Expert Opinion

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Design of fine particles for pulmonary drug delivery

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Particle design for inhalation is characterized by advances in particle processing methods and the utilization of new excipients. Processing methods such as spray drying allow control over critical particle design features, such as particle size and distribution, surface energy, surface rugosity, particle density, surface area, porosity and microviscosity. Control of these features has enabled new classes of therapeutics to be delivered by inhalation. These include therapeutics that have a narrow therapeutic index, require a high delivered dose, and/or elicit their action systemically. Engineered particles are also being utilized for immune modulation, with exciting advances being made in the delivery of antibodies and inhaled vaccines. Continued advances are expected to result in 'smart' therapeutics capable of active targeting and intracellular trafficking.

Keywords: absorption enhancer, aerosol, amorphous glass, anti-infective, dry powder inhaler, immunoglobulin, inhalation, particle engineering, peptide, porous particle, powder formulation, protein, pulmonary drug delivery, spray drying, spray freeze drying, supercritical fluid, systemic drug administration, vaccine

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1. Introduction

Formulations of asthma drugs have relied on 'top-down' manufacturing methods, wherein large, crystalline drug particles are milled (micronized) to produce fine crystals with a mean geometric diameter of 1 – 5 µm [1-4]. When used in pressurized metered-dose inhalers, the crystals are often combined with small amounts of surfactant to improve the quality of the suspension [2]. In dry powder inhalers, the crystals are mixed with large lactose carrier particles or pelletized to improve powder fluidization [5,6,135]. Owing to the potency and large therapeutic index of asthma drugs, these formulations, elegant in their simplicity, have provided safe and efficacious therapy for millions of asthmatics; excipients were avoided, given the chronic nature of the treatment and the simple fact that complex formulations were not needed.

The micronization process is analogous to smashing two crystal balls against one another. The crystal balls shatter into millions of pieces, with little hope of controlling the size, shape, and surface composition of the resulting fine particles. Moreover, milling processes often produce charged surfaces with amorphous character, resulting in particles with an increased tendency to agglomerate.

Owing to the strong interparticle cohesive and adhesive forces noted with fine crystals and their blends, lung delivery efficiencies of just 10 – 30% of the nominal dose are typically observed [7]. The percentage of carrier particles and the poor delivery efficiency limits the maximum lung dose that can be delivered to just a few milligrams [8]. This, in turn, restricts the choice of therapeutics that can be delivered effectively. In addition, significant variations in lung dose are noted with these blends as a function of the patient's peak inspiratory flow rate. Coupled with the large degree of variability associated with the low pulmonary delivery efficiencies and efficient oropharyngeal filtering, standard blending technology can only be used for drugs with a relatively large therapeutic index. Improved control of the inhaled

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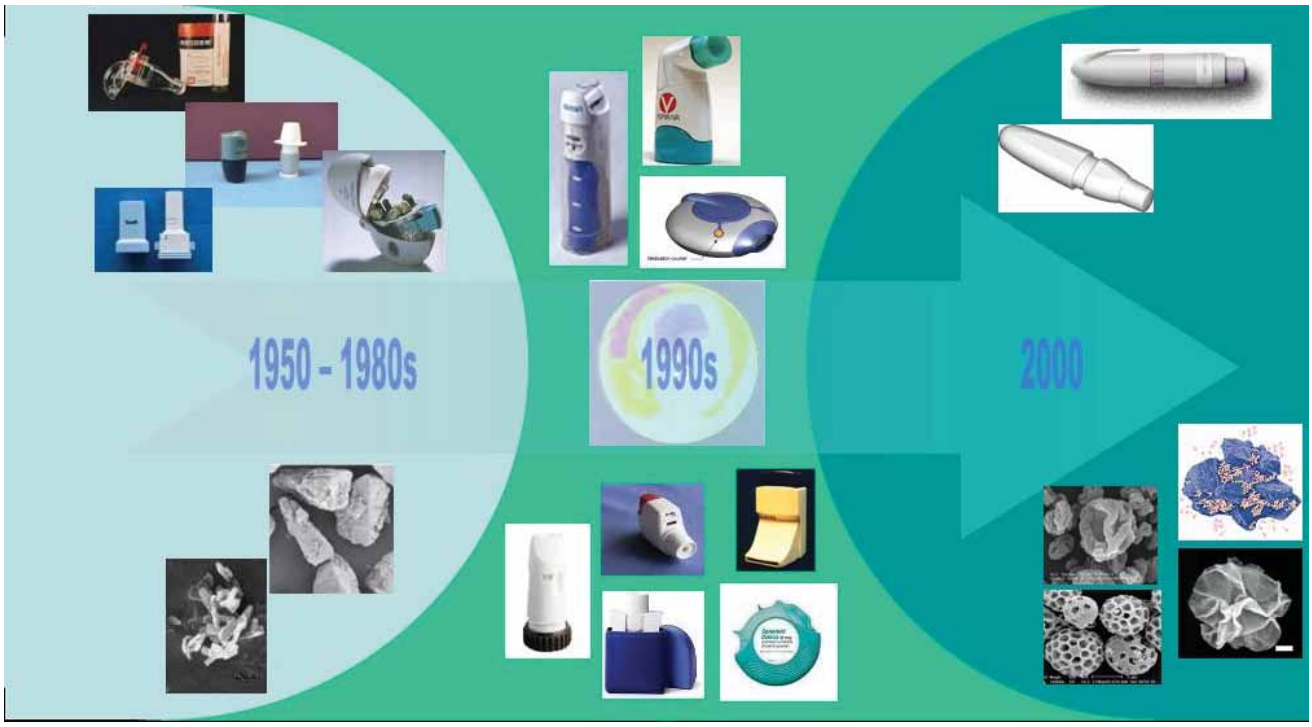

Figure 1. Evolution of the technology in dry powder inhalation products. First-generation dry powder inhalation products were composed of simple powders (micronized drug blends) and simple inhalers (portable capsule-based passive dry powder inhaler). The second generation of dry powder inhaler products were characterized by the same simple powders and more sophisticated devices. The devices included multi-dose, passive inhalers and active, dry powder inhalers. The third generation of dry powder inhaler products are characterized by sophisticated powders and a return to simple inhalers. The powders are encompassed by the engineered powders explored in this review.

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dose is required for therapeutics with a smaller therapeutic index (e.g., insulin).

