods are

Box 1. Polymers suitable for use in nanoparticle-based pulmonary vaccines.

Synthetic	*Natural*
Aliphatic polyesters	Albumin
Poly(caprolactone)	Alginic acid
Poly(glycolic acid)	Poly(glutamic acid)
Poly(lactic acid)	Polyhydroxyalkanoates
Block copolymers	Cellulose
Polydioxanone	Chitosan
Polyglyconate	Collagen
Poly(lactic-co-glycolic acid)	Gelatin
Other classes	Hyaluronic acid
Poly(alkyl cyanoacrylates)	Proteins
Poly(amino acids)	Starch
Polyanhydrides	
Poly(ortho esters)	
Polyphosphazenes	
Polyphosphoesters	

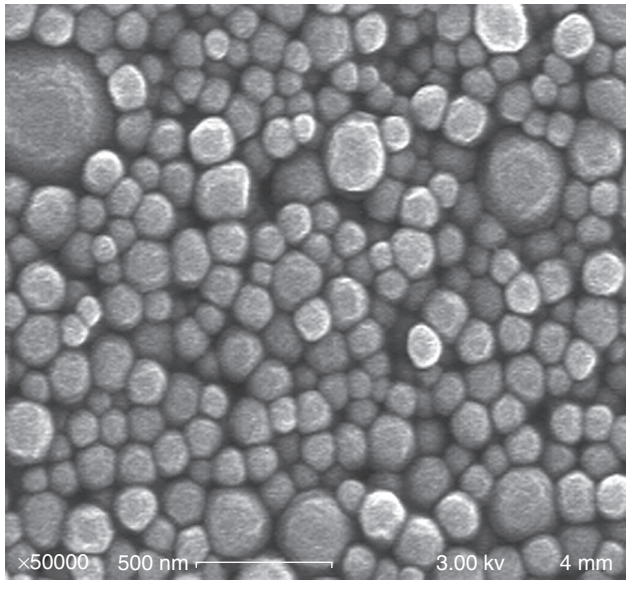

Figure 1. Scanning electron microscope image of PLGA nanoparticles created by a solvent-displacement method.
PLGA: Poly(lactic-co-glycolic acid).

potentially extremely scalable, and, where possible, represent perhaps the most attractive nanoparticle production methods, given that they avoid additional reaction steps that add to the cost of manufacture. **Figure 1** shows an example of mono-disperse nanoparticles created by a solvent displacement method.

3. Pulmonary delivery

As with many aerosol drugs, aerosol vaccines often target the gas exchange regions of the deep lung. A major aim of deep lung delivery for vaccines is avoidance of mucociliary clearance. It has been shown that depositing particles in the bronchiolar regions versus the upper airways increases particle retention time from < 24 h to several days [37]. By prolonging exposure to the antigen, the probability of generating a sustained immune response may be increased and the number of required boosting doses diminished.

3.1 Particle deposition

Particle deposition during inhalation occurs when an inhaled particle has sufficient linear momentum to deviate from the lines of airflow. The primary causes of deposition in the lungs are inertial impaction, sedimentation and diffusion, the importance of each varying with particle size and zone of penetration inside the respiratory tract [38]. A convenient intrinsic factor for expressing the deposition tendency of particles and lung retention times [39,40] is aerodynamic diameter – the diameter of an idealized particle of unit density (1.0 g/cm^3) with the aerodynamic behavior of the particle itself. The aerodynamic diameter (d_{aer}), is related to the geometric diameter (d_{geo}) and particle density (ρ) by the equation

$$d_{aer} = d_{geo}\sqrt{\rho_{particle}} \quad (1)$$

This equation, formally appropriate for spherical (or spherically isotropic) particles, can be expressed more generally by introducing a shape factor, as described elsewhere [41].

Particles with a mass median aerodynamic diameter (MMAD) of > 5 µm primarily deposit via inertial impaction in the mouth, nose and trachea, where they are rapidly cleared. Particles with a MMAD < 5 µm are capable of reaching the bronchiolar regions [42,43], where they are quickly cleared by mucociliary action. Very small particles (MMAD < 100 nm) will largely escape deposition in the upper and central airways and deposit by diffusion in the alveolar region [37], but such particles tend to require such large energy to create (as liquid droplets) or deaggregate (in dry powder form) that they are generally impractical delivery systems for vaccines [44]. Particles with a MMAD > 100 nm but < 2 µm are largely exhaled following inhalation [45], as they are too large for diffusive deposition and too small for inertial or sedimentation deposition. This practically leaves a small MMAD size window, in the range of 2 – 5 µm, where particles can achieve high deposition rates (**Figure 2**).

