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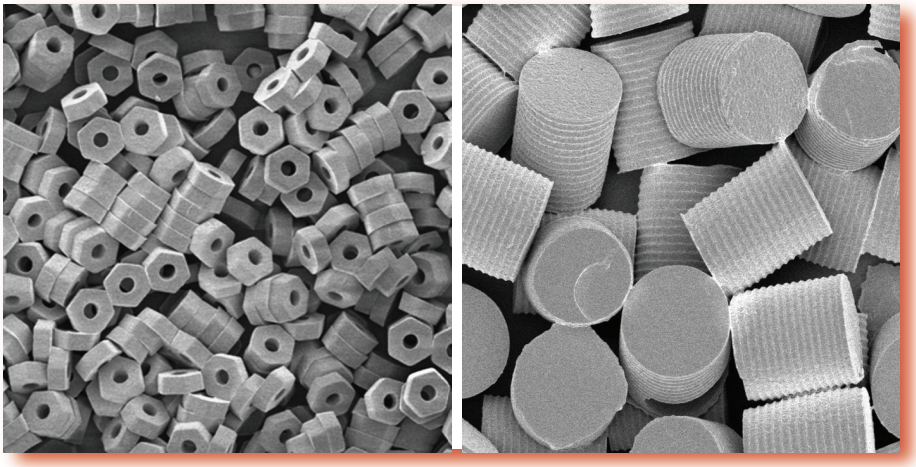
Particle engineering for inhalation formulation and delivery of biotherapeutics

Advances in particle engineering and micro-molding are enabling more control over inhalation powder chemical composition and aerodynamic size



Micro-molded PRINT particles, fabricated to sizes a fraction of the diameter of human hair, are being used in pharmaceutical development (as shown, 3 micron hexnuts, left, and 3 micron cylinders, right).

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Introduction

Inhalation as a drug delivery route has been in clinical practice for more than 50 years, with small molecules for the treatment of asthma/COPD capturing the majority market share of inhalation products. Since that time, protein therapeutics or biotherapeutics have emerged as a significant class of drugs in clinical development and on the market. Delivery of biotherapeutics directly to the lung via inhalation may hold several advantages over subcutaneous and intravenous drug delivery for both systemic and respiratory drug targets. For systemic therapeutics, like insulin, expansive alveolar surface area provides enhanced drug adsorption directly into the blood stream. For respiratory targets, inhalation offers direct drug delivery to the site of disease, potentially reducing the overall required dose and decreasing systemic exposure to the drug and formulation excipients. Development of inhalation formulations with biotherapeutics has nevertheless lagged behind other delivery routes in terms of the number of marketed products. Currently, nebulized recombinant human DNase (Pulmozyme, Genentech) for the treatment of cystic fibrosis is the only marketed oral inhalation biotherapeutic. The small number of currently-marketed inhaled biologics could partially be attributed to fewer respiratory targets being identified and pursued, but may also be related to the formulation and delivery challenges associated with maintaining protein and inhalation drug product stability.

When developing inhaled biotherapeutics, key considerations must be made in order to ensure 1) the biotherapeutic has maintained its chemical structure and bioactivity throughout the formulation processing steps and product storage and 2) the drug product maintains its physical stability during storage. A wide array of assays can be used to assess chemical stability and bioactivity of biotherapeutics; most commonly, size exclusion and reversed phase chromatography, gel electrophoresis, mass spectrometry and binding and/or cell-based assays that are specific to the molecule's mechanism of action. Physical characterization tools are used to measure the particle's aerodynamic diameter, such as time-of-flight analysis and cascade impaction. These methods allow for a population of particles to be sized based on mass median aerodynamic diameter (MMAD). Several factors influence an aerosol MMAD, including particle porosity, volume and shape. It is generally accepted that particles with an aerodynamic diameter of 1-5 μm (referred to as the "respirable range") tend to deposit in the lungs, while particles larger than 5 μm are trapped in the upper respiratory tract (primarily back of the throat and