The evolution of 'bottom-up' processing methods, wherein the drug substance is dissolved in a solvent and then precipitated to produce fine particles, affords greater control of particle properties, including particle size and distribution, morphology, porosity, density, microviscosity and surface energy. This, in turn, allows control of attributes such as powder fluidization, dispersibility, chemical stability and pharmacokinetic/pharmacodynamic properties, enabling new classes of therapeutics to be delivered via inhalation. This review focuses on advances in particle design (excipients and process), and their implication on the development of innovative new products for inhalation (Figure 1). The review presents examples in specific product areas (e.g., amorphous glasses for the delivery of peptides/proteins, porous particles for the delivery of small molecules and macromolecules with improved powder fluidization and dispersibility), where particle engineering has advanced the field, enabling new product opportunities. The manuscript is not intended to be a comprehensive review of all of the art related to particle engineering and inhalation. An in-depth assessment of particle processing technologies can be found in recent reviews [137-138]. Moreover, the authors have not attempted to address the large amount of work associated with the development of pulmonary sustained-release technologies. Once again, an excellent analysis of this area can be found in recent reviews [139-140].

Figure 2 provides a glimpse into the range of therapeutics that can be delivered when new particle engineering strategies are employed. Lung doses ranging from a few micrograms (e.g., potent asthma drugs such as formoterol) to tens of milligrams (e.g., anti-infectives such as tobramycin) can be delivered in a single puff. The mass of drug administered will depend on the nature of the delivery device and the nature of the engineered particles. Although also not addressed in this review, the design of the dry powder delivery device is critically important in achieving a successful drug/device combination product [141]. The evolution of drug/device combination products for the delivery of dry powders is chronicled in Figure 1.

2. Particle processing

Particle engineering has enabled particles with geometric sizes ranging from < 100 nm (ultrafine particles), to 100 µm to be considered for pulmonary drug delivery (Figure 3). This range of sizes comes with a striking diversity with respect to

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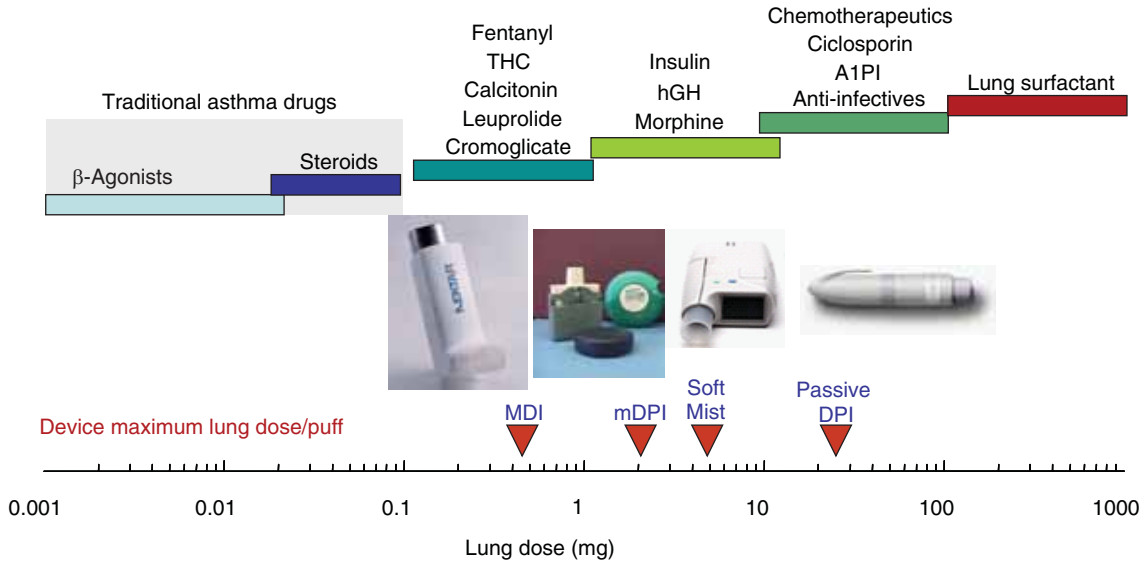

Figure 2. Approximate lung doses required for various therapeutics delivered via the pulmonary route. Also shown are the maximum lung doses that can be delivered in a single puff from portable aerosol devices.

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A1PI: α_1-antitrypsin; DPI: Dry powder inhaler; hGH: Human growth hormone; MDI: Metered dose inhaler; mDPI: Multidose dry powder inhaler; THC: Tetrahydrocannabinol.

physicochemical and biological properties. For example, ultrafine particles are able to bypass macrophages clearance [9,10] and readily translocate across the pulmonary epithelium into the bloodstream [9]. These factors are intriguing, but must be balanced by apparent increases in toxicity [9,11,12]. At the other extreme, large porous particles with geometric diameters of 5 – 30 µm are also able to avoid (perhaps slow) macrophage clearance [13]. Deposition efficiencies on the order of 60% have been reported [14]. Physicochemical properties, deposition patterns, safety and clearance all differ significantly with variations in particle size. Figure 3 captures a small number of the diverse particles which have been engineered with pulmonary therapeutics in mind. The particles range from crystalline drug particles (A, I) and carriers (C), to amorphous drug particles (D, G, H, J). These particles are discussed in more detail throughout the review.

Spray drying has become a popular bottom-up processing method for producing engineered particles for inhalation [15-51]. Its principal advantages include the ability to rapidly produce a dry powder, and to control particle attributes, including size, morphology, density and surface composition [15]. The spray drying process consists of three unit operations: i) atomization of micron-sized liquid droplets; ii) drying into powder form in a stream of hot, dry gas, and; iii) collection of the micron-sized particles from the moist gas stream. Relatively high gas temperatures are needed for spray drying (typically > 50 °C). However, most proteins and peptides are able to maintain activity through the process, due to the very short timescale of the drying process (in the order of milliseconds), and the reduced temperatures in the vicinity of the protein due to evaporative cooling [17-21,25]. In addition, the rapid formation of an amorphous glass locks the protein in a rigid matrix, limiting mobility and decreasing chemical reactivity [17-21,25]. The particle size and distribution of the atomized droplets can be controlled by choice of the atomizer (e.g., rotary, ultrasonic, twin fluid), the air to liquid ratio in the atomizer and the feedstock concentration. The nature of the feedstock to be spray dried is critical. The feedstock can be comprised of solutions in a variety of solvents [17-21] and mixed solvents [44-47], or can be complex dispersions, comprising emulsion droplets [34,37-43,89] or suspensions [33]. The surface composition and morphology of the particles will be determined by the solubility, diffusivity and surface activity of the drug and excipients during the drying process [88,89]. Drying conditions that are controlled include the gas feed rate, solution feed rate, the inlet temperature, outlet temperature and relative humidity in the drying chamber. Particle collection is typically accomplished with cyclone or baghouse capture. The small size of the particles required for inhalation has necessitated the development of custom atomizers and collection systems.

