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Inhalable Nanoparticle-based Dry Powder Formulations for Respiratory Diseases: Challenges and Strategies for Translational Research

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Abstract

The emergence of novel respiratory infections (e.g., COVID-19) and expeditious development of nanoparticle-based COVID-19 vaccines have recently reignited considerable interest in designing inhalable nanoparticle-based drug delivery systems as next-generation respiratory therapeutics. Among various available devices in aerosol delivery, dry powder inhalers (DPIs) are preferable for delivery of nanoparticles due to their simplicity of use, high portability, and superior long-term stability. Despite research efforts devoted to developing inhaled nanoparticle-based DPI formulations, no such formulations have been approved to date, implying a research gap between bench and bedside. This review aims to address this gap by highlighting important yet often overlooked issues during pre-clinical development. We start with an overview and update on formulation and particle engineering strategies for fabricating inhalable nanoparticle-based dry powder formulations. An important but neglected aspect in *in vitro* characterization methodologies for linking the powder performance with their bio-fate is then discussed. Finally, the major challenges and strategies in their clinical translation are highlighted. We anticipate that focused research onto the existing knowledge gaps presented in this review would accelerate clinical applications of inhalable nanoparticle-based dry powders from a far-fetched fantasy to a reality.

Keywords dry powder inhalation · pulmonary drug delivery · nanoparticles · particle engineering · translational research

Introduction

The burden of various respiratory conditions (e.g., coronavirus disease 2019 (COVID-19), lung cancer, tuberculosis, chronic obstructive pulmonary disease (COPD), etc.) has risen sharply over the past decades, warranting the

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development of highly effective and safe respiratory therapeutics. Pulmonary delivery of respiratory therapeutics is preferred over systemic (e.g., intravenous or oral) administration to achieve localized delivery of drugs to the lungs for improved treatment efficacy and reduced dose requirements, while simultaneously limiting off-target drug distribution and thereby reducing systemic adverse effects [1].

Recent advances in nanotechnology have attracted significant interest in utilizing nanoparticles (herein defined as particles < 1000 nm for purposes of this review) as carriers for pulmonary drug delivery. Compared to conventional inhalable formulations where the drug is present either in solution or suspension form (e.g., as formulations for use in nebulizers) or as micronized dry particles in conventional inhalable dry powder formulations, inhalable nanoparticle-based drug delivery systems possess multiple unique merits (Fig. 1) [2]. Firstly, nanoparticles can be decorated with ligands (e.g., antibodies, peptides, etc.) on the nanoparticle surface to target specific cell types within the lungs, e.g., lung tumor cells [3]. Secondly, while microparticles or sub-micron particles in the size range of

Fig. 1 Therapeutic merits of inhalable nanoparticle-based formulations over conventional inhalable formulations
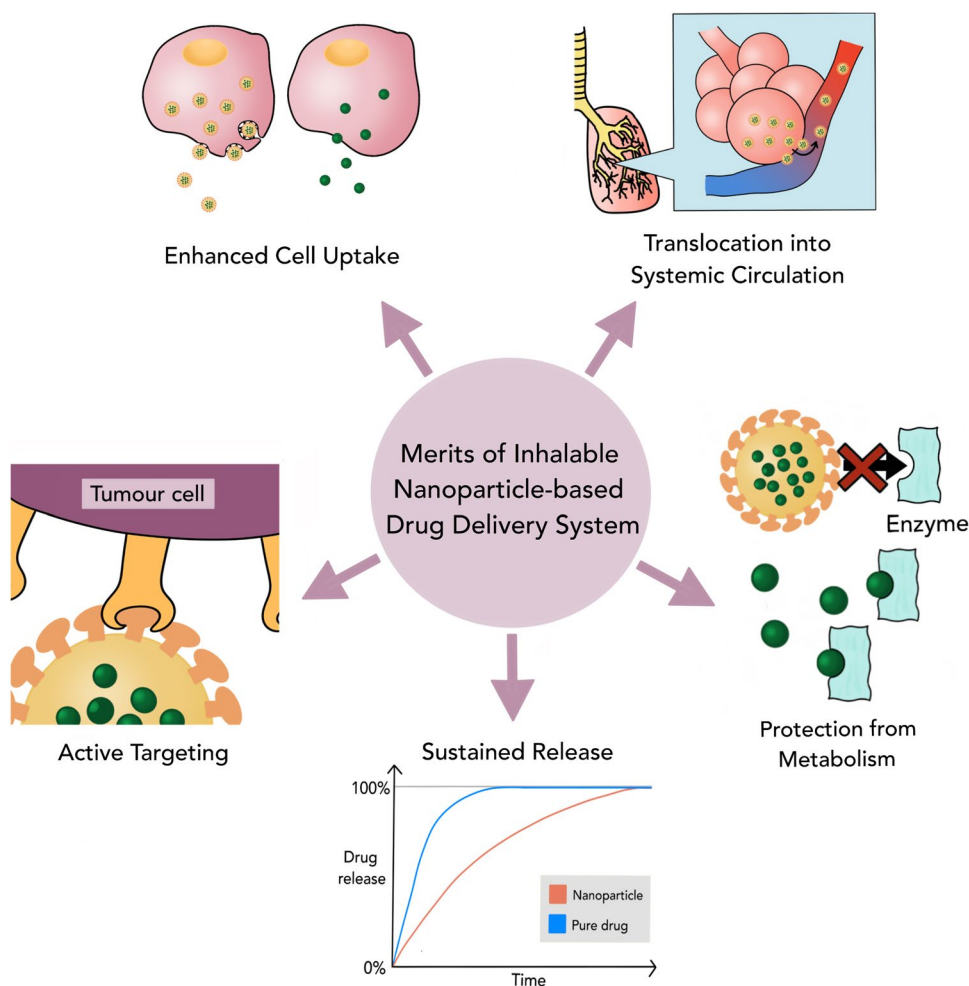
0.5 – 3 µm are readily phagocytosed by alveolar macrophages [4], nanoparticles below < 300 nm can escape macrophage uptake and protect the drug cargo from enzymatic degradation [5]. Thirdly, nanoparticles can improve drug uptake into cells compared to free drugs by various endocytosis-based pathways [6]. Fourthly, sustained release or even controlled release of drugs is possible via modulation of the nanocarrier properties [7]. Finally, and most notably, nanoparticles can translocate from the alveoli to the systemic circulation while microparticles cannot, thereby providing an alternative method for systemic delivery of nanoparticles without the need for invasive intravenous administration [8]. This feature is particularly useful for conditions with both lung and systemic manifestations (e.g., metastasized lung cancers, tuberculosis, etc.).

An important consideration for pulmonary delivery of nanoparticles is the choice of the aerosol-generating device. Various types of devices are currently commercially used for generating inhalable aerosols, including nebulizers, pressurized metered-dose inhalers (pMDIs), soft-mist inhalers, and dry powder inhalers (DPIs). However, the high surface energy of nanoparticles, particularly in suspension form, would promote their aggregation by Ostwald ripening and recrystallization [9].

DPIs are therefore more suitable for the delivery of nanoparticles by oral inhalation due to their greater physical stability. DPIs are also more portable compared to nebulizers and eliminate the need for hand-breath coordination with pMDIs, which makes them particularly attractive for long-term treatment of chronic pulmonary diseases [10]. This review thus focuses on inhalable nanoparticle-based dry powder formulations, i.e., powder formulations with nanoparticles either adsorbed onto the surface of a carrier powder, agglomerated into powder, or dispersed within the powder matrix (see Subsection "Formulation Strategies") which have suitable physicochemical properties for oral inhalation. Inhalable nanoparticle-based powders differ from conventional inhalable powder formulations (e.g., carrier-blend particles, large porous particles, etc. [11]) in that for conventional inhalable powder formulations drug particles are present in micronized form. Upon inhalation, the micronized drug in conventional inhalable powder formulations dissolve in lung lining fluid as drug molecules, while for inhalable nanoparticle-based powders the powder redisperses into primary nanoparticles upon contact with lung lining fluid, which is critical to the unique merits of inhalable nanoparticle-based formulations as discussed above.

Efficient pulmonary deposition of nanoparticles is a prerequisite for inhalable nanoparticle-based formulations to exert their therapeutic effect. However, direct oral inhalation of nanoparticles (including nano-sized powders) would result in ineffective delivery of nanoparticles to the lungs as particles with aerodynamic diameter $(D_A) < 1$ μm would likely escape impaction and sedimentation and remain suspended in the airways after inhalation before being exhaled [11]. As particles with D_A $1-5$ μm tend to deposit in the deep lungs by sedimentation while those with $D_A > 5$ μm would be trapped in the upper airways by inertial impaction, inhalable nanoparticle-based powders must be precisely engineered to fulfil the particle size requirement for deep lung deposition [11]. Once the powder particles deposit into the lungs, they should readily redisperse into primary nanoparticles upon contact with alveolar lung lining fluid. However, the nanoparticle suspension drying process exerts various stresses (e.g., shear and thermal stress) onto the primary nanoparticles which could damage their integrity, resulting in changes to their size distribution and/or morphology upon redispersion that could significantly affect their biological fate and therapeutic performance [2].