One of the challenges presented by the 'narrow aerodynamic size window' for the delivery of aerosol vaccines to the lungs lies in the fact that micron-sized

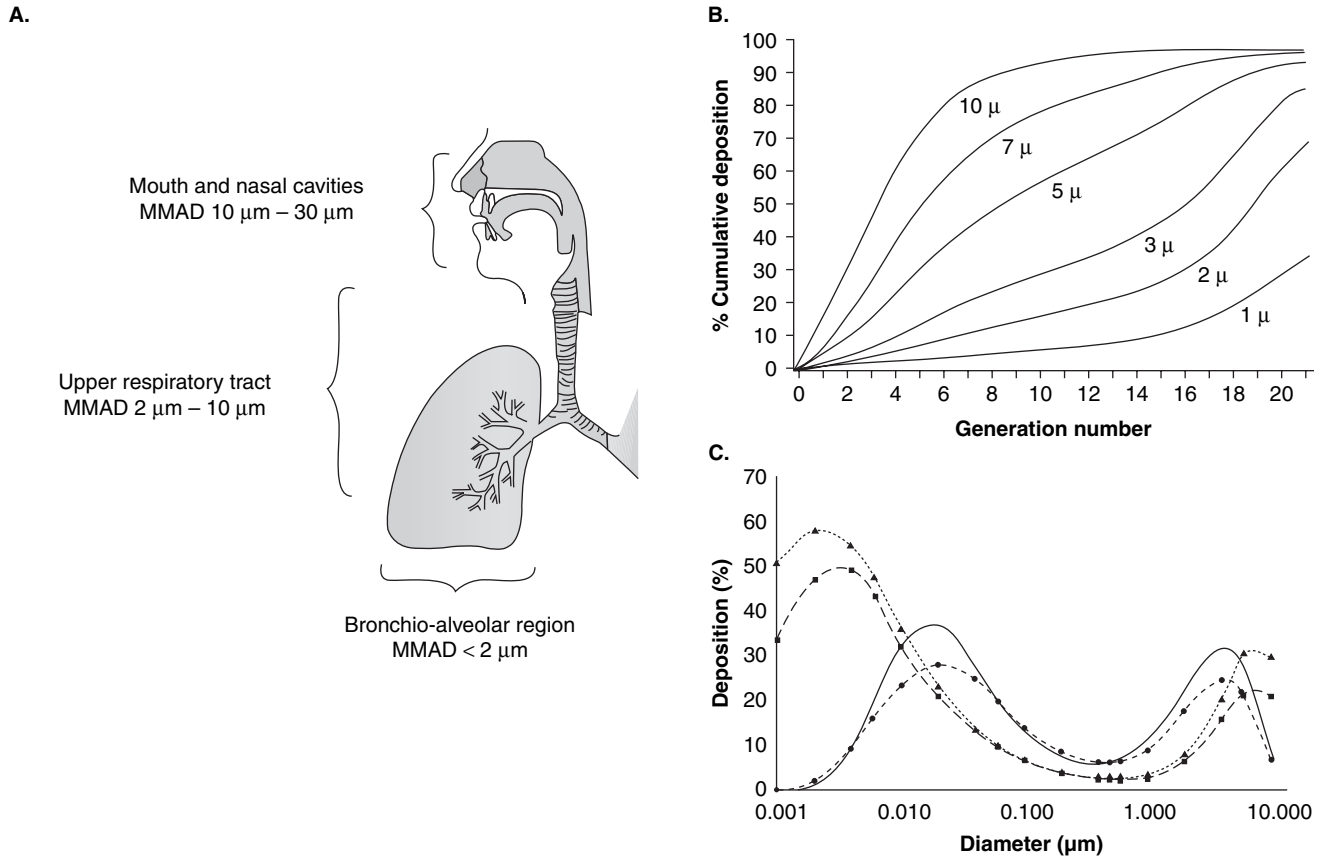

Figure 2. A. Illustration of the size constraints for aerosol particle deposition in the respiratory tract. B. Cumulative particle deposition in the lung of selected aerosols with a mass median aerodynamic diameter of < 10 μm. Cumulative deposition is plotted against airway generation number. Note that small particles deposit primarily in the deep lung, whereas larger particles deposit in the upper airways (reproduced with permission from Gerrity *et al.* [45]). **C.** Deep lung (alveolar) deposition as a function of particle size for various deposition models (USBG and ICRP). Note the bimodal distribution – particles with a mass median aerodynamic diameter > 100 nm but < 1 μm do not deposit efficiently.

Reproduced with permission from [37].