Supercritical fluid (SCF) processing methods [52-59] make use of the high solvation power of supercritical solvents such as carbon dioxide. SCF methods can typically be subdivided into two groups: those that use CO_2 as a solvent, and those that use CO_2 as an antisolvent. In the former, the therapeutic

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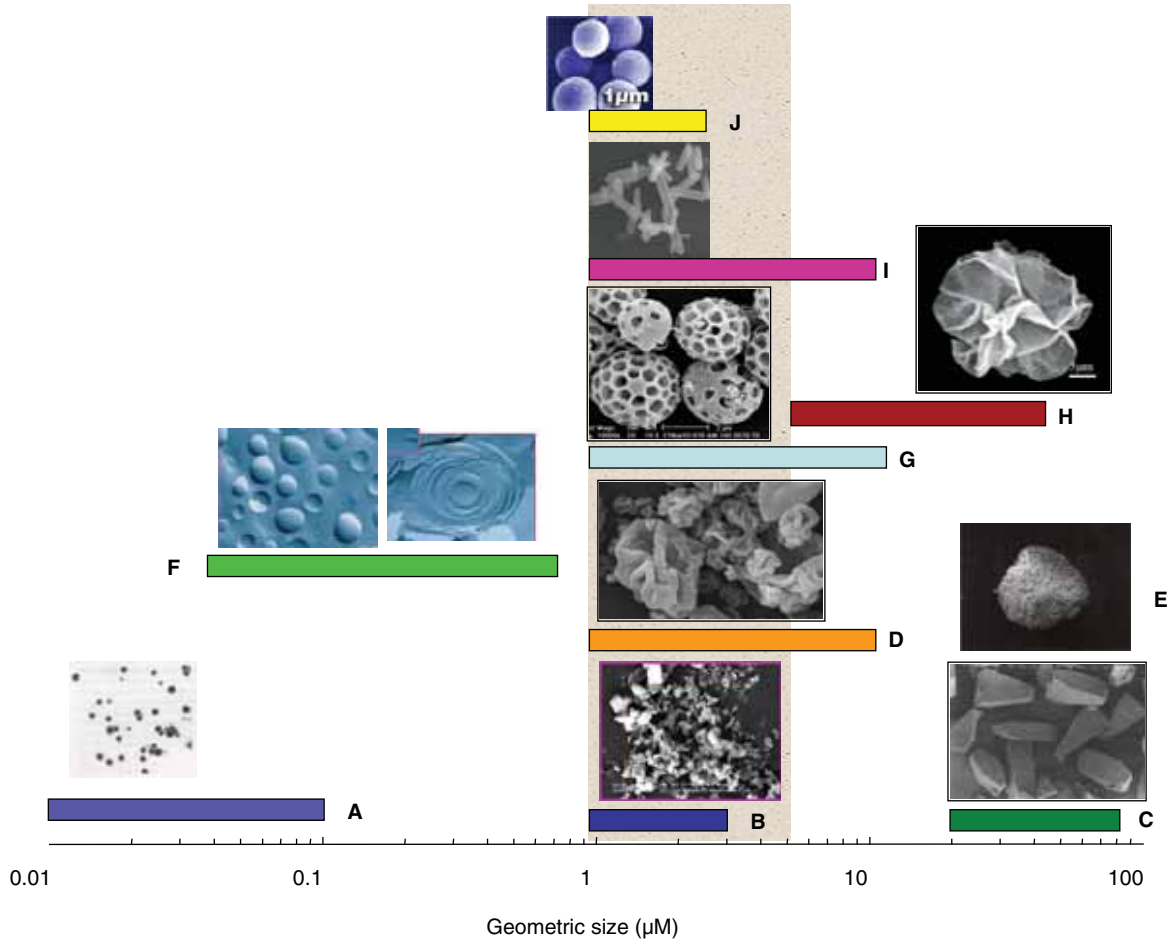

Figure 3. Size and morphology of a collection of engineered particles for inhalation. The grey shaded area represents the region 1 – 5 µm. **A**. Solid lipid nanoparticles [135]. **B**. Standard micronized drug. **C**. Lactose monohydrate carrier particle. **D**. Spray-dried amorphous glass (PulmoSol®, Nektar Therapeutics). **E**. Spheronized budesonide (Astra Zeneca). **F**. Small, unilamellar (left) and multilamellar (right) liposomes. **G**. Spray-dried, small, porous particles (PulmoSphere®, Nektar Therapeutics). **H**. Spray-dried, large, porous particles (Alkermes); **I**. SCF microcrystals (Nektar Therapeutics); **J**. Protein microspheres (Baxter).
SCF: Supercritical fluid.

is dissolved in the SCF, followed by rapid expansion of the SCF solution across an orifice to cause super saturation of the solute, homogeneous nucleation and particle formation. This process has been termed Rapid Expansion of Supercritical Solutions. The process of using CO_2 as an antisolvent involves making a solution of the therapeutic in an organic solvent. The resulting solution is sprayed through a capillary nozzle into a bulk of SCF. Given the high solubility of the SCF in the organic solvent, volume expansion occurs when the two solvents make contact, with particle formation. In an adaptation of the process termed Solution Enhanced Dispersion by Supercritical fluids, a coaxial design with a mixing chamber is used to improve mass transfer rates. SCF processes generally produce crystalline drug particles with greater control of physical and solid-state properties relative to micronization (narrow particle size distribution, reduced charge, increased powder dispersibility). Sievers and co-workers [60-63] have developed a hybrid technique that they term Carbon dioxide Assisted Nebulization with a Bubble Dryer (CAN-BD). Aqueous solutions comprising a bioactive agent are atomized in near-critical carbon dioxide at ~ 80 bar and dried with nitrogen gas at ~ 50 °C. The high pressure in the atomizer enables smaller atomized droplets to be formed.

In spray freeze drying, a feedstock is atomized into liquid nitrogen, and precipitated particles are harvested [64-66]. The process is able to create highly dispersible porous particles. Relative to spray drying, the process is more efficient in terms of product recovery, but it is time consuming, inconvenient (handling of liquid nitrogen) and costly [64].

Foam drying [201-203] involves drying solutions or suspensions of sensitive biologicals in a freeze-drying chamber at non-damaging temperatures. The sample is first boiled under vacuum (e.g., 5 °C, 0.5 torr) to form a mechanically stable foam, and then dehydrated in a conventional freeze

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dryer at elevated temperature (~ 25 – 50°C) to reduce moisture content and increase the glass transition temperature. The process may also be advantageous because of the longer timescale (like freeze drying) for removal of water. The foam-drying process results in a highly relaxed surface. For inhalation, foam drying requires a second step to mill the powder into respirable sizes.

Media milling and wet milling have been used to produce nanocrystalline drug particles [67-69]. Owing to their high surface area and surface energy, milled nanoparticles have a tendency to agglomerate. As a result, the attrition process is usually conducted in the presence of surfactants or block copolymers (e.g., Pluronic F-68). A wide range of novel processing methods are being developed to produce nanoparticles [70-76].