Over the past decades, significant research has been dedicated to overcoming the abovementioned challenges in developing inhalable nanoparticle-based dry powder formulations, i.e., the engineering of nanoparticle-based powders with (1) d_A between 1 and 5 μm and (2) satisfactory redispersibility back into primary nanoparticles upon contact with aqueous medium. Despite such efforts, the mainstream of inhalable nanoparticle-based formulations tested in clinical trials and subsequently approved (e.g., Arikayce®) were used in combination with nebulizers [12–15]. Only few inhalable nanoparticle-based dry powder formulations have entered early-stage clinical trials [16, 17] with none having successfully obtained regulatory approval to the best of our knowledge, indicating that there is a significant translational barrier for these formulations. The main objective of this review is to address the existing gap for inhalable nanoparticle-based dry powder formulations between bench and bedside. We begin with providing an update on formulation and particle engineering techniques to fabricate inhalable nanoparticle-loaded dry powders formulations with relevant literature examples. Considerations in *in vitro* characterization methodologies to improve the correlation between *in vitro* performance and biological fate are then discussed. Finally, the major knowledge gaps in their clinical translation and research directions are identified and addressed with our opinion.

Considerations in Development of Inhalable Nanoparticle-Based Dry Powders

The development of inhalable nanoparticle-based dry powders requires the judicious selection of (1) drying adjuvants in the formulation to protect nanoparticles from drying stresses and (2) particle engineering technique to form nanoparticle-loaded dried powder with suitable aerosol performance for deep lung delivery. Various approaches have been attempted in past research, and a summary of such studies is provided in Table I.

Nanoparticle design is the first important consideration in the development of inhalable nanoparticle-based powders. Various types of nanoparticles, e.g., polymeric nanoparticles [83], liposomes [84], solid lipid nanoparticles [7], mesoporous silica nanoparticles [85], etc., have been extensively researched as nanocarriers for pulmonary delivery, with polymeric and lipid nanoparticle systems being the most popular types of nanocarrier investigated in inhalable nanoparticle-based powders (Table I) due to their relative abundance of clinical safety data. Extensive reviews on the respective advantages, limitations, and design considerations of different types of nanoparticles are available elsewhere [7, 83–85]; in this review, the focus is mainly on the formulation and particle engineering strategies for fabricating inhalable nanoparticle-based powders.

Formulation Strategies

While various terminologies have been used to describe inhalable nanoparticle-loaded dry powders, they can generally be divided into three types: Nano-embedded microparticles, nanoagglomerate microparticles, and nanoparticle-carrier systems. A schematic diagram of these designs and their dispersion mechanisms within the respiratory tract is depicted in Fig. 2, and their pharmaceutical properties are summarized in Table II.

Nano-Embedded Microparticles

Nano-embedded microparticles (also known as nano-in-microparticles) consist of drug-loaded nanoparticles embedded or dispersed within a micron-sized matrix, produced by drying of nanosuspensions with dissolved bulking or shell-forming agents. During drying, the bulking or shell-forming agent(s) form a matrix to protect nanoparticles from physical stresses. The morphology of the produced particles is generally spherical in shape with observable dispersion of nanoparticles within the matrix (Fig. 3a). Upon deposition into the lungs, the matrix structure degrades within the lung lining fluid to release nanoparticles (Fig. 2a). Due to the high proportion of excipients required by mass to form the matrix, the overall drug loading is generally low. This strategy therefore is more suitable for the delivery of nanoparticles encapsulating highly potent drugs.

Disaccharides (e.g., lactose, trehalose) and alcohol sugars (e.g., mannitol) are frequently used as bulking or

Table 1 Summary of Studies on the Development of Inhalable Nanoparticle-based Dry Powders

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Small molecules										
Spray drying										
Simvastatin	Pulmonary arterial hypertension	Nanocrystal (Lecithin)	Mannitol	N.A	105.5	N.A	1.33±0.18	19.75±0.45	Aerolizer®/NGI/60	[18]
5(6)-carboxyfluorescein	N.A	Liposome (Cholesterol)	Trehalose, leucine	1:36.3–72.6	143±1	94±7	1.75±0.04	65±3	N.A./ACI/60	[19]
Methotrexate	Lung cancer	Polymeric nanoparticle (Gelatin)	Leucine	1:1.5	180.31±10.10	160±9.78	2.59±0.31	49.53±2.10	Cyclohaler®/NGI/60	[20]
Itraconazole	Pulmonary aspergillosis	Polymeric nanoparticle (TPGS, cholesterol)	Methylcellulose	2.2:10	88.4±1.8	1.02±0.04*	2.16±0.02	65.35±1.68	Osmohaler®/NGI/90	[2]
Cyclosporine A	Immuno-suppressant	Polymeric nanoparticle (Lecithin, lactose)	Mannitol	1:0–1:1	225.7±4.9–294.7±1.2	N.A	N.A	≈10	Rotahaler®/NGI/60	[21]
								≈60	Aerolizer®/NGI/100	
Gefitinib	Lung cancer	Solid lipid nanoparticle (Lecithin, cholesterol, glucosamine-PEG-stearic acid, Pluronic F127®)	Mannitol	1:0.025	187.23±14.08	N.A	4.48±0.02	44.41±0.28	Aerolizer®/ACI/60	[22]
			Lactose			N.A	5.70±0.07	26.45±0.61		
Ethambutol	Tuberculosis	Solid lipid nanoparticle (Compritol®)	N.A	N.A	57.65±0.23	N.A	5.629±0.15	23.98±0.38	N.A./NGI/60	[23]
			Mannitol	1:0.04			4.148±0.02	30.91±0.77		
Levofloxacin	Tuberculosis	Targosphere liposome	Lactose, leucine	N.A	≈100	<1*	N.A	≈60	Handihaler®/NGI/60	[24]
Benzothiazinone 043		Mesoporous silica nanoparticle			≈470	>1*	N.A	≈60		
Meloxicam	Cystic fibrosis/COPD/NSCLC	Polymeric nanoparticle (PVA)	Leucine	4.9:0–4	138±5	N.A	1.55±0.06–2.33±0.08	72.81±1.46–75.67±3.46	Breezhaler®/ACI/28.3	[25]

Table 1 (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Ciprofloxacin	Pulmonary infections	Polymeric micelle (Chitosan, linoleic acid)	Mannitol, phenylalanine	52.95:100:20	N.A	291.1	N.A	60.3	Cyclohaler®/TSI/60	[26]
Chloramphenicol palmitate	MDR pulmonary bacterial infections	Polymeric nanoparticle (PLGA)	Lactose, leucine	1:0.6–0.85	160±4	N.A	3.3±0.5	27±13	Handihaler®/NGI/41	[27]
		Pure drug nanoparticle			159±6		3.1±0.2	33±10		
Thiamphenicol palmitate		Polymeric nanoparticle (PLGA)			178±12		2.8±0.3	36±10		
		Pure drug nanoparticle			177±6		2.9±0.2	47±9		
Ivacaftor	Cystic fibrosis	Polymeric nanoparticle (PHEA-RhB-PLA-PEG/PHEA-PLA-Tat)	Mannitol	1:15	78.6±3.8	N.A	5.74±0.07	24.1±3.19	Turbospin®/NGI/60	[28]
			Mannitol, cysteamine	1:11.25:3.75	80.7±4.4		12.0±4.13	9.44±2.52		
Docetaxel	NSCLC	Polymeric nanoparticle (Cholesterol-PEG)	Leucine	1:0.1	182.3±3.2	189.4±2.7	4.20±0.12	54.39±2.30	Cyclohaler®/NGI/b	[29]
Salbutamol	Asthma	Liposome (Soya phosphatidyl choline (SPC), cholesterol)	Maltodextrin	1:36.3–72.6	167.2±0.170	N.A	3.72±0.17	44.68±0.57	Rotahaler®/ACI/60	[30]
			Lactose				3.49±0.12	64.01±0.43		
Doxorubicin	Lung adenocarcinoma	Dendrimer (Polyamidoamine)	Mannitol	1:10	9.9±0.5	10.0±0.6	1.80±0.11	62.75±3.79	Rotahaler®/ACI/28.3	[31]
Vancomycin-adsorbed MNP	Bacterial community-acquired pneumonia	MNP (Coated with lauric acid)	Lactose, dextran	1:4:1	19.2±5.8	N.A	≈2.9	≈60	Unspecified Plastiape DPI/NGI/60	[32]
Ketotifen	Asthma	Core–shell complex (Dextran sulfate, Poloxamer 407)	Leucine	1:2	70±0.3	103.7±7.92	1.23±0.32	67.4	N.A./TSI/60	[33]