ICRP: International commission on radiological protection; MMAD: Mass median aerodynamic diameter; USBG: University of Salzburg.

particles are energy intensive to produce in liquid form and tend to aggregate excessively when prepared in dry powder form. The delivery of such particles with high efficiency to the lungs has required the development of sophisticated expensive inhalers [46,47] and poses a barrier to the development of an inhaled vaccine therapy of practical use. In recent years, responding to the need for low-cost aerosol delivery systems with high efficiency, a new kind of material form, referred to as 'large porous particles', has been developed. This particle form, first described in 1997 [48], exploits the fact that particles deposit in the lungs principally by inertia, as opposed to particle size. Reducing particle mass by rendering particles more porous allows the delivery of relatively large geometric sizes (≥ 5 μm) with small aerodynamic diameters (≤ 5 μm), thereby allowing penetration into the appropriate regions of particles that, by virtue of their large geometric size, aggregate with less tenacity. Examination of Equation 1 reveals that this can be accomplished by reducing the density of the particle below unit density (typically < 0.1 g/cm^3).

3.2 Aerosol formulation

Nanoparticles can be formulated into aerosols though spray-drying [49,50], a process where dissolved solids are atomized into 50 – 100 μm sized droplets and exposed to rapidly circulating hot air. The volatile components then rapidly evaporate resulting in dry, respirable-sized particles that are stable over considerable periods of time [51]. Biocompatible excipients (amino acids, lipids and sugars) are typically added to the feed solutions to provide dry powders with bulk and to encourage the formation of desirable aerodynamic qualities.

To achieve a low density particle, solutions are spray dried in an environment where the rate of evaporation is faster than the diffusion of nanoparticles in the drying droplet. The governing mass transfer process is characterized by the

dimensionless Peclet number, which expresses the ratio of the rates convection (evaporation) to diffusion, and can be expressed by the equation

$$Pe = \frac{V_f R}{D} \quad (2)$$

V_f is the velocity of the drying front, R the radius of the drying droplet, and D is the diffusion coefficient of the solutes (including nanoparticles). At high Peclet numbers, nanoparticles in the drying droplet diffuse slower than the rate of movement of the droplet surface driven by evaporation. This causes the nanoparticles to gather and eventually coalesce at the evaporation surface, where they are held in place until drying ceases. In the dried state they are held stationary by cohesive Van der Waal and ionic forces or are dispersed within the excipient matrix, depending upon the proportion of nanoparticles. The dried particles are typically hollow and of low density and have been termed porous nanoparticle-aggregate particles (PNAPs). Nanoparticle concentrations and excipient properties affect the density of the final aerosols [52]. It has been suggested that the degree of porosity may be controlled by varying the concentration of phospholipids in the spray-drying feed solution [53]. As the excipients are generally soluble in physiological fluids the aerosol particles readily dissolve upon contact with the lining of the lung, releasing the nanoparticles (**Figure 3**).

As drying is a function of both heat and mass transfer, the temperature and relative humidity at which spray drying is carried out play critical roles in the formation of the final product. The thermal exposure of polymers in spray drying must be carefully controlled, as nanoparticles with low glass transition temperatures (T_g) may aggregate, melt and fuse during the process [50]. This occurs even though the drying of atomized droplets is extremely fast (in the order of seconds) as the powder remains in contact with air at high temperatures throughout a process run. PLGA is particularly susceptible to this problem, with a T_g of < 40°C, limiting the temperatures at which spray drying can be carried out. Collection or 'outlet' temperatures above the polymer T_g produce aerosols with poor aerodynamic properties and unfavorable nanoparticle recoveries, as well as lower yields (due to the adhesion of particles to the inner surface of the drying drum). **Figure 4** illustrates the difference in PNAP surface morphology in 170-nm polystyrene (T_g > 110°C) and 170 nm PLGA (T_g < 40°C) nanoparticle powders dried at approximately the same outlet temperatures. This melting problem can be overcome by both drying in humidity-free air, which allows a reduction in effective spray drying temperatures, and decreasing the relative mass of nanoparticles in the feed solution (< 25% total mass) to reduce the number of nanoparticle–nanoparticle contacts.

For bacterial-based vaccines, such as BCG, spray drying can lead to a novel kind of nanoparticle for pulmonary delivery. In this case [54], dried bacteria assume an elongated dried form. The long axis of the dried particle, which is of a micron size, tends to contribute to relatively good flowability, and the narrow (nano-dimension) axes of the dried bacteria provide aerosol features that resemble nanoparticles. In the case of BCG, the live organism is cylindrical, with a radius of approximately a few hundred nanometers and a length of two or three microns. Once dried through spray drying, the BCG organism contributes to a flowable dry powder, owing to the long axis, as well as to the presence of small dried non-active particles, such as leucine [55]. However, these BCG particles have a far better propensity for aerosolization than dry powders lacking the narrow nanoparticle dimension, owing to the particles' high aspect ratio which confers the ability to 'fly like arrows', a phenomenon previously commented upon at length in the context of (otherwise inherently toxic) materials such as asbestos [56].