3. Porous particles

Just as a child's whiffle ball moves much slower in an air stream than a solid baseball, so too do the aerodynamic properties of porous drug particles differ significantly from standard micronized drug formulations. The aerodynamic diameter (d_{aer}) depends not only on the geometric size of the particles (d_{geo}), but also on their envelope mass density (ρ_{env}), which is expressed as Equation 1.

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

Edwards and co-workers [13-14,44-47] hypothesized that powder fluidization and dispersibility could be improved by increasing d_{geo}, but that a small d_{aer} could be maintained if the particles were engineered to have a low density. They hypothesized that these large, porous particles (d_{geo} = 5 – 30 µm, ρ_{env} < 0.4 g/cm^3) would have three principal advantages relative to micronized drug. First, the improved powder fluidization and dispersibility would enable high lung delivery efficiencies with a simple, portable, passively operated dry powder inhaler. Indeed, lung deposition as high as 59% of the nominal dose was reported with a vehicle formulation [14]. Second, the larger particles will be poorly phagocytosed by alveolar macrophages, enabling the development of pulmonary, controlled-release formulations [13,45,46]. Finally, the improved powder flow and fluidization afforded by the larger particles will eliminate the need for carrier particles, thereby enabling larger doses (nominal powder dose of ~ 30 mg) to be delivered in a single puff [207]. Alkermes has a number of large porous particle formulations in clinical development, including formulations of insulin [142,143], and human growth hormone, in collaboration with Lilly. The inhaled insulin programme has advanced to Phase III.

Independent studies by Weers, Tarara and co-workers demonstrated that a large geometric size is not a prerequisite to achieve improved powder fluidization and dispersion with porous particles [8,33-34,37]. They demonstrated that small porous particles (d_{geo} = 1 – 5 µm) could achieve comparable lung delivery efficiencies (58% of the nominal dose) [37], with high emitted doses (> 80%), and excellent uniformity in dose.

The ability of porous particles to bypass the throat and deliver a high dose into the lungs leads to a decrease in interpatient variability, even when the powder is administered from a simple, portable inhaler [14,34,37,39,47,134]. The variances observed in clinical pharmacoscintigraphy studies with porous particles were typically 10 – 20% [14,34,37,39,47], versus 20 – 60% for standard micronized drug formulations [134]. The high delivery efficiencies noted with porous particles in passive dry powder inhalers have also been shown in multiple studies to be remarkably independent of variations in peak inspiratory flow rate (PIF) [14,37,47]. The dependence of *in vivo* lung delivery with PIF depends on the interplay of two factors: the variation in powder dispersion with PIF, and the increase in inertial impaction in the throat with PIF. For micronized powders, the variations in powder dispersion often swamp the variations in inertial impaction, leading to increased lung deposition at higher PIFs. For porous particles, the particles disperse at lower energies, and the two competing effects balance one another. The independence of lung deposition with PIF may also contribute to more reproducible delivery *in vivo*.

The ability to efficiently deliver large percentages of small porous particles into the lungs without blending enables new classes of therapeutics with higher lung doses to be delivered as dry powders [8,33-36]. Presently, inhaled tobramycin (TOBI®, Novartis) is administered twice daily to cystic fibrosis (CF) patients by nebulization of an aqueous solution of drug over a 15- to 20-min period. Administration of tobramycin inhalation powder (TIP; Novartis, Nektar Therapeutics) was demonstrated in Phase I clinical studies to cut the administration time to 5 min, resulting in potential improvements in patient compliance and quality of life. *In vitro* studies simulating the inspiratory flow patterns of a young adult CF patient have demonstrated dose linearity for TIP up to a 65 mg fill mass (No. 2 capsule) [8,36]. Similarly, simulations reveal that powder doses as high as 50 mg can be efficiently delivered to pediatric CF patients [8,36]. So far, the large powder doses have been well tolerated by CF patients [35]. Cough- and throat-related effects were the most common adverse events, and were not considered serious. TIP is in Phase III clinical studies. Other high-dose, anti-infective products in development include amphotericin B inhalation powder (Nektar Therapeutics) for treating fungal infections in immunocompromised patients, and ciprofloxacin inhalation powder (collaboration between Bayer and Nektar Therapeutics).

Porous particles have also shown utility in improving suspension stability in metered dose inhalers [38-40], and in drug delivery in conjunction with liquid ventilation [41,42]. The improved suspension stability leads to improvements in

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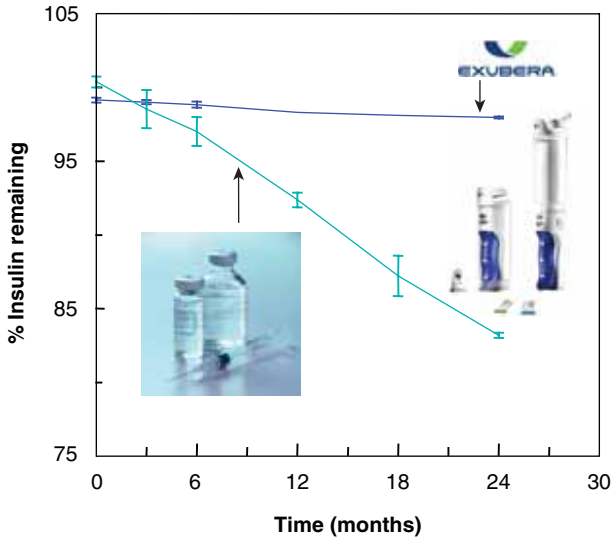

Figure 4. Room temperature (25 °C) stability of amorphous glass formulations of Exubera® (insulin human [rDNA origin] inhalation powder; Pfizer) versus a standard injectable insulin formulation.
Parenteral data from [133].

dose content uniformity, especially for highly potent actives, such as formoterol [38]. Excellent suspensions were prepared either when the drug was dissolved in the aqueous phase of the spray-drying feedstock [38,39], or when the drug was dispersed as microcrystals and coated with a porous phospholipid layer [40]. A gamma scintigraphy study was conducted comparing a small porous particle formulation of albuterol sulfate with the Ventolin Evohaler® (GlaxoSmith-Kline) [39]. The small porous particles were twice as efficient in terms of lung delivery compared with the commercial Evohaler product. Stable suspensions of small, porous particles were also prepared in long chain perfluorocarbons used in liquid ventilation (e.g., perfluorooctyl bromide [PFOB]) [41,42]. The delivery of drugs in conjunction with liquid ventilation may be able to overcome limitations associated with the delivery of aerosols to severely ill patients with obstructed lungs [41,42]. PFOB has been shown to be an effective liquid for lavaging material from the lungs without removing lung surfactant, and because of its positive spreading coefficient, is able to penetrate and deliver drug into non-ventilated regions of the lungs.