Table 1 (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_r	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Doxorubicin, methotrexate	Lung cancer	PEGylated magnetic particle (MNP (Fe$_3$O$_4$), silica, PEG)	Mannitol	1:4	<50	N.A	3.96±0.23	21.94±3.38	Aerolizer®/NGI/60	[34]
Rapamycin	Airway inflammation	Polymeric nanoparticle (PHEA-g-RhB-g-SUCC-PCL-g-PEG)	Mannitol	1:13	51.1	162.1	N.A	10.6±2.8	Turbospin®/NGI/60	[35]
							7.09±1.25	42.5±4.98	RS01/NGI/90	
Paclitaxel	Lung cancer	Polymeric micelle (TPGS)	Lactose	1:62	255.2±21.2	310	3.8±0.98	60.1±10.2	Spinhaler®/ACI/60	[36]
Iron oxide MNP	N.A	N.A	Mannitol	1:4	145.7±2.9	159.0±3.8	4.5±0.2	34.3±3.8	Handihaler®/NGI/60	[37]
				1:19		144.2±4.5	4.5±1.0	31.2±2.0		
SPIONs (Free DOX encapsulated in microparticles)	Lung cancer	N.A	Lactose	20:2.8:77.2	56±6	N.A	3.27	>90	DP4 dry powder insufflator/NGI/30	[38]
Rhodamine	Pulmonary infections	Polymeric nanoparticle (PLGA, PVA, alginate)	Lactose	1:20	≈280	≈350	3.4±1.7	52±1	Turbospin®/MSLI/N.A	[39]
		Polymeric nanoparticle (PLGA, chitosan, alginate)		N.A	≈300	≈340	5.7±1.8	38±1		
Alendronate	Osteoporosis	Polymeric nanoparticle (Poloxomer F68)	N.A	N.A	44.11	N.A	3.45	43.85±0.52	Rotahaler®/ACI/60	[40]
Sodium cromoglicate	Allergy/inflammation	Pure drug nanoparticle	N.A	N.A	≈100	N.A	4.46±0.14	62.02±2.12	RS01/MSLI/60	[41]
Rifampicin	Tuberculosis	Polymeric nanoparticle (PLGA)	Leucine	2:3	195 4	N.A	N.A	35.3±2.1	Unspecified Plastiape DPI/ACI/28.3	[42]
				4:1				44.7±2.3		
N.A	N.A	Polymeric nanoparticle (Ethyl cellulose)	Maltodextrin, PVP	1:5:5	111.4±10.2	2.9*	3.1±0.1	≈35–40	Handihaler®/NGI/45	[43]

Table I (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (µm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
		Polymeric nanoparticle (EDRL)			78.3±19.2	≈1*	3.4±0.5			
		Polymeric nanoparticle (PLGA)			81.7±11.5	5.2*	3.3±0.1			
Levofloxacin	Tuberculosis	Polymer-lipid hybrid nanoparticle (PLGA, lecithin)	PVA, leucine	5:1.5:3.5	420±30	≈1*	7.0	12	PET/NGI/85	[44]
				5:1:4		1.1*	6.6	23		
Ciprofloxacin	Bronchiectasis	Nanoparticle complex (Dextran sulfate)	Mannitol, leucine	10:10:1	290±25	1.0±0.2*	≈6.5	≈23	PET/NGI/85	[45]
Spray freeze drying										
Cyclosporine A	Immunosuppressant	Polymeric nanoparticle (TPGS)	Mannitol	1:2.5–20	≈160	≈180	2.70–3.40	42–66	Osmohaler®/NGI/100	[46]
Ivacaftor (IVA) and colistin (COL)	Cystic fibrosis	Polymeric nanoparticle (DSPE-PEG)	N.A	N.A	199.3±4.3	N.A	N.A	61.4±3.4 (IVA) 63.3±3.3 (COL)	RS01/NGI/100	[47]
Levofloxacin	Lung biofilm infection	Polymeric nanoparticle (PCL)	PVA	3:2	290±40	≈1.3*	≈3.5	N/A	PET/NGI/85	[48]
Curcumin	Cystic fibrosis	Nanoparticle complex (Chitosan)	Mannitol, leucine	1:1:0	290±50	5.2±1.0*	1.37±0.18	27±6	PET/NGI/85	[49]
				1:1:0.1		3.8±0.2*	≈1.0	≈40		
				1:1:0.25		10.1±0.9*	1.07±0.18	49±1		
				1:1:0.5		18.5±0.9*	1.16±0.06	40±2		
Cefixime	Respiratory tract infections	Polymeric nanoparticle (PVP K-30)	Mannitol, leucine	1:0.5–1 (sugar) + 10–20% leucine#	244	N.A	N.A	18.96±0.76–40.17±1.25	Cyclohaler®/TSI/60	[50]
			Trehalose, leucine					47.00±2.22–79.28±0.45		
			Raffinose, leucine					54.37±2.22–64.73±1.78		
N.A	N.A	Polymeric nanoparticle (ethyl cellulose)	Maltodextrin, PVP	1:5:5	111.4±10.2	≈1*	2.4±0.1	≈43–46	Handihaler®/NGI/45	[43]

Table 1 (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_r	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
		Polymeric nanoparticle (EDRL)			78.3±19.2	≈1*	3.2±0.2			
		Polymeric nanoparticle (PLGA)			81.7±11.5	≈1*	3.4±0.3			
		Lipid nanocapsule (Kolliphor® HS15)	Trehalose, PVP		441.8±7.5	<1.5*	14.2±2.6	2		
		Solid lipid nanoparticle (Witepsol®)			36.2±2.6	≈1*	7.1±2.3	13		
Levofloxacin	Inflammation	Polymer-lipid hybrid nanocomplex (PLGA, lecithin)	PVA, leucine	5:1.5:3.5 5:1:4	420±30	≈1*	5.6	26	PET/NGI/85	[44]
Ciprofloxacin	Bronchiectasis	Nanoparticle complex (Dextran sulfate)	Mannitol, leucine	4:4:1	290±25	2.2±0.4*	≈2.8	≈29		[45]
Freeze drying Isoniazid (INH), pyrazinamide (PZA)	Tuberculosis	Polymeric nanoparticle (Chitosan)	Mannitol	10%#	249.72±5.00 – 576.44±36.13	N.A	3.37±0.05–3.44±0.16 (INH) 3.28±0.07–3.53±0.08 (PZA)	35.75±0.10–43.95±1.34 (INH) 30.81±0.06–41.03±0.92 (PZA)	In-house glass inhaler/ACI/60	[51]
Thymoquinone	COVID-19	Polymer-amino acid nanoparticle [L-arginine-poly(ester amide)]	N.A	N.A	187.41±18.32	N.A	1.679±0.087–1.911±0.065	22.7–23.7	Aerolizer®/NGI/60	[52]
Afatinib (AFT) (Free paclitaxel (PTX) encapsulated in microparticles)	NSCLC	Solid lipid nanoparticle (Stearic acid, poloxamer 188)	PLGA, PVP K-12, PVA	8:45:13.5::30	358.3±3.38	≈500	3.26±0.11 (AFT), 3.25±0.20 (PTX)	23.04±0.47 (AFT) 24.07±2.27 (PTX)	Turbospin®/NGI/90	[53]

Table I (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Isoniazid	Tuberculosis	Polymeric nanoparticle (Mannosylated chitosan, hyaluronic acid)	Trehalose	4%#	N.A	303±16.2	2.7	35	Breezhaler®/NGI/90	[54]
N-acetylcysteine	Tuberculosis	Polymeric nanoparticle (PLGA) / Polymeric nanoparticle (PLGA+Pluronic® F127)	N.A	N.A	307.50± 9.54 / 382.63± 6.42	N.A	2.35±0.17 / 2.57±0.12	55.33±3.51 / 62.67±2.08	Osmohaler®/NGI/30	[55]
Ciprofloxacin	Lower respiratory tract infections	Polymeric nanoparticle (PEtOx)	N.A	N.A	159.4± 4.5– 199.0± 1.8	200.0± 22.0– 353.0±55.1	N.A	35.0±0.7– 39.1±0.4	Breezhaler®/TSI/60	[56]
Freeze drying followed by lactose blending (nanoparticle-carrier systems)										
Bedaquiline	Tuberculosis	Polymeric nanoparticle (Chitosan)	Lactose	10%# 9.75:0.25+	109.7±9.3	N.A	3.38±0.02	28.27±0.9	Rotahaler®/ACI/28.3	[57]
Rifampicin	Tuberculosis	Polymeric nanoparticle (Chitosan)	Lactose	10%# 95.5+	124.1± 0.2	N.A	3.3±0.18	33.27±0.87	N.A/ACI/N.A	[58]
Prothionamide	Tuberculosis	Polymeric nanoparticle (Chitosan)	Mannitol, lactose	2%# 1:1+	301.9	N.A	1.76±1.96	81.19	In-house DPI/ACI/28.3	[59]
Linezolid	Tuberculosis	Polymeric nanoparticle (PLGA)	Mannitol, lactose	1:3+	45.2	N.A	3.78	N.A	N.A/ACI/28.3	[60]
Biologics *Spray drying*										
Mimetic peptide	Cardiomyopathy	Inorganic nanoparticle (Calcium phosphate)	Mannitol	1:0.07–1	80±15	85.4±6.0– 1992.0±21.0	N.A	31–74.3	RS01/FSI/60	[61]
miR-146a	COPD	Polymeric nanoparticle (PGA-co-PDL)	Mannitol, leucine	1:1.5:0 – 1:0:1.5	244.80±4.40	409.7±10.05	5.28±0.721	51.33±2.90	Cyclohaler®/NGI/60	[62]