3.3 Vaccine manufacturing

A major advantage of spray drying, relative to many other methods of creating nanoparticle formulations (see **Box 2**), relates to its scalability for manufacturing purposes. The potential for producing antigenic nanoparticles in-line with the aerosols is an additional and related advantage. These advantages offer the possibility to reduce the cost of manufacturing to industry norms [57,58].

Techniques such as encapsulation with chitosan, which requires only the addition of tripolyphosphate [59], may potentially be carried out simultaneously and fed directly to the spray-drying process. For antigens that can be passively adsorbed to the surface of nanoparticles, solvent displacement techniques may be similarly used as they rely only on the solubility parameters of polymer-solvent systems where, in certain solubility regimes, nanoparticles form spontaneously and are thermodynamically stable [60]. Thus, they are naturally scalable, requiring only the addition of small amounts of stabilizing agents such as biocompatible polyvinyl alcohol. Nanoparticles created in this manner have already been shown to be easily sterile filtered in vaccine formulations [61], suggesting that it may be possible to eliminate the expensive sonication and centrifugation steps necessary in other techniques. Solvent displacement methods have not been aggressively pursued in the past because the achievable polymer-to-solvent mass ratios are typically too low for many manufacturing purposes; however, these requirements lie well within the solid contents of aerosol spray-dry feed solutions (typically < 3% w/v). Such a manufacturing process is idealized in **Figure 5**.

4. Invoking an immune response

Pulmonary delivery of vaccines targets pulmonary immune cells with the goal of establishing mucosal as well as systemic protection against respiratory infectious diseases [62]. Freely soluble antigens are poorly immunogenic and therefore

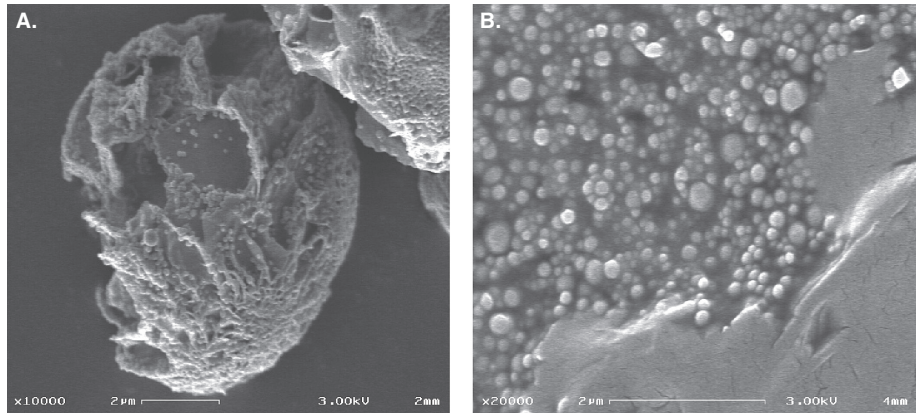

Figure 3. A. Scanning electron microscope image of a typical PNAP. The particle is hollow and composed of L-leucine (75% w/w) and 170 nm PLGA nanoparticles (25% w/w). Note the nanoparticles are clustered at the surface. **B.** Scanning electron microscope image of recovered nanoparticles after the dry powder has been suspended in water.
PLGA: Poly(lactic-co-glycolic acid); PNAP: Porous nanoparticle-aggregate particle.

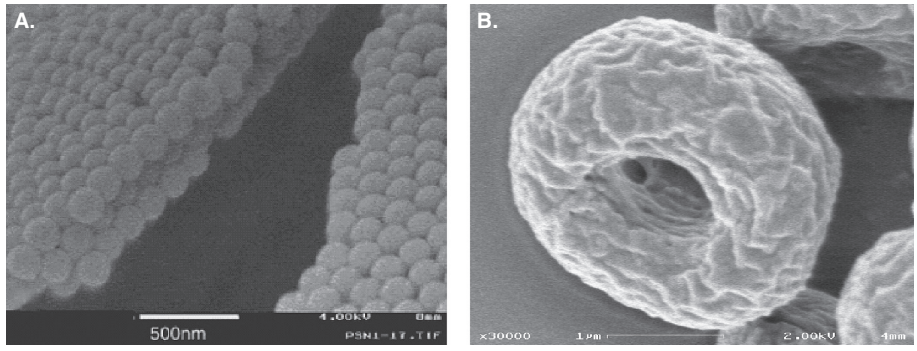

Figure 4. Scanning electron microscope images of the surface morphology of PNAPs composed of different types nanoparticles spray dried at high temperature (> 50°C). A. 170-nm polystyrene nanoparticles (100% w/w; from Tsapis *et al*. [49]). **B.** 170-nm PLGA nanoparticles (100% w/w). The nanoparticles have fused during the spray drying process.
PNAP: Porous nanoparticle-aggregate particle.