Porous particles have been prepared by a number of bottom-up manufacturing methods. The large, porous particles of Edwards and co-workers are prepared by spray drying from an ethanol/water solution at relatively low solids content (< 0.5% w/w) [13,44]. The process is akin to blowing a balloon up and then popping it to create a highly wrinkled surface. Large, porous, polymeric microspheres for inhalation have been prepared by standard emulsion- and double emulsion-based manufacturing processes [13]. The small porous particles of Weers and co-workers [33,38], and Steckel and Brandes [43] are prepared by spray drying a fluorocarbon-in-water emulsion. The fluorocarbon serves as a pore-forming agent to help create hollow/porous particles and the drug can be incorporated either dissolved in the continuous phase or as fine crystals dispersed in the continuous phase. Variations in the volume fraction of the pore-forming agent and processing conditions enable exquisite control of the particle density and geometric size, and control of the emulsion droplet size determines pore size. Acusphere utilizes solid pore-forming agents that can be removed by sublimation or dissolution in a secondary process to create porous particles [208,209]. Porous particles have also been prepared by spray freeze drying [57,58,64,65], CAN-BD [60-63] and by various supercritical fluid processing methods [52-56].

4. Peptide and protein delivery

Macromolecules, notably proteins and peptides, are large, labile materials that are usually administered only by injection. Scientists have explored less invasive forms of delivery, including formulations for ocular, oral, nasal, gastrointestinal and transdermal administration. The large surface area of the alveolar epithelium (~ 100 m^2 in adults), its small thickness (0.1 – 0.2 µm), and dense network of endocytic vesicles make the lung a preferred non-invasive route of administration for peptides and proteins [77-79]. Bioavailabilities on the order of 10 – 25% of the subcutaneous dose have been observed in the absence of penetration enhancers [77,78]. Years of research by Nektar Therapeutics (formerly Inhale Therapeutic Systems) and Pfizer culminated in the recent approval of Exubera® (insulin human [rDNA origin] inhalation powder) [80].

4.1 Room temperature stability

The engineered particles in Exubera were designed to stabilize labile macromolecules against physical and chemical degradation in an amorphous glass (Figure 4). Unlike small molecules, where the crystalline state provides optimal chemical stability, maximal chemical stability for many peptides and proteins is observed in amorphous glass formulations. Glass stabilization involves the use of specially selected carbohydrates, polyols, amino acids, polymers or organic salts, which, when dried in combination with the macromolecule form rigid amorphous matrices [19-25,60-65,81-89]. This technology is embodied in Nektar's PulmoSol®, and Innovata's Q-4Tsys® technologies. Innovata has also developed modified, oligosaccharide, glass-forming excipients that may provide controlled release.

Two mechanisms for glass stabilization of peptides and proteins on desiccation have been proposed. The first involves replacement of the hydrogen bonds that water forms with the protein in solution with hydrogen bonds to hydroxyl-rich

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Table 1. Dry glass transition temperature of excipients.

Excipient	Dry T_g (°C)
Glycerol	-93.0
Sorbitol	-3.0
Glucose	38.2
Maltose	100.6
Trehalose	117.0
Sucrose	73.4
Raffinose	112.6
Lactose	111.8
Mannitol	11.0
Trehalose esters	42.0 – 110.0
Leucine	140.0
Trileucine	70.0 – 100.0 depending on pH
Sodium citrate dihydrate	170.0 (pH > 7), 11.0 (pH < 3)
Ficoll	110.0
Dextran	83.0
Maltotriose	76.0
Galactose	10.0
Xylose	-10.0
Ribose	-10.0

T_g: Glass transition temperature.

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excipients, such as sugars and polyols [85]. The second involves the formation of a highly viscous 'vitreous' liquid that immobilizes the protein [86]. It is likely that both mechanisms are operative in many formulations.

An optimal glass-forming agent has the following characteristics. First, it should be unreactive. Reducing sugars, such as glucose and lactose, should be avoided, as they can react with amino groups on proteins (Maillard reaction), often leading to protein inactivation and a characteristic brown discoloration in the powder. Second, the stabilizer should be a good glass former, with a high glass transition temperature (T_g). Table 1 catalogs the dry T_g of a number of glass-forming agents. T_g values are typically measured by differential scanning calorimetry [87]. Glasses exhibit a transition from the glassy state to a more disordered rubbery state with increasing temperature. Below T_g, there is a gradual loss in configurational entropy to the point of zero mobility (T_0), where motion stops and the glass enters into 'molecular gridlock'. The microviscosity below T_0 can be as high as 10^{13} Poise, and as a result all chemical reactions, including those requiring little motion of the protein (e.g., oxidation and hydrolysis), slow to a crawl or stop altogether. Reactions requiring significant molecular motion (e.g., aggregation) slow effectively at temperatures above T_0, but below T_g. The T_0 has been linked with the hydration limit (W_m), where the degrees of freedom of water mobility within the glass are also limited. Molecular mobility in amorphous glasses is assessed using calorimetric (e.g., moisture-induced thermal activity traces) or dilatometric measurements [87]. Finally, preferred glass-forming agents should tend to form supersaturated solutions on concentration, as opposed to crystallizing. Nonetheless, some glass-forming agents (e.g., mannitol, leucine) have a propensity to crystallize. In order to overcome this limitation, they are often utilized in combination with other glass-forming agents, which help to prevent cystallization and maintain the formulation in an amorphous state.

Amorphous glass formulations should have a T_g that is significantly higher than the proposed storage temperature, preferably below T_0. For room-temperature-stable formulations of proteins and peptides, the T_g after manufacture should ideally be ~ 50°C higher than the storage temperature to ensure that a reasonable shelf-life for the product can be achieved. Even short-term excursions above T_g can lead to irreversible phase transformations (e.g., crystallization) of excipients. The glass transition temperature depends critically on the moisture content of the powder. As such, amorphous formulations must be packaged to protect the powder from the deleterious effects of moisture ingress. For example, Exubera is packaged in foil–foil blisters to minimize water permeation [80]. Ideal glass-forming excipients include trehalose and sodium citrate, which both have T_g values well in excess of 100 °C (Table 1).

Although carbohydrates, polyols and organic salts are excellent glass formers, they are often hygroscopic with high surface energies, and, as such, may be suboptimal from the standpoint of aerosol delivery. As a result, amorphous glass formulations have continued to evolve and relatively complex core/shell particles are being advanced. These particles utilize multiple excipients that differ in their solubility and surface activity. During the drying process, the more soluble materials with low surface activity will diffuse away from the interface, leaving more hydrophobic, surface-active materials at the interface [19,20,204]. Such materials drive improved fluidization and dispersibility for the engineered particles. The development of core/shell particles also helps to maintain the stability of the peptide/protein during the dehydration process, as proteins often degrade when exposed to the air/water interface. Elversson and Millqvist-Fureby [88,89] also demonstrated that 'surface competition' could be used to create *in situ* polymer-coated particles with encapsulated protein.