Table I (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
TNF-α siRNA	Inflammatory lung disease	Lipid-polymer hybrid nanoparticle (L5N12 lipidoid, PLGA)	Trehalose, dextran, leucine	100:6:54:40	205.1±9.2	256.1±10.07	2.57±0.13	65.67±2.06	Aerolizer®/NGI/100	[63]
BSA (adsorbed)	N.A	Polymeric nanoparticle (PGA-co-PDL)	Leucine	1:1.5	299.03±32.02	282.46±2.17	1.21±0.67	76.95±5.61	Cyclohaler®/NGI/60	[64]
		Polymeric nanoparticle (PGA-co-PDL, DMAB)			348.36±14.02	356.73±33.83	2.80±0.21	70.67±4.07		[65]
BSA (encapsulated)		Polymeric nanoparticle (PGA-co-PDL)			203±5.4	N.A	1.71±0.1	78.57±0.1		[66]
Lysozyme	N.A	Pure drug nanoparticle	N.A	N.A	77.4±14.8 – 545.7±319.8	31.0±8.1–53.8±9.8	N.A	<20 ; 60–80	Rotahaler®/NGI/60 ; Aerolizer®/NGI/100	[67]
Pneumococcal surface protein A	Pneumonia	Polymeric nanoparticle (PGA-co-PDL)	Leucine	1:1.5	322.83±4.25	N.A	1.70±0.03	74.31±1.32	Cyclohaler®/NGI/60	[68]
GFP siRNA	N.A	TPP dendriplex (G4NH$_2$-TPP)	Mannitol	1:30000 (siRNA: mannitol)	363±55	340±150	4.8±0.3	38.5±3.1	Rotahaler®/ACI/28.3	[69]
GFP/GATA3 siRNA	Asthma	Polyplex [(mPEG-b-PCL)-g-PEI]	Mannitol ; Trehalose	5–10%#	≈80–120 ; ≈50–80	≈100–170 ; ≈90–110	4.77±0.15–5.50±0.29 ; 4.65±0.14–5.19±0.47	22.5±2.4–32.3±5.3 ; 14.0±2.4–18.3±5.0	Handihaler®/NGI/30	[70]
Bulk DNA	N.A	Polyplex (PEI)	Mannitol ; Trehalose	10%#	≈200 ; ≈170	≈180 ; ≈210	4.67±0.13 ; 3.17±0.21	67.5±1.3 ; 72.6±3.4	Handihaler®/ACI/28.3	[71]
siRNA	N.A	Lipid nanoparticle (Ionizable cationic lipid, helper lipid, cholesterol, PEG-DMG)	Lactose	5%#	≈80–130	≈130–280	2.85±0.35–2.90±0.42	28.1±3.80–29.5±0.60	Handihaler®/NGI/N.A	[72]

Table I (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Papain	N.A	Solid lipid nanoparticle (Glyceryl dibehenate)	Mannitol	1:3	174±7	163±6	N.A	≈33	Rotahaler®/TSI/60	[73]
			Trehalose			182±8		≈40		
		Solid lipid nanoparticle (Glyceryl tristearate)	Mannitol		508±16	541±4		≈32		
			Trehalose			478±7		≈50		
Lysozyme	N.A	Nanocomplex (mPEG-lin-GA/mPEG-mik-GA)	Mannitol	1:9	109±71 – 183±111	82±54-111±111	N.A	≈20–40	Unnamed Vectura DPI/FSI/60	[74]
			Mannitol, leucine	1:8:1		74±43–112±76		≈5–15		
			Trehalose	1:9		86±60- 132±96		≈10–25		
			Trehalose, leucine	1:8:1		61±59- 218±184		≈55–65		
Ovalbumin	N.A	Polymeric nanoparticle (PLGA, PVA)	N.A	N.A	264±5	278±9	3.1	49	Unihaler/NGI/59	[75]
							3.3	51	Cyclohaler®/NGI/100	
			Leucine	0.01%#		N.A	3.2	42	Unihaler/NGI/59	
							3.5	45	Cyclohaler®/NGI/100	
mRNA	N.A	Nanocomplex (PEG$_{12}$KL4)	Mannitol	1.1:98.9	467.93±24.93	N.A	5.54±0.81	≈36	Breezhaler®/NGI/90	[76]
				5.5:94.5		432.03±13.62	4.45±0.36	≈42		
TNF-α siRNA	N.A	Solid lipid nanoparticle (Lecithin, cholesterol)	Mannitol	1:30	136.3±22.8	178.1±33.5	5.97±1.73	22.42±12.88	RS01/NGI/60	[77]
Spray freeze drying										
mRNA	N.A	Nanocomplex (PEG$_{12}$KL4)	Mannitol	1.1:98.9	467.93±					
24.93	N.A	2.13±0.08	≈64	Breezhaler®/NGI/90	[76]					

Table 1 (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Plasmid DNA	N.A	Polymeric nanoparticle (Chitosan, TPP)	Mannitol	5.5:94.5		375.03±9.90	1.53±0.15	≈70	PET/NGI/60	[78]
				1:9	121±43	N.A	1.7±0.5	22.2±2.2		
Luciferase siRNA	N.A	Polymer nanoplex (PEI)	Mannitol, leucine	47.2:893:50	N.A	N.A	1.17±0.12	64.1±11.3	Jethaler®/ACI/28.3	[79]
				141.5:789:50	186±9	189±10	N.A	N.A		
siRNA	N.A	Liposome-protamine-DNA complex (DOTAP, DOPE, protamine, hyaluronic acid)	Mannitol, leucine	N.A	128.3±2.3	2.12*	N.A	34.7±3.0	Jethaler®/ACI/28.3	[80]
		Liposome-protamine-DNA complex (DOTAP, DOPE, Protamine)			191.9±8.7	1.08*		39.2±5.4		
Thin-film freeze drying										
CRISPR-Cas9 plasmid (PX458)	Cystic fibrosis	Polymer nanoplex (PEGylated chitosan)	Mannitol	1:10	184.1±6.6	≈350	4.8±0.3	60±2.2	RS01/NGI/60	[81]
			Mannitol, leucine	1:10:0.34		≈280	4.6±0.4	62.3±4.4		
			Sucrose	1:10		≈210	N.A	N.A		
			Sucrose, leucine	1:10:0.34		≈195		24±0.6		
			Trehalose	1 10		≈205		21.3±0.6		
			Trehalose, leucine	1:10:0.34		≈190		32.4±3.2		
TNF-α siRNA	N.A	Solid lipid nanoparticle (Lecithin, cholesterol)	Mannitol	1:30	136.3±22.8	165.3±48.3	3.96±0.97	37.01±4.52	RS01/NGI/60	[77]

Table 1 (continued)

Diagnostic/therapeutic agent(s)	Indication(s)	Type of nanoparticle (nanocarrier)	Excipients	NP:excipient ratio (w/w)	S_i	S_f	MMAD (μm)	FPF/respirable fraction (%)	Inhaler device/impactor apparatus/airflow rate (L/min)	Ref
Supercritical fluid drying siRNA										
(Free DOX encapsulated in microparticles)	Lung cancer	Polymeric nanoparticle (Chitosan)	PLLA	N.A	≈100	N.A	4.58	59.16	N.A./ACI/28.3	[82]