Box 2. Common methods for creating nanoparticles.

	Ref.
Ionic cross-linking	[85,86]
Desolvation	[85,87]
Flash-precipitation	[58]
Extrusion/gelation	[88]
Salting out	[32]
Solvent displacement	[18,32,57,60,89]
Solvent evaporation/emulsification	[32,35,89]
Spray-drying	[90]

require co-administration with adjuvants – substances that amplify immune reactions – in order to elicit sufficient responses.

Adjuvants can generally be segregated into three categories: i) mineral salts; ii) molecules that stimulate the immune system through innate chemical recognition; and iii) substances that allow for the persistent presentation of antigens to the immune system [63]. Aluminum salts are powerful systemic adjuvants, providing both an antigen depot and the ability to stimulate expression of the antigen-presenting and co-stimulatory molecules [64]. These types of adjuvants are the only ones approved by the FDA for human use; however they suffer from a profound limitation in that they are known to be deficient in eliciting mucosal IgA responses [65]. Vaccines administered parenterally generally fail to induce mucosal immunity [66], and although

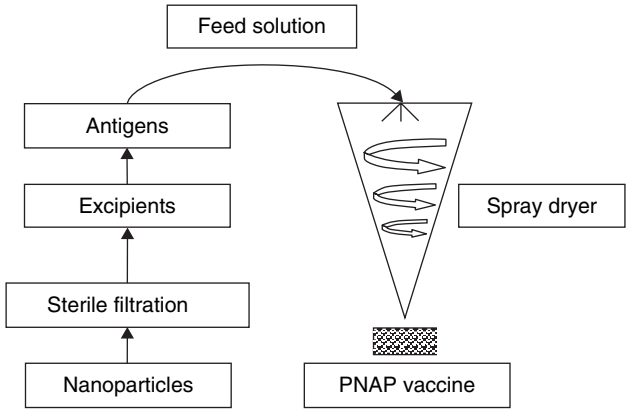

Figure 5. Idealized vaccine manufacturing process for highly scalable nanoparticle formulations.
PNAP: Porous nanoparticle-aggregate particle.

immunostimulatory molecules such as bacterial toxins, lipopolysaccharides, DNA motifs and saponins are all extremely powerful mucosal adjuvants, they can have toxic and adverse side effects, which could be severely compounded in the lung. These factors suggest that particulate systems that act as antigen delivery vehicles, including those of viral or cellular origin, may be the most suitable for inclusion into pulmonary vaccines.

4.1 The role of nanoparticles

It was demonstrated over 40 years ago that polymer-based carriers of antigens are capable of invoking strong immune responses [67]. The adjuvant-like effect of nanoparticles likely relies on the stimulation of specialized antigen-processing dendritic cells that effect T-cell responses and induce B-cell differentiation and antibody production [68]. Dendritic cell-specific uptake of PLGA nanoparticles has been shown in a variety of tissues and cell types. For instance, it has been shown that PLGA nanoparticles (350 nm) are readily taken up by dendritic cells after intradermal injection in mice and to induce expression of MHC class II molecules [69]. Immature dendritic cells have also been shown to phagocytose PLGA nanoparticles (500 nm) with and without the adjuvant monophosphoryl lipid A *in vitro* [70]. In this case, the dendritic cells phagocytosed nanoparticles as efficiently as macrophages – the primary phagocytic cells of the immune system. PLGA nanoparticles (250 – 350 nm) loaded with the cancer-associated antigen (MUC1 mucin peptide BLP25) and monophosphoryl lipid A comprised a vaccine delivery system targeting dendritic cells, providing superior delivery to and internalization by dendritic cells, with increased activation of naive T cells as compared to the soluble antigen [71].