mouth).[1] Traditional inhalation powder formulation technologies (e.g., jet milling) are often limited by their difficulty in formulating biotherapeutics. As a result, nebulization is commonly the choice drug product form for delivering proteins to the lung. This is the case of the marketed product Pulmozyme and other inhaled proteins in clinical development such as alpha-1 antitrypsin (AAT) (Kamada Biopharmaceuticals). Dry powder inhaler (DPI) delivery, however, is generally acknowledged to be more efficient and convenient for patients compared to nebulization and often more compatible with biotherapeutics than metered dose inhaler (MDI) formulation components and propellants. For these reasons, inhalation powders delivered by DPIs are a promising inhalation drug product form for the pulmonary delivery of proteins. Recent innovations in particle engineering, a term used to describe particle generation techniques driven by rational design of particle size, morphology and chemical composition, have made important advances for this field by enabling several inhalation powder biotherapeutics to reach clinical development (Table 1).

Particle engineering for inhalation

Emulsion, supercritical antisolvent and spray drying are three examples of particle engineering systems being adopted for the generation of inhalation powder therapeutics.[2] Emulsion systems have been shown to be capable of generating solid spherical insulin, alpha-1-antitrypsin and lipid particles, but have difficulty controlling particle size and homogeneity.[2] With this approach, solid spherical particles are formed using non-miscible solvent emulsions generated through sonication or homogenization. Particles thereby form in the dispersed phase and are isolated by evaporating the continuous phase (typically a volatile solvent) to produce discrete particles. Similarly, supercritical or near-critical fluid antisolvent and expansion systems have been used to generate inhalation powder formulations with an anti-CD4 antibody, alpha-1-antitrypsin, and trypsinogen.[3] Particles developed with this method are formed through precipitation of protein from a supercritical solution (usually supercritical CO_2) or precipitation of protein from saturated solutions using supercritical fluids as anti-solvents.[4] Supercritical CO_2-Assisted Nebulization with a Bubble Dryer (Supercritical CAN-BD) is another method that creates protein particles by passing emulsions of drug, solvents and supercritical CO_2 through a nozzle to generate liquid droplets that are then dried by nitrogen gas.[4] Spray drying, a third particle engineering approach, is one of the most common technologies used to generate inhalable particles containing proteins. During spray drying, a feed solution of protein is atomized through a nozzle using compressed air or gas. As the gas cools, the atomized liquid droplets phase transition to solid protein particulates.[5] The chemical composition of the solid particulates is dictated by the content within the feed solution and the variables that control particle size and morphology are beginning to be better understood.[5] One variation of spray drying is spray freeze drying, a technique that avoids producing air-liquid interfaces by atomizing particles into a cryogenic liquid followed by lyophilization to produce discrete particles.[6]

As with all particle engineering systems, the ability to "scale-up" laboratory processes to industrial or commercial levels should be a critical consideration. Scale-up of emulsion-based systems can be complex, as the evaporation phase is generally slow and produces large solvent waste streams.[2] Supercritical CAN-BD conducted as a continuous process can be scaled in correlation with the liquid emulsion flow rate into the atomizer nozzle.[3] Spray drying is amenable to producing large quantities of material and has been used in both marketed and clinical phase products (see Table 1), but often requires different spray drying units depending on the desired

Table 1

Clinical development of inhalation powder biotherapeutics using particle engineering

Developer	Molecule/Indication	Particle Engineering Technology	Furthest Phase of Development
Pfizer / Nektar (Exubera)	Insulin / Diabetes	Spray drying	Marketed (terminated)
Eli Lilly / Alkermes (AIR)	Insulin / Diabetes	Large porous particles	Phase III (terminated)
Mannkind (Afrezza)	Insulin / Diabetes	Technospheres	Phase III
Aerovance (Aerovant)	Pitrakinra, IL-4/IL-13 receptor antagonist / Asthma	Spray drying	Phase II
NexBio (Fludase)	Sialidase fusion protein/ Influenza virus	Temperature-controlled organic solvent assisted precipitation (TOSAP)	Phase II
Mannkind (MKC253)	GLP-1 (Glucacon-like peptide)/Diabetes	Technospheres	Phase I

scale. Scale-up of spray freeze drying processes, on the other hand, is expected to be problematic, as large volumes of cryogenic liquids can introduce safety hazards.[2]