Abbreviations: *ACI* Anderson cascade impactor, *BSA* bovine serum albumin, *COPD* chronic obstructive pulmonary disease, *COVID-19* Coronavirus Disease 2019, *DMAB* didodecyldimethyl ammonium bromide, *DOPE* 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, *DOTAP* 1,2-dioleoyl-3-trimethylammonium-propane, *DPI* dry powder inhaler, *DSPE* 1,2-disterol-sn-glycerol-3-phosphorylethanolamine, *EDRL* Eudragit® RL, *FSI* fast screening impactor, *G4NH$_2$-TPP* generation four, amine-terminated, poly(amidoamine)-triphenylphosphonim, *GFP* green fluorescent protein, *MDR* multiple drug resistant, *MNP* magnetic nanoparticle, *(mPEG-b-PCL)-g-PEI* polyethylenimine-graft-(polycaprolactone-block-methoxy-polyethylene glycol), *mPEG-lin-GA* linear methoxy polyethylene glycol-poly(glutamic acid) copolymers, *mPEG-mik-GA* miktoarm methoxy polyethylene glycol-poly(glutamic acid) copolymers, *mRNA* messenger RNA, *MSLI* multi stage liquid impinger, *N.A.* not available/applicable, *NGI* Next Generation Impactor, *NP* nanoparticle, *NSCLC* non-small cell lung cancer, 407, *PCL* poly(caprolactone), *PEG* polyethylene glycol, *PEG-DMG* 1,2-dimyristoyl-sn-glycero-3-methoxypolyethylene glycol, *PEI* polyethyleneimine, *PET* powder entrainment tube, *PetOx* poly(2-ethyl-2-oxazoline), *PGA-co-PDL* poly(glycerol adipate-co-ω-pentadecalactone), *PHEA-g-RhB-g-SUCC-PCL-g-PEG* α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide-graft-rhodamine B-graft-poly-ε-caprolactone-succinate-graft-polyethylene glycol, *PHEA-PLA-Tat* α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide-D,L-poly(lactic acid)-Tat protein graft copolymer, *PHEA-RhB-PLA-PEG* α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide-rhodamine B-D,L-poly(lactic acid)-polyethylene glycol graft copolymer, *PLGA* poly(lactic-*co*-glycolic) acid, *PLLA* poly(L-lactic acid), *PVA* polyvinyl alcohol, *PVP* poly(vinylpyrrolidone), S_f size of redispersed nanoparticles after reconstitution of dry powder in aqueous medium, S_i initial nanoparticle size, *siRNA* small interfering RNA, *SPION* superparamagnetic iron oxide nanoparticles, *TPGS* D-α-tocopheryl polyethylene glycol 1000 succinate, *TPP* tripolyphosphate, *TSI* twin-stage impinger

*Redispersibility index (RdI)

#Drying adjuvant concentration in nanosuspension (w/v)

+Freeze-dried nanoparticle:lactose carrier blend ratio
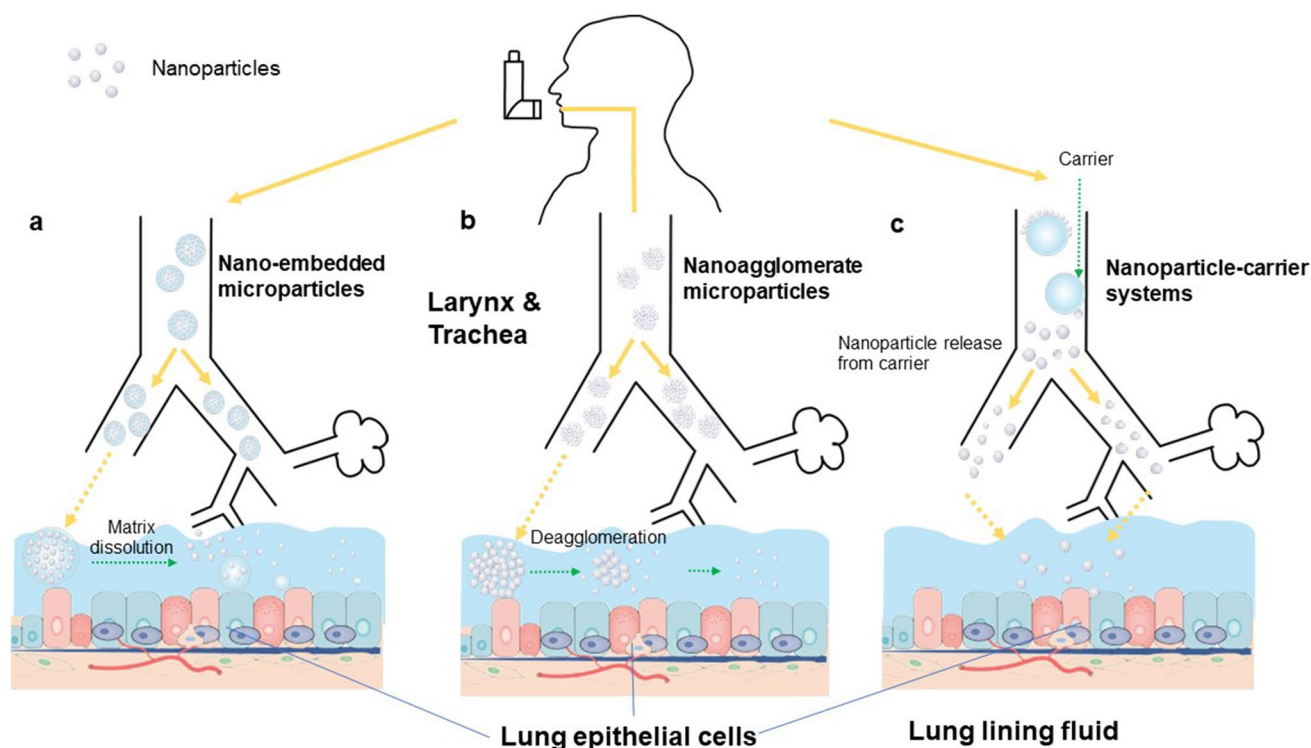
Fig. 2 Dispersion mechanism of nanoparticles loaded in **a** nano-embedded microparticles, **b** nanoagglomerate microparticles, and **c** nanoparticle-carrier systems in the respiratory tract

shell-forming agents, with lactose and mannitol being the most common choices as regulatory authorities have approved their use in oral inhalation products. It is worth noting that lactose is unsuitable for protein-carrying formulations as its reducing properties could trigger undesired Mailliard reaction [10]. Furthermore, the high hygroscopicity of lactose may induce significant moisture sorption and thus deteriorate aerosol performance. For inhalable nanoparticle-based powders, high hygroscopicity may also induce destabilization of nanoparticles (e.g., by Ostwald ripening) upon contact with absorbed moisture. This could be overcome with the incorporation of dispersion enhancers,

which are usually hydrophobic amino acids (e.g., leucine [87] and phenylalanine [26]). Mannitol is an alternative that can circumvent issues associated with lactose as it is non-reducing and relatively less hygroscopic. Mannitol also is more suitable for patients with diabetes mellitus as it is passively absorbed into the body [88], and its mucolytic properties make it a preferable carrier for conditions involving excessive mucus production (e.g., cystic fibrosis, for which it is an FDA-approved treatment to improve pulmonary function [89]). However, some patients may be hypersensitive to mannitol, and a mannitol tolerance test is recommended prior to treatment initiation [90]. Furthermore, its long-term

Table II Characteristics of Inhalable Nanoparticle-based Dry Powder Formulations

	Nano-embedded microparticles	Nanoagglomerate microparticles	Nanoparticle-carrier system
Morphology	Spherical	Hollow/porous	Spherical
Nanoparticle dispersion mechanism in respiratory tract	Dissolution of bulking/shell-forming agent	Dissolution of excipient bridges between agglomerated nanoparticles	Physical detachment of nanoparticles from carriers during inhalation
Production method	Spray drying/(spray) freeze drying of nanosuspension with bulking/shell-forming agent(s)	Spray drying/(spray) freeze drying of nanosuspension with protectant(s)	Blending/coating of dried nanoparticles with micron-sized carrier particles
Drug loading	Low	High	Very low
Flow rate dependence	Low	Low	High
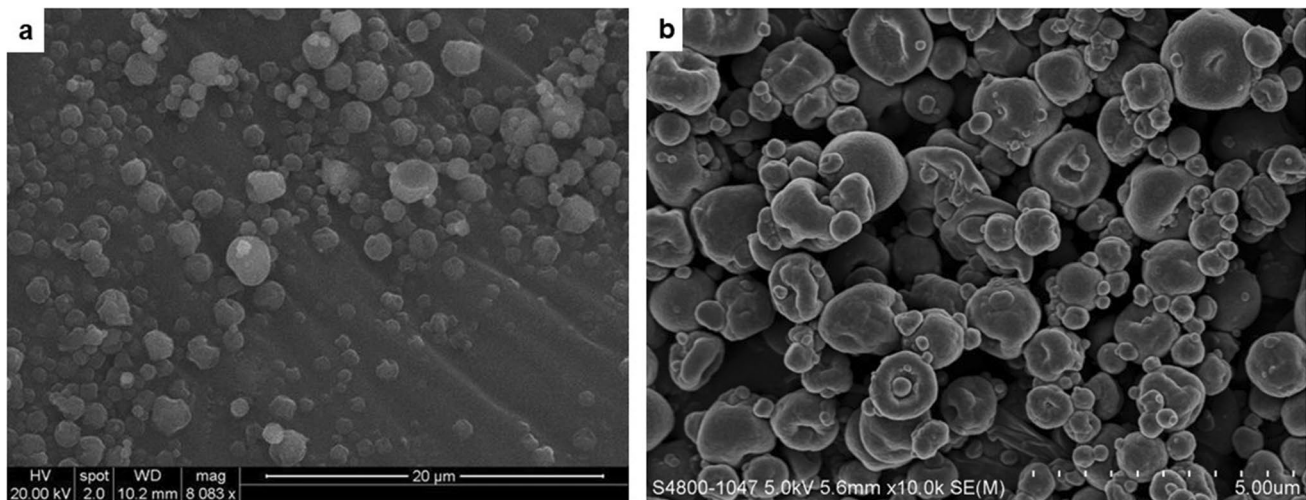
Fig. 3 SEM images of **a** itraconazole nano-embedded microparticles (reprinted with permission from [2]) and **b** remdesivir nanoagglomerate microparticles [86]