Nanoparticle size may also play an important role in determining the potency of particle-based vaccines. In Caco-2 cells, 100-nm nanoparticles were shown to have 2.7×10^3 higher cellular uptake (by number) than 1 μm particles [72] and in orally administered microparticles, only particles less then 5 μm in size have been found in the mesenteric lymph nodes [73]. In DNA vaccine studies, when the sOVA-C1 plasmid, encoding chicken egg ovalbumin (OVA), was delivered to mice intradermally on poly(L-lysine) coated polystyrene nanoparticles, 50 nm carriers were shown to be far more effective than 20 nm or 1000 nm carriers in eliciting OVA specific CD8 T-cell responses [74].

Surface properties also play an important role in particle-based vaccines. It has been shown that increasing amounts of hydrophobic stabilizer bound to the surface of nanoparticles drastically reduces cellular uptake [75]. Surface properties are known to affect the transport of particles across mucous membranes [76] and modifying PLGA microspheres with chitosan has been shown to increase mucosal residence time and enhance immunogenicity [77]. Nanoparticles may potentially target specific cell types through the use of surface markers like mannose receptors, antibodies, and toll-like receptors [78]. Such pathogen "mimicking" nanoparticles have been created with monophospholipid A, toll-like receptor 4 ligands, and CpG motifs and have been shown to be ingested by dendritic cells and activate strong T-cell responses [79].

Given that relatively few studies have been performed to investigate pulmonary administration of nanoparticle-based vaccines studies such as those described above might be pursued in the lung environment. The size and surface characteristics of nanoparticles make it plausible that the adjuvant-like effect will be found independent of the route of administration. Presumably, nanoparticles delivered to the bronchoalveolar region avoid the rapid clearance of scavenging alveolar macrophages [80] and thereby become available for uptake by the epithelial microfold (M) cells. These cells, as a component of the bronchus-associated lymphoid tissues, sample the environment for antigens and relay antigenic information to macrophages and dendritic cells housed in follicles and facilitate local IgA production [81]. The nanoparticles may then be transported to secondary lymph-nodes where they help initiate the systemic response. **Figure 6** illustrates the proposed mechanism of nanoparticle delivery to pulmonary cells via PNAP carriers.

4.2 Pulmonary DNA vaccines

Steps toward validating such an approach have been made in the application of nanoparticle aerosols to the delivery of DNA vaccines. DNA vaccines are designed to establish an immune response by delivering the DNA plasmid to lung epithelial cells that then express the protein. Biodegradable nanoparticles have been shown to incorporate into airway epithelium cells. Non-viral gene carriers composed of PLGA nanoparticles with surface polyethyleneimine (PEI, a cationic polymer) loaded with DNA (225 nm) and with varying ratios of PLGA:PEI and PEI:DNA were internalized and DNA transferred to the endolysosomal compartment in human airway submucosal epithelial cells (Calu-3) *in vitro*