Most particle engineering approaches with biotherapeutics tend to require formulation excipients in order to enhance both the particle population aerodynamic characteristics and preserve the biotherapeutic chemical structure. A wide range of formulation excipients have been shown to enhance inhalation powder formulations with biotherapeutics, as summarized in Table 2. In general, proteins are thought to be de-stabilized by the stresses associated with drying at air-liquid interfaces, upon exposure to elevated temperatures and during freeze-thaw cycles. Stabilizing proteins and peptides as dry powders has been achieved in two ways: 1) replacing hydrogen bonds that would normally be made with water while in an aqueous solution with hydroxyl-rich excipients and 2) locking the protein in a viscous mixture with excipients that have a high glass transition temperature.[7] Non-reducing sugars, such as trehalose, sucrose and raffinose, meet both requirements while reducing sugars such as lactose will react with free amines on the protein or peptide.[8] Sugar alcohols (e.g. sorbitol), metal ions (e.g. zinc), buffer salts and surfactants (e.g. polysorbates) have also been proposed to assist in maintaining protein structure in the dried state, while amino acids have been observed to enhance the aerodynamic performance of dry powder formulations.[6,8,9] Optimizing inhalation powder formulations and excipient combinations, in general, is a molecule-specific process and often empirically identified. The greater the flexibility a particle engineering platform has in its capability to screen excipient combinations, the more likely an acceptable formulation will be identified that can be advanced towards product development. Moreover, despite improvements in inhalation powder formulations with current particle engineering technologies, precise control of particle size and morphology for the optimization of dose uniformity and lung deposition remains a challenge.[10]

Micro-molding engineered particles

Through adaptations of advanced technologies from the microelectronics industry, Liquidia Technologies has recently applied a mold-based particle engineering platform, termed PRINT, which stands for Particle Replication In Non-wetting Templates,[14] to the generation of inhalation powders.[15] Unlike other particle engineering technologies, this platform achieves uniform particle shape, size, and morphology defined by the input mold feature. The PRINT particle engineering platform has broad life science applications, including the development of next generation inhalation powder formulations and has previously been shown to be compatible with biotherapeutics such as proteins and oligonucleotides.[15,16] A schematic of the PRINT process is illustrated in Figure 1. Drug or drug blended with excipients flow into a fluoropolymer micro-mold of a precise size and shape. The molded particles, taking on the geometric dimensions of the mold cups, are isolated as stable dispersions of particles or free-flowing powders. The control over particle characteristics afforded by the PRINT micro-molding approach leads to narrow aerodynamic distributions around a pre-defined particle size and chemical composition.[15] In addition, the PRINT micro-molding approach has been adapted for roll-to-roll

Table 2

Common excipients used in inhalation powder particle engineering formulations

Protein	Technique	Excipients	MMAD (μm)	Article Reference
Recobminant human DNase; Anti-IgE antibody	Spray drying; spray freeze drying	Mannitol, trehalose, sucrose	2.6-11.0	(6)
IgG1	Spray drying	Mannitol, trehalose, sucrose, leucine	4.2-6.3	(9)
Lysozyme	Spray drying, porous	Trehalose, raffinose	2.8-2.9	(11)
Anti-CD4 antibody	Supercritical CAN-BD*	Saccharide, surfactant	1.4-1.8	(3)
Alpha-1-antitrypsin	Supercritical CAN-BD	Trehalose, Tween-20	1.9-2.2	(3)
Trypsinogen	Supercritical CAN-BD	Trehalose, sucrose	0.9-1.4	(3)
Recombinant human DNase	Spray drying	Sodium chloride	3.1-5.5	(12)
Salmon calcitonin	Spray drying	Human serum albumin, mannitol, citric acid, sodium citrate	3.3	(13)

* CAN-BD = CO_2-Assisted Nebulization with a Bubble Dryer

manufacturing and has reached a scale of cGMP-compliant production appropriate to support preclinical and clinical studies. This roll-to-roll process is continuing to be developed in order to achieve commercial scales.[15] These features of the PRINT particle engineering platform position it as a useful tool for advancing the development of inhalation powder formulations and products with biotherapeutics that are suitable for respiratory delivery.