safety remains unestablished due to its only recent approval by the US FDA.

Nanoagglomerate Microparticles

Nanoagglomerate (also known as nanoaggregate, nano-matrix [67], or Trojan [91]) microparticles consist of nanoparticles agglomerated with each other in a controlled manner after drying. The morphology of inhalable nanoagglomerate powder formulations is normally either hollow or porous, facilitating its low particle effective density (ρ_{eff}) for superior aerosolization (Fig. 3b). Upon dispersion as aerosols from the DPI and deposition into the lungs, the nanoagglomerate microparticles would redisperse into primary nanoparticles to exert their therapeutic effects (Fig. 2b).

Nanoagglomerate microparticles are engineered by drying nanosuspensions with dissolved protectants and optional dispersion enhancers (e.g., leucine) in low quantities. The protectant(s) not only protect primary nanoparticles from structural damage due to drying stresses, but also form "bridges" between agglomerated nanoparticles to facilitate redispersion of primary nanoparticles upon contact with the lung lining fluid [48]. Similar to nano-embedded microparticles, lactose and mannitol are the most frequently employed protectants, yet both have their respective drawbacks. Mannitol undergoes recrystallization upon heating/freezing, which not only reduces its protective function, but also further aggravates mechanical stresses exerted on primary nanoparticles during drying. This results in deteriorated aqueous redispersibility of the resultant dry powders [2, 48]. Mannitol may also undergo polymorphic transformations by interacting with nanoparticle stabilizer [46], which may affect the aerosol performance of the resultant dried

powder [92]. While lactose remains amorphous throughout the drying process and thus offers stronger protection over mannitol, the resultant powder would likely suffer from poor flowability and aerosol performance as mentioned above. The use of polymers as protectants has emerged as a promising alternative to overcome such drawbacks. For instance, Cheow *et al.* reported that using polyvinyl alcohol (PVA) as protectant for spray-freeze-drying polycaprolactone (PCL) nanoparticles resulted in dry powder formulations with superior aqueous redispersibility and similar aerosolization characteristics compared to formulations with mannitol as protectant [48], while Wan *et al.* demonstrated that co-spray-drying itraconazole nanosuspensions with methylcellulose, a gel-forming polymer that undergoes *in situ* thermal gelation upon heating within a spray dryer to entrap and protect nanoparticles could yield inhalable dry powder with excellent aqueous redispersibility [2]. However, the use of polymers over lactose and mannitol as protectants remains less common (see Table I) as such polymers have not been used as excipients in approved oral inhalation products, and their safety requires further investigation (see Sect. 4.3).

Nanoparticle-Carrier Systems

A less commonly used method to deliver nanoparticles into the lungs as inhalable dry powders is physical adsorption of dried nanoparticles onto the surface of a coarse inert carrier (usually lactose) via either coating or blending. Unlike nano-embedded and nanoagglomerated microparticles where nanoparticle release into the lung lining fluid is based on dissolution of the adjuvant bridges/matrix, physical detachment of the nanoparticles from the micron-sized carrier, which requires a high flow rate that may not be achieved

by patients with impaired pulmonary function, is necessary (Fig. 2c) [93]. Furthermore, a large quantity of the carrier relative to nanoparticles is required (i.e., very low drug loading), and the large geometric particle size of the coarse inert carrier results in substantial deposition of powder within the throat by inertial impaction [94], which severely limits nanoparticle deposition in the deep lungs. Therefore, nanoparticle-carrier systems are less preferable compared to nano-embedded and nanoagglomerate microparticles.

Particle Engineering Techniques

The most common nanoparticle-based dry powder formulations (i.e., nano-embedded microparticles and nanoagglomerate microparticles) are engineered by drying of nanosuspension with dissolved drying adjuvants by various drying techniques, with spray drying, spray freeze drying, and freeze drying being the most common techniques employed. This section only serves to provide a brief overview on these techniques and relevant considerations for inhalable nanoparticle-based powders; extensive reviews on the particle engineering of inhalable dry powders are available in the literature [88, 95].

Spray Drying (SD)

Spray drying is the most commonly used method to produce inhalable nanoparticle-based powders (see Table I), in which the feed liquid containing nanosuspension and dissolved drying adjuvants is atomized into droplets and dried with heated gas to result in nanoparticle-based dried powder. The morphology of spray-dried powder normally is hollow and wrinkled or dimpled, which facilitates its low density and therefore appropriate d_A for inhalation [96]. Nano-sized powders could also be directly produced using a nano spray dryer for subsequent blending with carriers [97].

The main advantage of spray drying is its facilitation of precise control of particle size and therefore *in vitro* aerosol performance of inhalable dry powders by manipulating spray drying processing parameters [2]. However, it must be emphasized that the correlation between spray drying parameters and *in vitro* aerosol performance for inhalable nanoparticle-based powders is also formulation dependent, and thus the results could deviate from expected trends. For example, while an increase in feed pump rate would be expected to result in larger droplet (and therefore particle) size, Wan *et al.* reported absence of a clear trend between the feed pump rate and the geometric median diameter/*in vitro* aerosol performance metrics of spray dried itraconazole nanoagglomerate microparticles [2]. As both processing and formulation parameters could significantly affect the aqueous redispersibility of inhalable nanoparticle-based powder formulations [2], rational optimization of these parameters,

for example via design of experiments (DoE), is warranted to produce inhalable nanoparticle-based powders with both good redispersibility and aerosol performance [98]. Another unique merit of spray drying is its shorter processing time, which can minimize the risk of causing colloidal instability issues of nanoparticles during drying. Spray drying is also a scalable and continuous process that is highly suitable for pre-clinical and clinical development of relatively costly nanoparticle-based DPI products.

One key disadvantage of spray drying is that it is less preferred for heat-sensitive materials. These materials not only include heat-labile drugs and biologics but also low melting point polymers, e.g., polycaprolactone and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) [2, 99] which are commonly used as nanocarriers and stabilizers. A novel solution to overcome this issue is the use of supercritical fluid-assisted spray drying (SASD), where carbon dioxide (CO_2) in supercritical fluid state is mixed and solubilized within the feed liquid prior to spray drying upon which the CO_2 vaporizes. The spray drying process operates at a lower drying temperature (typically $< 60°C$ [95]), thereby minimizing the thermal stress on the nanoparticles and energy input. The SASD technique has been successfully applied to the drying of 5(6)-carboxyfluorescein-encapsulating liposomes into inhalable nano-embedded microparticles with good aqueous redispersibility [19]. Another disadvantage of spray drying is its relatively low yield as 30–50% (*w/w*) of dry powders would be retained in the cyclone or other components of the spray dryer, with collection efficiency particularly poor for particles < 2 μm [100]. To overcome this limitation, nano spray drying has been developed where droplets are produced using a vibrational mesh and fine particles are collected electrostatically [97]. The nano spray drying technique not only improves sample recovery and yield (especially particles < 2 μm) but also is capable to produce powders with superior aerosol performance relative to conventional spray drying [97].

Spray Freeze Drying (SFD)

Unlike spray drying which involves heat input, spray freeze drying involves the freezing of droplets atomized from an atomizer or a spray nozzle using a cryogen and lyophilization of the frozen droplets in a freeze dryer. Similar to spray drying, particle size control in spray freeze drying could be achieved by controlling the processing and formulation parameters. However, there are limited reports on how various processing parameters (e.g., primary drying temperature) as well as their interactions could influence the aqueous redispersibility of spray-freeze-dried inhalable nanoparticle-based dry powders. More studies are warranted to guide future development of these formulations.