The nature of the drying process has been found to be important in maintaining the stability of peptides and proteins, as well as live viruses and cells. There are two key aspects to the drying process: time and temperature. The freeze drying process requires temperatures below the freezing point of water. This can have a negative impact on the viability of sensitive biologicals. Also, spray drying, which dries particles on the timescale of milliseconds, can remove water too rapidly to preserve activity. Some new bottom-up

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processing methods may provide improved stability. For example, foam drying makes use of the best properties of both freeze drying (longer timescale) and spray drying (drying temperatures above the freezing point of water). Foam drying [201-203] has demonstrated excellent utility in stabilizing complex macromolecules, live viruses and cells. Other techniques being explored that have also demonstrated utility in reducing activity loss in labile biologicals include spray freeze drying [64,65] and CAN-BD [60-63].

Protein microspheres have also been prepared by temperature-controlled coacervation of proteins in the presence of polyethylene glycol (PEG). The PEG is removed in subsequent processing steps, resulting in microspheres comprising nearly 100% protein. The process produces microspheres with a geometric size of 1 – 3 µm (Figure 3J). Formulations of insulin [127] and α_1-antitrypsin [144] have been reported. The inhaled insulin product is in preclinical development.

4.2 Improving bioavailability

Inhalation remains the only route for peptide and protein administration that does not require enhancers to achieve effective systemic absorption. With that said, the relative bioavailability associated with pulmonary delivery (10 – 25% of subcutaneous injection) places a significant burden on the cost of goods of inhaled peptide therapeutics. Accordingly, the search continues for ways to improve the bioavailability of inhaled proteins and peptides. To this end, scientists are exploring the next generation of permeation enhancers with the aim of achieving an acceptable safety profile [90-98]. In addition, methods for slowing enzymatic degradation via molecule engineering (e.g., protecting labile residues from attack by PEGylation [99]) or the use of protease inhibitors [90] are also being explored.

A wide range of excipients have been explored as permeation enhancers for aerosols. These were recently summarized by Okamoto *et al.* [90]. First-generation enhancers included bile salts, surfactants, fatty acids, short-chain phospholipids and citric acid, to name a few. Many of these molecules exerted their effect by altering the curvature of the phospholipid-based epithelial membrane and concerns centred on irreversible damage to the epithelium. Even short-term loss of integrity of the pulmonary epithelium could have negative implications. Work continues with the development of new alternative enhancers. High molecular weight polymers that have bioadhesive properties (e.g., chitosan and polyacrylates) have been explored [91]. The bioadhesive nature of the polymer allows them to remain concentrated in the area of absorption. Chitosans have been shown to open tight junctions through the interaction of positively charged amino groups with the negatively charged sialic acid groups of membrane-bound glycoproteins. Yamada *et al.* [91] utilized bioadhesive chitosan oligomers to improve the pulmonary bioavailability of IFN-α in rats. Positive safety findings included a lack of membrane damage to the rat pulmonary tissues, as determined by absence of leakage of protein and lactate dehydrogenase in broncho-alveolar lavage fluid. Concerns remain with respect to the long-term safety of this approach, as well as the clearance of the high molecular weight polymer from the lungs.

Detergents have long been explored as permeation enhancers [90,95-98]. Tetradecyl-β-maltoside is the latest in this line of permeation enhancers [95-98]. Being nonionic, tetradecyl-β-maltoside has a very low critical micellization concentration relative to bile salts and other charged surfactants. Permeation activity is observed at lower concentrations. Significant permeation enhancement has also been observed for a number of biological therapeutics, including insulin [96] and low molecular weight heparin [97]. Tetradecyl-β-maltoside was shown to open tight junctions for a finite period of time, without evidence of adverse effects. Again, these were acute studies and do not address the concerns that would be associated with the development of chronic inhalation treatments containing enhancers.

Farthest along the development path (Phase III) is an inhaled insulin formulation being developed by MannKind Corp., which utilizes fumaryldiketopiperazine (FDKP) to alter the pharmacokinetic profile of insulin relative to other inhaled insulin formulations [93,94]. The Technosphere® particles exhibit a more rapid onset of action, and a more rapid return to baseline glucose levels post-meal. The mechanism by which FDKP enhances the rate of insulin absorption has not been established. Explanations attributing the rapid absorption to differences in insulin aggregation state (i.e., monomer versus hexamer) are not compelling, as multiple laboratories have shown that there is no increase in absorption rate when Lispro® (rapid-acting monomeric insulin; Eli Lilly) is given via inhalation compared with hexameric insulin [96,205]. Another hypothesis is that FDKP may alter the distribution of charges on the surface of insulin, thereby facilitating more rapid transport through the epithelial membrane [206]. Additional studies are required to better assess the mechanism of absorption enhancement. An increase in biopotency is noted relative to inhaled insulin formulations without an enhancer. Technosphere particles are prepared by controlled precipitation of FDKP followed by adsorption of insulin in its monomeric form onto the surface of the particles. The particles exhibit a high degree of porosity (like a three-dimensional sphere constructed from a deck of cards). Long-term clinical safety studies are in progress.

5. Immune modulation

5.1 Pulmonary vaccines

Despite the fact that a large number of pathogens enter via the respiratory tract, no commercial vaccines are presently administered via oral inhalation. The inability of parenterally administered vaccines to induce local immunity is considered to be a serious limitation [100].

The World Health Organization (WHO) estimates that ~ 1.7 million vaccine-preventable childhood deaths occurred in

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the year 2000, of which 777,000 (46%) were attributable to measles [101-104]. Since the early 1980s, a number of trials conducted in Mexico by the groups of Sabin and de Castro [105-107], have demonstrated the utility of delivering the measles vaccine via inhalation from a nebulizer. Pulmonary administration has been shown to be safe and immunogenic as a first dose among infants < 9 months of age, and able to induce mucosal and cellular immunogenicity among school-age children when they are provided with a booster dose.

The WHO, in collaboration with the Gates Foundation, is conducting an aerosolized measles vaccine campaign with the ultimate goal aimed at eradicating the disease [103]. Although the initial focus is on nebulizer systems that mimic the original Mexican nebulizer, there are additional Gates Foundation grants aimed towards the development of dry powder delivery systems for measles vaccines [301].

The inhalation of dry powders would be the preferred means for administering vaccines (e.g., measles) in developing nations [23,26,102]. In addition to the more robust immune response afforded by inhalation, dry powder vaccines provide two additional benefits of critical importance. First, aerosol administration eliminates the needle. This is expected to improve patient compliance, reduce the potential for disease transmission, and eliminate costs associated with sharps disposal. Second, the ability to formulate dry powder vaccines as amorphous glasses with high T_g eliminates the need for a cold chain of refrigerators to maintain stability on distribution. This is especially critical in remote arid areas, such as subSaharan Africa or India.