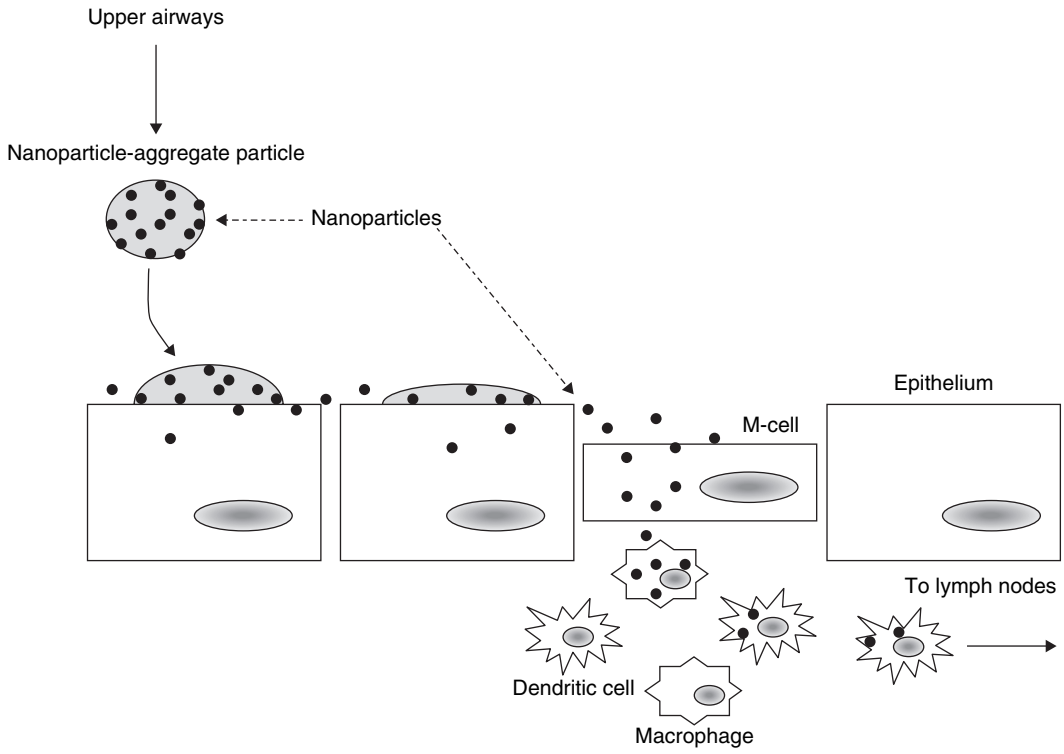

Figure 6. The mechanism of nanoparticle delivery via PNAPs. Aerosols with antigen-associated nanoparticles penetrate to the deep lung where they sediment to the lung lining. The excipient matrix of the PNAP dissolves, releasing nanoparticles for uptake by immune-competent cells.
PNAP: Porous nanoparticle-aggregate particle.

Table 1. Recent studies (non-exhaustive list) on particle-based vaccine delivery*.

Disease	Antigen	Polymer	Size	Model	Route	Ref.
Hepatitis B	HBsAg	Alginate-PLL	400 µm	M	SC	[91]
Hepatitis B	HBcAg	PLGA	3 – 5 µm	M	O, SC	[92]
Hepatitis B	HBcAg	PLGA	300 nm	M	SC	[93]
Malaria	P30B2, $(NANP)_6$P2P30	PLA, PLGA	1 – 20 µm	M	SC	[94]
Malaria	SPf66	PLGA	800 nm – 2 µm	M	ID, SC	[95]
Influenza	Hemagglutinin	PLGA	200 – 350 nm	N/A	N/A	[96]
Tuberculosis	Mtb8.4 peptide	PLGA	2 µm	M	ID	[97]
Tuberculosis	HLA-A*0201 plasmid	Chitosan	350 nm	M	ET, IM	[84]
Diphtheria	DT	Chitosan	2 – 3 µm	GP	IT, SC	[98]
Diphtheria	DT	Poly(acryl starch)	< 2.5 µm	M	O, IM	[99]
Tetanus	TT	PLGA	< 5 µm	GP	SC	[100]
Tetanus	TT	PLGA	1 – 80 µm	M	SC	[101]
Rotavirus	FRRV	Poly(acryl starch); PLGA	3 – 5 µm	M	IM, O	[102]

*Such systems may be easily adapted to dry powder nanoparticle-based formulations.
DT: Diphtheria toxoid; ET: Endotracheal; FRRV: Formalin-inactived rotovirus; GP: Guinea pig; ID: Intradermal; IM: Intramuscular; IT: Intratracheal; M: Mice; N/A: Not applicable; O: Oral; PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); PLL: Poly(L-lysine); SC: Subcutaneous; TT: Tetanus toxoid.

Table 2. Ideal characteristics of nanoparticle-based pulmonary vaccines.

Physical properties	Degradable and biocompatible components, small aerodynamic diameter (2 – 5 μm), small nanoparticles (100 – 300 nm) to facilitate uptake by cells, ability to incorporate different types of antigens, structural integrity of antigen preserved; dry and stable (< 5% moisture)
Administration	Safe with no toxicity or side effects, age and health status insensitive, easy to administer with dry powder inhalers
Immunity	Mucosal and systemic protection, effective at low doses, understood mechanism of action, no development of tolerance, sustained response to reduce the need for additional doses
Manufacturing	Easy to purify and sterilize, scalable, low costs to facilitate vaccination in developing nations, stable in range of temperatures to eliminate cold chain requirements

after 6 h [82]. The cytotoxicity of these nanoparticles was detected, but was found to be dependent upon the PEI:DNA ratio where lower ratios were able to reduce cell toxicity. Porcine gelatin, human serum albumin and poly(alkylcyanoacrylate) nanoparticles (100 – 300 nm) were incorporated into primary airway epithelium cells and the cell line 16HBE14o cells *in vitro* after 6 h [83]. In addition, little or no cytotoxicity and no inflammation (measured by IL-8 release) were detected for the protein (gelatin and albumin) nanoparticles. However, poly(alkylcyanoacrylate) nanoparticles demonstrated toxicity for airway epithelium cells with toxicity dependent upon alkyl sidechain length with shorter sidechains being more cytotoxic than longer ones.

DNA vaccine delivery to the lungs has been investigated utilizing chitosan, for its mucoadhesive properties, to formulate 350-nm nanoparticles with a DNA plasmid encoding eight HLA-A*0201-restricted T-cell epitopes from *Mycobacterium tuberculosis* [84]. The nanoparticles induced the maturation of dendritic cells in culture. Furthermore, pulmonary administration of the nanoparticles induced increased levels of IFN-γ secretion, indicating systemic T-cell activation, compared with pulmonary delivery of plasma alone or intramuscular injection.