Head-to-head studies comparing spray drying *versus* spray freeze drying revealed superior *in vitro* aerosol

performances of inhalable nanoparticle-based powders produced by the latter technique [43–45, 48], possibly due to the highly porous nature of the particles produced upon interstitial sublimation of water from the frozen droplets. However, whether spray freeze drying is superior to spray drying with regard to aqueous redispersibility of these formulations remains controversial, with multiple head-to-head studies reporting that spray freeze drying is superior [43, 44, 48] while Yu *et al.* reported an opposite trend, attributing such observation to formation of ice crystals during freezing [45]. Obviously, spray freeze drying is more suitable for heat sensitive nanoparticles, and the production yield is significantly higher compared to spray drying. However, spray freeze drying suffers from low throughput due to the long lyophilization process, and significant challenges remain in the translation of the spray freeze drying technique into production scale. This is likely why despite the abovementioned merits, spray freeze drying remains less evaluated for production of inhalable nanoparticle-based powders compared to spray drying (Table I).

Freeze Drying (FD)

Freeze drying involves the freezing of nanosuspensions with cryoprotectants followed by sublimation of the solvent under low pressure and temperature (primary drying), and then heating to remove the remaining solvent content (secondary drying). As freeze drying does not involve spraying of the feed liquid into droplets, particle size control of dry powders is more difficult compared to spray drying and spray freeze drying techniques. Indeed, freeze drying of nanosuspensions often results in powder with inferior *in vitro* aerosol performance due to the heterogeneous particle size distribution. Furthermore, irreversible aggregation may occur during the freezing of nanosuspensions in bulk, resulting in dry powder with poor aqueous redispersibility [101]. Combined with the inherit disadvantage of long processing times, freeze drying is less common in the particle engineering of inhalable nanoparticle-based dry powders.

Characterization and Quality Attributes for Inhaled Dry Powder

As with other dosage forms, the quality of DPI formulations must be carefully evaluated for their therapeutic potential. Table III summarizes the critical quality attributes and related characterization techniques of DPI formulations.

While the critical quality attributes listed in Table III are universal to all inhalable dry powder formulations, attention should be paid to certain attributes for inhalable nanoparticle-based powders. Drug loading of the powder is a major

concern as a low drug loading would result in the patient being required to inhale a high quantity of powder per dose for therapeutic effect which may not be feasible in the clinical setting. As presented in Table I, certain inhalable nanoparticle-based powder formulations require a high portion of excipient (nanoparticle loading of $< 10\%$ (*w/w*) powder mass) for sufficient protection of nanoparticles. During drug development, the formulation should be optimized such that only a minimal amount of excipient is required to provide satisfactory nanoparticle protection during drying. Inhalable nanoparticle-based powders should also be non-hygroscopic to minimize moisture sorption. Finally, certain processes used in fabricating nanosuspensions (e.g., anti-solvent precipitation) require the use of organic solvent which pose a concern of toxicity upon inhalation. Residual solvent content thus must be checked with references to pharmacopoeial and other regulatory guidelines to avoid potential safety issues.

In addition to routine characterizations, extra studies should be carried out to demonstrate the clinical utility of inhalable nanoparticle-based drug delivery systems, as stated below:

Redispersibility and Stability of Redispersed Primary Nanoparticles

To fully take advantage of nanotechnology for pulmonary drug delivery, inhaled nanoparticle-based powders must readily redisperse into primary nanoparticles upon contact with lung lining fluid. The redispersed primary nanoparticles should retain a similar particle size distribution and morphology to that prior to drying. The redispersibility index (RdI) is used as a simple indicator to evaluate powder redispersibility in an aqueous medium [101]. It is defined as the ratio of particle size of primary nanoparticles after powder reconstitution in aqueous media (S_f) over the size of nanoparticles prior to drying (S_i) (i.e., S_f/S_i). A RdI of unity indicates perfect redispersibility without particle size change. An acceptable S_f/S_i is considered to be within the range of 0.7–1.3 [2]. A review of literature (Table I) indicates that there is a lack of report on RdI and S_f of inhaled nanoparticle-based dry powder formulations in the scientific community, and thus their clinical potential remains unknown.

There is a degree of variability in the scientific literature on the methodology in determining aqueous redispersibility of inhalable nanoparticle-based powders. Firstly, various methods to disaggregate the nanoparticles upon addition of powder to the aqueous medium were adopted, including sonication [104], hand agitation [46], etc. However, such high-intensity processes do not mimic the actual redispersion of powder in the human body as powders

Table III Critical Quality Attributes of Inhalable Dry Powder Formulations and Their Characterization Methods and Requirements

Quality attributes	Characterization techniques	Clinical impacts	Desired requirements
Geometric particle size distribution	Laser diffraction particle size analyzer	Particle deposition in airways	Optimization for MMAD in the range of $1 - 5$ μm, maximized FPF and minimized GSD [11]
			Small geometric particle size, low tapped density
			(< 0.4 g/cm^3 [102]) and large shape factor favors reduced particle D_A [11]
Particle morphology (shape factor)	Scanning electron microscopy (SEM)		
Particle tapped density	Volumeter; Graduated cylinder		
Flowability			Carr index < 15;
			Angle of repose $< 35°$
Electrostatic charge	Electrometer		Minimized (correlation with airflow rate should be established) [103]
Drug loading	HPLC; LCMS/MS	Dose and dosing frequency	Normally maximized for flexible dose control
Drug assay and impurities	HPLC; UV; LCMS/MS	Product safety and efficacy	Compliance with pharmacopeia requirement
Residual solvent content	Thermogravimetric analysis (TGA)	Product quality and safety	Minimized; Compliance with pharmacopeia requirements
Crystallinity	Powder X-ray diffraction (PXRD)	Product quality	Determined based on drug release and stability requirements
Hygroscopicity	Dynamic vapor sorption (DVS)		Minimized
Stability	Stability chamber		Fulfillment of ICH and local regulatory guidelines

come into contact with lung lining fluid without external mechanical agitation. It is recommended to adopt a spontaneous aqueous re-dispersion method, i.e., adding the powder to the reconstitution medium and let the suspension set until no visible particulates are observed prior to sizing. Secondly, there is significant variation in the reconstitution medium used. While reconstitution in pure water is most straightforward to assess redispersibility and has been adopted by many studies [2, 43, 49, 61], it may not truly represent the fate of nanoparticle-based powders upon redispersion of primary nanoparticles in lung lining fluid, which has a different pH from pure water and contains various types of soluble phospholipids and proteins [105]. These components may alter nanoparticle stability by complex bio-nano interactions, thereby hindering their intended therapeutic function. Therefore, it is highly recommended that redispersibility studies are performed in both deionized water and biologically relevant media such as stimulated lung fluid (SLF) [105] and phosphate-buffered saline (PBS) [106].

It must be emphasized that redispersibility studies could only detect changes in particle size, and other relevant physicochemical changes could also occur, e.g., drug degradation or changes in particle morphology during the stressful drying processes and subsequent storage. However, only few studies have evaluated these attributes. Suitable techniques should be used to confirm the colloidal and chemical stability of redispersed primary nanoparticles, including but not limited to transmission electron microscopy (TEM) for observation of redispersed nanoparticle morphology and nanoparticle separation techniques (e.g., centrifugal ultrafiltration) to determine drug loading and encapsulation efficiency of redispersed primary nanoparticles [107]. Significant deviations of such properties from the nanosuspension before drying would require further optimization of the formulation (e.g., an increase in protectant amount). The long-term storage stability of the inhalable nanoparticle-based powders should also be assessed according to ICH or relevant pharmacopoeial conditions, and verification of relevant parameters such as redispersibility, aerosol performance, drug loading and encapsulation efficiency, redispersed nanoparticle morphology, etc. As the protein corona is known to alter the colloidal stability of nanoparticles and may affect the results of *in vitro* cellular or *in vivo* studies, it is suggested to monitor the colloidal stability of redispersed primary nanoparticles in biological media (e.g., complete cell culture medium) to improve *in vitro-in vivo* correlation (IVIVC) [106]. Modification of nanocarrier surface (e.g.,

PEGylation or protein adsorption) could enhance colloidal stability in the biological environment [106].