Engineered measles vaccines comprising the Zagreb strain have been prepared by multiple bottom-up techniques including spray freeze drying [65] and foam drying [201], and by traditional top-down methods involving powder blends [102]. The viability of the live virus has been shown to be maintained through the manufacturing process. Inhalation as a dry powder also circumvents the need to reconstitute a freeze-dried vaccine powder for injection.

Recent studies have also demonstrated that oral inhalation may be suitable for the administration of the trivalent MMR (measles, mumps, rubella) vaccine. The development of inhaled formulations of multivalent vaccines is a critical factor for the potential utilization of inhaled vaccines in the developed world [106].

Mucosal vaccination may also be superior for non-replicative vaccines (e.g., split subunit influenza vaccines) endowed with limited intrinsic immunity, which are presently administered parenterally [26,100,108]. This was demonstrated by Smith *et al.* [26], who spray-dried Fluzone® (Aventis Pasteur) in a phospholipid-based microparticle. Exposure of the bronchial associated lymphoid tissue to the spray-dried vaccine was more effective than parenteral or nasal administration in triggering specific immunity. In particular, pulmonary administration resulted in a more robust local response with respect to both humoral and cellular-based immunity. Surprisingly, pulmonary administration also gave a more robust systemic response. Indeed, the robust immune response and protection afforded by pulmonary administration of inactivated influenza vaccine was clinically demonstrated by Waldman *et al*. in 1969 [108]. Relative to live influenza vaccines, a formulated subunit vaccine for respiratory delivery may also circumvent concerns regarding side effects in young children and the elderly, as well as inherent manufacturing and regulatory issues. In addition, dry powders for inhalation do not require preservatives. A recent study by Lombry *et al*. [32] suggested that pulmonary administration of DNA vaccines may also induce a more robust immune response than parenteral administration.

A variety of vaccines against biowarfare agents are being developed [109]. It is likely that these vaccines may be more effective if administered via aerosol. In this regard, it is interesting to note that aerosol vaccination was routinely conducted in the former Soviet Union to protect scientists from the biowarfare agents (e.g., anthrax, plague, tularemia, smallpox) they were developing [110].

5.2 Therapeutic antibodies

Therapeutic antibodies are being developed for pulmonary diseases, such as respiratory infections (e.g., respiratory syncytial virus, *Pseudomonas aeruginosa*), allergic and inflammatory diseases (e.g., asthma and atopic conditions) and cancer (e.g., adenocarcinoma, epithelioma) [111]. Presently, antibodies are administered by intravenous injection. Their effectiveness depends on the ability to achieve therapeutic concentrations in the target organ. Pulmonary administration of antibodies enables efficient targeting to the lungs, at concentrations 10- to 100-fold higher than can be achieved by injection [27,28]. Although inhalation enables these high local concentrations of immunoglobulin to be achieved, effective delivery requires uptake across the epithelium into the pulmonary interstitium. Achieving therapeutic concentrations of antibodies in the pulmonary interstitium requires an understanding of the dynamics of particle versus macromolecule clearance. One can envision two possible scenarios. First, 'retentive' particles with slow IgG release may be associated with improved interstitial IgG targeting by avoiding saturation of putative IgG transporters and prolonging antibody release. Conversely, particles that release the immunoglobulin rapidly ('non-retentive' particles) may circumvent particle clearance on the mucociliary escalator and phagocytosis by alveolar macrophages. Dellamary *et al.* [27] demonstrated that non-retentive particles were preferred, and that concentrations as high as 60% of the administered IgG dose could be deposited in the interstitial tissue. In contrast, retentive particles were rapidly cleared by alveolar macrophages in an Fc receptor-mediated scavenging process over a period of 15 – 30 min. Dellamary *et al.* also demonstrated that the non-retentive particles loaded with anti-influenza antibody were effective in curbing virus replication, with a resulting positive clinical outcome in mice. Once in the interstitium, the IgG molecules were absorbed very slowly into the systemic circulation

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over a period of days, suggesting that a depot of immunoglobulin can be achieved in the pulmonary interstitium.

An inherent problem of particulate formulations is the enhancement of the immune response to determinants expressed by incorporated proteins. One way to minimize the potential impact is to use humanized antibodies or fully human monoclonal antibodies. Even in this case, anti-idiotypic antibodies may be induced. The development of these neutralizing antibodies may trigger unresponsiveness to subsequent administrations. Dellamary *et al.* demonstrated that this issue could be overcome with administration of an intravenous priming dose [27]. This may be important for chronic administration of therapeutic antibodies.

Therapeutic antibodies of IgE have been prepared in amorphous glasses by spray drying [146,147]. Unfortunately, local delivery of IgE antibody to the lungs failed to show efficacy following allergen challenge in asthmatics [145].

5.3 Targeting therapeutics

The rapid clearance of 'retentive' particles comprising immunoglobulins by alveolar macrophages may be used to actively target therapeutics to alveolar macrophages by Fc receptor-mediated endocytosis. Rojanasakul *et al.* [112] utilized formulations comprising a polylysine–IgG conjugate and plasmid DNA, and demonstrated improved transfection in cultures of alveolar macrophages. In contrast, pulmonary epithelial cells lacking the Fc receptor showed no transfection. Curiel *et al.* [113] utilized transferring–polylysine conjugates as a means to transfect lung epithelial cells. Although successful, the ubiquitous presence of transferrin receptors in various cell types in the lung may result in non-specific targeting. Bot *et al.* [29] also demonstrated effective targeting to alveolar macrophages of a phospholipid-based dry powder influenza vaccine loaded with IgG. Cryan [114] recently completed an excellent review of carrier-based strategies for targeting protein and peptide therapeutics to the lungs. The review goes into detail regarding active targeting (e.g., ligands), intracellular trafficking, endosomal release and nuclear localization of drugs – factors that will drive continued advances in particle and formulation engineering in the next decade.

5.4 Facilitating systemic delivery of protein therapeutics

The potential for antibodies to achieve a depot in the pulmonary interstitium may enable the sustained delivery of peptides and proteins. Syntonix's SynFusion® technology links the Fc region of the antibody to a macromolecule in a novel manner [115-117]. When administered via inhalation, the therapeutics are transported across the pulmonary epithelium by receptor-mediated transcytosis via the neonatal FcRn receptor. The FcRn receptor is expressed in both the bronchial and alveolar epithelium. There may be advantages, however, to administration of antibodies to the bronchial airways, as there are more abundant FcRn receptors and less potential for macrophages clearance. *In vitro* cell studies have demonstrated that IgG's absorptive transcytosis process is controlled by high affinity and low capacity binding kinetics, with a maximal absorption of ~ 80 ng/h [119,120]. As such, it may be difficult to utilize the lung to transport many therapeutic antibodies into the systemic circulation, as higher systemic concentrations are required than are readily achievable. With that said, Syntonix has demonstrated that they can achieve therapeutic concentrations of several peptides and proteins in the systemic circulation, with prolonged absorption kinetics and high bioavailabilities (20 – 50%) [115-117]. Syntonix is in clinical development with erythropoietin, follicle stimulating hormone (with Serono), IFN-β (with Serono) and a range of peptides (Boehringer Ingelheim). Further studies are required to better understand the dose limitations associated with the technology. So far, the drugs have been administered as a liquid via nebulization. Formulation as a dry powder may enable higher efficiencies of lung delivery to be achieved, further enhancing the utility of the technology. Syntonix was recently acquired by Biogen Idec.