5. Conclusion

The pulmonary delivery of vaccines provides an opportunity to remove needle injection from traditional vaccination procedures, improve vaccination efficacy, and significantly impact healthcare in developing world regions where control of infectious diseases remains a major global health problem. Nanoparticles may play a key role in the realization of this opportunity. With new manufacturing methods, they can now be formed on a large scale from a number of biocompatible polymers, including PLGA and chitosan. New highly respirable material forms, such as PNAPs, provide vehicles for delivering antigens to the lung with good efficiency and from simple inexpensive inhalers. These trends argue for more safety and efficacy studies on nanoparticle-based dry powder vaccines, particularly those that have the potential to provide protection against a wide range of respiratory and infectious diseases (**Tables 1** and **2**).

6. Expert opinion

Although research to explore pulmonary vaccination via nanoparticles appears promising, with particular relevance to present international efforts to improve developing world healthcare, many scientific obstacles remain before the potential for broad public use can be realized.

Among the obstacles to needle-less vaccine technology targeted to the lungs are those related to establishing safety in animals and humans and protective immunity for nanoparticle-based antigen vaccines with adjuvants that are demonstrably safe. Most studies so far evaluating the safety of nanoparticles as vaccine delivery vehicles have focused on environmental and industrial exposure to ultrafine particles (< 100 nm), with nanoparticles composed of insoluble or non-degradable materials such as carbon-black or heavy metals that do not represent the characteristics of medical nanoparticles. Safety studies with degradable nanoparticle systems will be needed involving relatively soluble systems, such as those based on biodegradable polymers or liposomes. Polyanhydride and poly(ortho ester) systems are particularly interesting as many of these polymers have been intensely investigated and have or are near to having FDA approval for use in a variety of medical products. Such polymers can provide increased matrix stability for sensitive antigens, faster degradation rates if particle retention proves problematic, or higher thermal stability to allow more particles to survive the drying process. Although high temperatures and shear stresses encountered during spray drying have hampered the exploration of liposomes and other lipid-based carriers in dry powder formulations, novel methods that increase stability (e.g., chemical crosslinking) have emerged that may allow lipid-based nanoparticle designs as well. Whatever the composition of biodegradable nanoparticles, the development of a class of inexpensive, easily prepared biodegradable nanoparticles with (e.g., florescent) tracking capability would be highly useful in order to improve our present understanding of nanoparticle fate in the lungs with biodegradable systems.

Other frontiers relate to nanoparticle design. Research on environmental nanoparticulate material has revealed that nanoparticles with mean geometric sizes of less than ~ 100 nm tend to be absorbed by the epithelium of the lungs and be

transported systemically to other parts of the body. Insoluble nanoparticles with mean geometric sizes > 100 nm tend rather to be phagocytosed. The design of nanoparticle delivery systems for antigens with optimal size, and appropriate surface chemistry, can allow the effective targeting of antigens to appropriate cells of the body, whether local to the lungs or located elsewhere in the body. Although early work on DNA vaccines has explored the role of nanoparticle size for improving needle-less vaccine potential, much more work is needed before it will be possible to design pulmonary nanoparticle vaccines for optimal safety and efficacy.

Finally, low cost and large scale manufacturing processes are needed for the ultimate success of nanoparticle delivery systems as vectors for needle-less vaccines. Spray drying, a low-cost unit operations process highly developed for the pharmaceutical (as well as foods, cosmetics and chemical) industry, may provide a special opportunity. Developing spray-drying operations that allow aseptic conditions, easy filling of appropriate quantities, while maintaining low cost, will be essential to the successful development of a low-cost noninjectable vaccine.

Declaration of interest

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Affiliation

Brian Pulliam[1], Jean C Sung[2] & David A Edwards[†3]
[†]Author for correspondence
[1]Graduate Program in Biophysics, Harvard University Faculty of Arts and Sciences, 40 Oxford Street, ESL 406, Cambridge, MA 02138, USA
[2]Graduate Program in Bioengineering, Harvard School of Engineering and Applied Sciences, 40 Oxford Street, ESL 406, Cambridge, MA 02138, USA
[3]Gordon McKay Professor of the Practice of Biomedical Engineering, Harvard School of Engineering and Applied Sciences, 322 Pierce Hall, 29 Oxford Street, Cambridge, MA 02138, USA
Tel: +1 617 495 1328; Fax: +1 617 495 9837; E-mail: dedwards@seas.harvard.edu