Generation of PRINT particles containing functional proteins is possible as either pure protein particle designs or blended with a broad range of formulation excipients. Enzymes, serum proteins, engineered proteins and full-length human monoclonal antibodies have been formulated using the PRINT platform. A wide range of excipients for protein formulation have also been incorporated into PRINT particles, including non-reducing sugars, amino acids and surfactacts, for the purpose of improving the biotherapeutic chemical stability and the dry powder aerosol performance. As one example, the PRINT micro-molding technology has been used to generate shape-specific particles with the biotherapeutic bovine DNase. This has been done both as a protein blended at an equal ratio with the non-reducing sugar trehalose (Figure 2, left) and as pure protein (Figure 2, right).[15] The particle geometries displayed in Figure 2 have previously been demonstrated with other drug-excipient combinations to possess an MMAD of less than 3 µm (data not shown) and are thus expected to be suitable for lower respiratory tract delivery. In addition to achieving the desired particle size and shape, the chemical structure and bioactivity of protein formulations are preserved throughout the PRINT particle fabrication process, as assessed here for bovine DNase by size exclusion chromatography for the excipient blended particle formulation (Figure 3) and previously using a methyl green DNA degradation assay for the pure protein particle formulation,[15] respectively. These examples demonstrate the capability of micro-molding particle engineering, via the PRINT technology, to generate inhalation powder formulations with biotherapeutics that are suitable for direct pulmonary delivery.

Outlook for inhaled biotherapeutics

Recent advances in particle engineering technologies are enabling the development of biotherapeutics for pulmonary delivery today. These technologies control key particle attributes such as excipient composition and porosity, while the PRINT particle engineering platform additionally has the unique ability to precisely define particle size and shape using its micro-molding approach. Production scale for particle engineering technologies is an important consideration that needs to be addressed on a technology-by-technology basis. Nevertheless, as use and acceptability of particle engineering for the development of inhalation powder biotherapeutics becomes more widespread, the potential benefits in terms of patient convenience, drug safety and efficacy should be realized along with the ultimate goal of improving patient care.

Figure 1

Schematic of Particle Replication in Non-wetting Templates (PRINT) micro-molding methodology

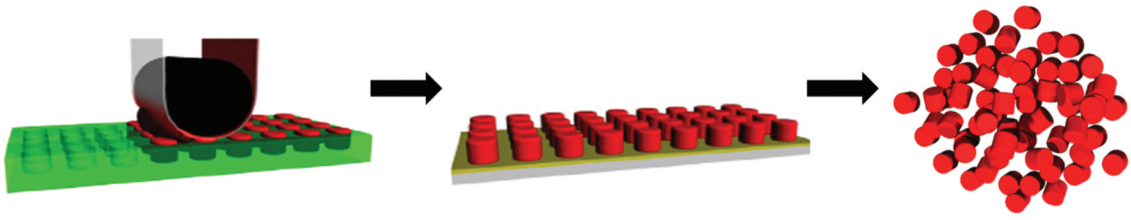


Figure 2

Micro-molded protein particles of defined size and shape

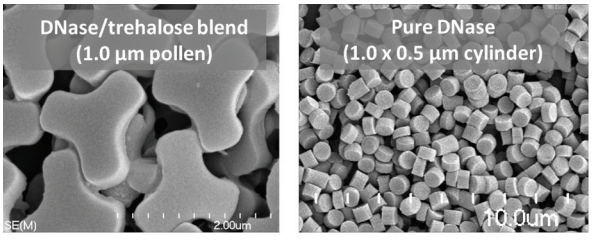
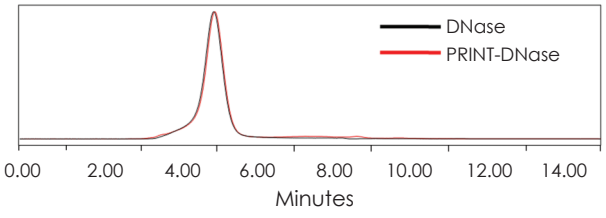


Figure 3

Retention of protein chemical structure after micro-molding particle fabrication by size exclusion chromatography



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