In a similar fashion, Sacaan *et al.* (Arizeke) [118] have developed a chimeric fusion protein consisting of a ligand that binds to the polymeric immunoglobulin receptor (pIgR) and a therapeutic protein. The high capacity pIgR efficiently delivers IgA dimers (mw = 450 kDa) and IgM pentamers (mw = 900 kDa) from the basolateral to the apical surface of the lung epithelium.

6. Conclusions

A decade ago, noted aerosol scientists Richard Dalby and Tony Hickey stated: '*The forces governing dispersion are well documented and consist mainly of electrostatic, van der Waals, and capillary forces. Knowing these forces exist has not facilitated aerosol generation to any extent*' [121]. Since that time, advances in particle engineering (e.g., porous particles) have enabled particles with decreased interparticle cohesion and improved fluidization to be advanced. In turn, these formulations have enabled new therapeutics requiring larger delivered doses (e.g., anti-infectives) and narrower therapeutic indices (e.g., insulin) to be developed. Stabilization of peptides, proteins, live viruses and cells has been achieved by development of amorphous glass formulations coupled with sophisticated process engineering. These advances in particle design have enabled non-invasive delivery of proteins and peptides to be achieved. Immune modulation represents a huge opportunity of pulmonary delivery, affording non-invasive vaccine and antibody delivery without the need for the cold chain. The future looks bright for engineered particles in pulmonary delivery, and continued advances will pave the road towards targeted delivery of therapeutics.

7. Expert opinion

The long timeline associated with inhaled insulin development led some to question whether engineered

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particles would gain acceptance in inhalation products. Indeed, engineered particles have yet to penetrate into life cycle management opportunities for asthma therapeutics. Asthma therapeutics are low cost, potent molecules with a large therapeutic index. As a result, the improvements in delivery efficiency and reductions in interpatient variability afforded by particle engineering are not compelling drivers for reformulation. Instead, life cycle management activities for asthma therapeutics have focused on the development of fixed-dose combination products and convenient delivery devices. Blend technologies have also continued to evolve [122-125,210], as new analytical techniques (e.g., atomic force microscopy) have improved understanding of inter-particle cohesive and adhesive forces [5,6]. It remains unlikely that advanced particle engineering technologies will be utilized in the formulation of existing asthma therapeutics. More likely, the technologies will enter into the asthma space with the development of new chemical entities (NCEs).

With that said, the pharmaceutical industry has also been reticent to stack the risk of a new drug delivery technology on top of the risks associated with the development of a NCE. With the approval of Exubera, and the ascension of many other inhalation products based on engineered particles into late-stage development, the opportunities to develop NCEs with these technologies is at hand. Interestingly, as the particle engineering technologies are reaching commercialization, the technology providers are transitioning from being drug delivery companies to fully integrated specialty pharmaceutical companies. As such, providing a service on a potential partner's NCE may no longer fit within their business models.

To advance forward, engineered particles have had to come in the back door, driving significant innovation and opportunity in the field. The approval of Exubera [80,126] brought many firsts: i) the first non-invasive method of insulin delivery; ii) the first room-temperature-stable insulin formulation; iii) the first active dry powder inhaler. Exubera required the development and large scale commercialization of multiple new technologies including: i) spray drying to produce 1 – 3 µm particles for inhalation; ii) autofilling of 1 mg doses of fine particles; iii) development of packaging technology designed to protect the amorphous powder from the deleterious effects of moisture. The competition in the inhaled insulin marketplace will become intense in the timeframe of 2010 – 2015, as multiple products and technologies emerge. The products following Exubera are attempting to differentiate themselves based on features (e.g., device size, more rapid onset of action, titratable dose, reservoir device) [13,93,94,127-129,142,143]. As additional products emerge, cost will become a major driver.

Because of the relatively low bioavailability of inhaled macromolecules, researchers continue to search for formulation alternatives to reduce cost of goods. The recent advances in particle design (e.g., porous particles) have dramatically improved delivery efficiencies, enabling significant increases in bioavailability. In addition, active transport [114-118] may enhance bioavailability for potent macromolecules, especially higher molecular weight proteins. The use of enhancers in inhalation products continues to be explored, although the ability to formulate in the absence of enhancers remains a key advantage for pulmonary delivery compared with other modes of delivery. A number of additional macromolecular drugs (e.g., parathyroid hormone, follicle stimulating hormone, human growth hormone, erythropoietin) are in development, and one can expect that additional peptides and antibody products will follow, as additional long-term safety studies of inhaled macromolecular drugs are completed. One item holding back the development of inhaled proteins and peptides are difficulties associated with sourcing. The development of biogenerics and/or biosimilars across the globe may help to facilitate product development of inhaled macromolecules.

After inhaled insulin products, the next wave of engineered particles has been designed to combat respiratory tract infections. Low cost dry powder vaccines are being developed for immunization in developing nations [102-107]. These vaccines will someday help to eradicate the world of many infectious diseases. In addition, dry powder inhalation of anti-infectives with engineered particles is in late stage development [34-36]. These high-dose products (lung dose > 10 mg), are enabled by the improved delivery efficiency and high drug loading achievable in porous particle formulations. It is anticipated that additional high-dose products will be developed (e.g., antivirals, α_1-antitrypsin, ciclosporin, lung surfactant, low molecular weight heparin) as these new technologies move through to commercialization. Finally, advances in the ability to deliver discrete nanoparticles into the lungs may enable new opportunities [67-75].

The reduced interpatient variability afforded by porous particles is also expected to enable improved pulmonary delivery of therapeutics with a narrow therapeutic index.

As a result of the highly vascularized alveolar epithelium, inhalation is a preferred route of administration for the rapid delivery of therapeutics to the heart or brain (e.g., in the treatment of migraine, emesis and anxiety). So far, spray dried amorphous particles have been utilized in the development of room-temperature-stable formulations of macromolecules. Amorphous glass particles may also facilitate ultra-rapid systemic delivery, as the amorphous phase has been demonstrated to have more rapid dissolution than crystalline drug particles. Inhalation may also have a role in the avoidance of first-pass metabolism and food effects for orally administered drugs. Advances in our ability to target drugs to cells and control cellular trafficking are expected to expand upon our ability to deliver complex macromolecules such as DNA and RNA [130-132].

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