In Vitro Drug Release

Unlike typical dissolution where drugs are present in molecular form and their concentrations can be directly determined by UV, HPLC, or LC/MS/MS, separation of nanoparticles from release medium should be performed in order to obtain an accurate *in vitro* drug release profile. Currently, there are no compendial methods for assessing either dissolution profile of inhaled dry powders or *in vitro* drug release of nanoparticles, resulting in substantial variations in the methodology among literature reports. This not only causes erratic correlations between *in vitro* release of drug nanoparticles and *in vivo* pharmacokinetics, but also impedes the systematic head-to-head comparison among the reported systems. To set a stage for IVIVC for inhalable nanoparticle-based powders, several factors should be taken into consideration for the experimental design. Firstly, a bulk sample of powder was directly dispersed into the release medium in most studies on inhalable nanoparticle-based powders. While this is the most convenient approach, only particles with $d_A < 5$ μm can practically reach the lungs. Therefore, it is recommended that the FPF of powders should first be separated from the bulk sample, e.g., using a fast-screening impactor (FSI) [1] or modifying the powder collection procedure of the pharmacopoeial apparatus used to assess *in vitro* aerosol performance [108], prior to dispersion into release medium. Secondly, as mentioned, the presence of drug in the release medium could either be in dissolved form or as redispersed nanoparticles, and separation of these two forms is needed to avoid overestimating *in vitro* drug release. The most common methods to determine *in vitro* nanoparticle drug release include continuous flow, dialysis membrane and sample & separate methods [107], and with continuous flow [30, 46] and dialysis membrane [20, 26] methods most commonly seen in studies on inhalable nanoparticle-based powders. However, each method has their own limitations. The continuous flow methods are costly with complicated experimental setups. Dialysis membrane methods may overestimate release kinetics of drug-loaded polymeric nanoparticle systems due to interactions between the dialysis membrane and nanoparticles [107]. The major challenge for sample & separate method is in the separation of nanoparticles from free drugs after sampling. While Weng *et al.* [107] have developed a new sample & separate method to determine drug release from polymeric nanoparticles with higher accuracy and precision, the appropriateness of this technique for inhalable nanoparticle-based powders requires further investigation. Finally, there is significant variation in the volume of the release medium across literature. Some studies have utilized a very high volume (e.g., 200 mL [21,

29]) of release medium which significantly deviates from the volume of human lung lining fluid; 10 – 30 mL is generally considered for better correlation with the clinical application of inhalable nanoparticle-based powders [109]. As with redispersibility studies, *in vitro* drug release studies should be performed in biorelevant media such as PBS or SLF.

In Vitro Aerosol Performance

Examination of the *in vitro* aerosol performance of nanoparticle-based dry powders is critical for predicting their lung deposition efficiency upon inhalation *in vivo*. The primary parameters required to be reported include the fine particle fraction (FPF) (normally defined as fraction of particles with $d_A \leq 5$ μm), the mass median aerodynamic diameter (MMAD), and the geometric standard deviation (GSD). Several types of pharmacopoeial apparatuses are available for this purpose, including multi-stage liquid impingers (MSLI), Andersen cascade impactor (ACI), Next Generation Impactor (NGI), etc. The NGI is often preferred over other apparatuses as it offers superior resolution of the aerodynamic particle size distribution with minimal inter-stage overlap across a wide range of airflow rates (30 – 100 L/min) [110].

Experimental considerations in *in vitro* aerosol performance testing of any dry powder formulations include the choice of inhaler device used and the range of airflow rate tested. For passive inhalers (the most common type of inhalers available on the market), effective powder dispersion from the device relies on the achievement of a sufficient airflow rate (Q) across the device, requiring patients to generate a sufficient inspiratory effort or pressure drop (ΔP, usually 1 – 6 kPa) against the intrinsic resistance (R) of the device (i.e., $\sqrt{\Delta P} = Q \times R$) [111]. Various commercial inhaler devices with different intrinsic resistances are available and classified into low-resistance (e.g., Breezhaler®, Aerolizer®), medium-resistance (e.g., Ellipta®, Accuhaler®), and high-resistance (e.g., Handihaler®) devices [112]. While low-resistance devices require a smaller inspiratory effort to achieve higher flow rates across the device, they simultaneously have a higher flow rate requirement to disperse the formulations compared to high-resistance devices [111]. It is observed that most studies on inhalable nanoparticle-based powders only tested the formulations using a single type of inhaler as proof-of-concept (Table I). However, during commercial development, it is recommended to evaluate the *in vitro* aerosol performance of the formulation using a range of inhaler devices to identify the device with best aerosolization efficiency of the formulation.

Furthermore, optimal flow rates for different devices are established based on their commercial formulations, which may not apply when used with other formulations, especially

since the morphology of dry powders affects their dependence on airflow rate for effective dispersion [113]. However, our literature review (Table I) finds that most studies on inhalable nanoparticle-based powders only performed *in vitro* aerosol performance testing using a fixed flow rate, which do not take account of the large inter-patient variability of inspiratory effort across patients of varying pulmonary function. During pre-clinical development of an inhalable nanoparticle-based DPI product, it is suggested that a wide range of inspiratory flow rates should be covered, with a careful consideration of the diseases and patient conditions. Most patients across various respiratory conditions (e.g., asthma [114], COPD [114], pulmonary arterial hypertension [115], and cystic fibrosis [116]) can achieve a peak inspiratory flow rate in the range of approximately 40 – 100 L/min using most commercially available passive inhaler devices; thus, selection of tested flow rates within this range (e.g., 45, 60, and 90 L/min) is advised. In later stages of pre-clinical development, the use of breathing simulators in impactor testing that mimics actual patient respiratory patterns may result in stronger IVIVC [117]. If significant variations in aerosolization efficiency (e.g., FPF) across varied flow rates or respiratory profiles occur, the formulation should further be optimized.

Challenges and Knowledge Gaps for Clinical Translation

The successful clinical translation of any pharmaceutical product requires the product to demonstrate satisfactory efficacy, safety, and quality. However, thorough evaluation of these attributes for nanomedicine is complicated as there is a lack of standardized regulatory guidance available with great inconsistency among guidelines issued by regulatory agencies across the globe [118]. For inhalable nanoparticle-based powders, unique regulatory issues arise due to their intended route of administration (i.e., oral inhalation). While regulatory challenges for nanomedicines have been summarized concisely in a recent review by Foulkes *et al.* [118], in this section important knowledge gaps and relevant future research directions to the clinical translation of inhalable nanoparticle-based dry powders are addressed (Fig. 4) to set the stage for future regulatory guidance and successful commercialization.

Manufacturing Scale-up and Process Optimization

A major challenge in the clinical translation of nanomedicines is their reproducible and scalable production, which is particularly complex for inhalable nanoparticle-based dry powders as such requirements are pertinent for both nanosuspension fabrication and drying processes. Conventional nanoparticle fabrication techniques are conducted in batch with significant batch-to-batch variability in nanoparticle properties, particularly particle size [119]. Further variability is often observed during scaling-up of nanoparticle production [120] as changes in production scale likely bring about variations in mass transfer rate and momentum of building blocks that dictate nanoparticle assembly [121]. It is critical to achieve a stringent particle size control of primary nanoparticles as it not only affects their biological fate (see Sect. 4.2) upon redispersion, but also impacts the aerosol performance of the dried powder [122]. Similarly, batch-to-batch variations in aerosol performance of the inhalable dry powder may occur during the drying process, e.g., variations in temperature throughout the drying chamber for (spray) freeze drying [123], and scaling up of (spray) freeze drying remains difficult due to the large equipment footprint and stringent conditions required. Product variability could be reduced by the adoption of the pharmaceutical Quality-by-Design (QbD) approach to enhance product and process understanding, with the ultimate aim of deriving a control strategy for the consistent production of nanomedicines [98]. Batch-to-batch variations and scalability issues may further be minimized by the development of a continuous manufacturing platform combining a continuous nanoparticle fabrication process (e.g., flash nanoprecipitation [FNP] [120] and microfluidic mixers [124]) with a continuous drying operation (e.g., spray drying) [125]. Scale-up (or scale-down) is easily achieved by adjusting the mass flow rate through the platform without the need for large-scale equipment, hence reducing manufacturing costs.

Bio-Fate and Its Correlation with Nanoparticle Physicochemical Properties

While *in vitro* aerosol performance and redispersibility are major CQAs in the development of inhalable nanoparticle-based dry powders, the physicochemical properties of primary nanoparticles, i.e., particle size, surface charge, and shape, are equally important and should be optimized for maximal therapeutic effect. However, unlike the former where defined requirements exist, there is significant ambiguity in what is considered "optimal" for various nanoparticle physicochemical properties. As shown in Table I, most studies on the inhalable nanoparticle-based powders had a primary nanoparticle size < 300 nm. This size range was generally accepted to be suitable for evading phagocytic clearance and enhancing lung retention of nanoparticles [5]. However, there is few, if any, available studies that systematically investigated particle size effect of inhaled nanoparticles within the 20–200 nm size range of pharmaceutical interest. It is worth mentioning that a smaller particle size within such range does not guarantee superior therapeutic performance. For example, Valsalakumari *et al.* demonstrated that 120 nm paclitaxel-loaded lipid nanocapsules enhanced cellular uptake into

Fig. 4 Challenges and knowledge gaps for clinical translation for inhalable nanoparticle-based dry powders
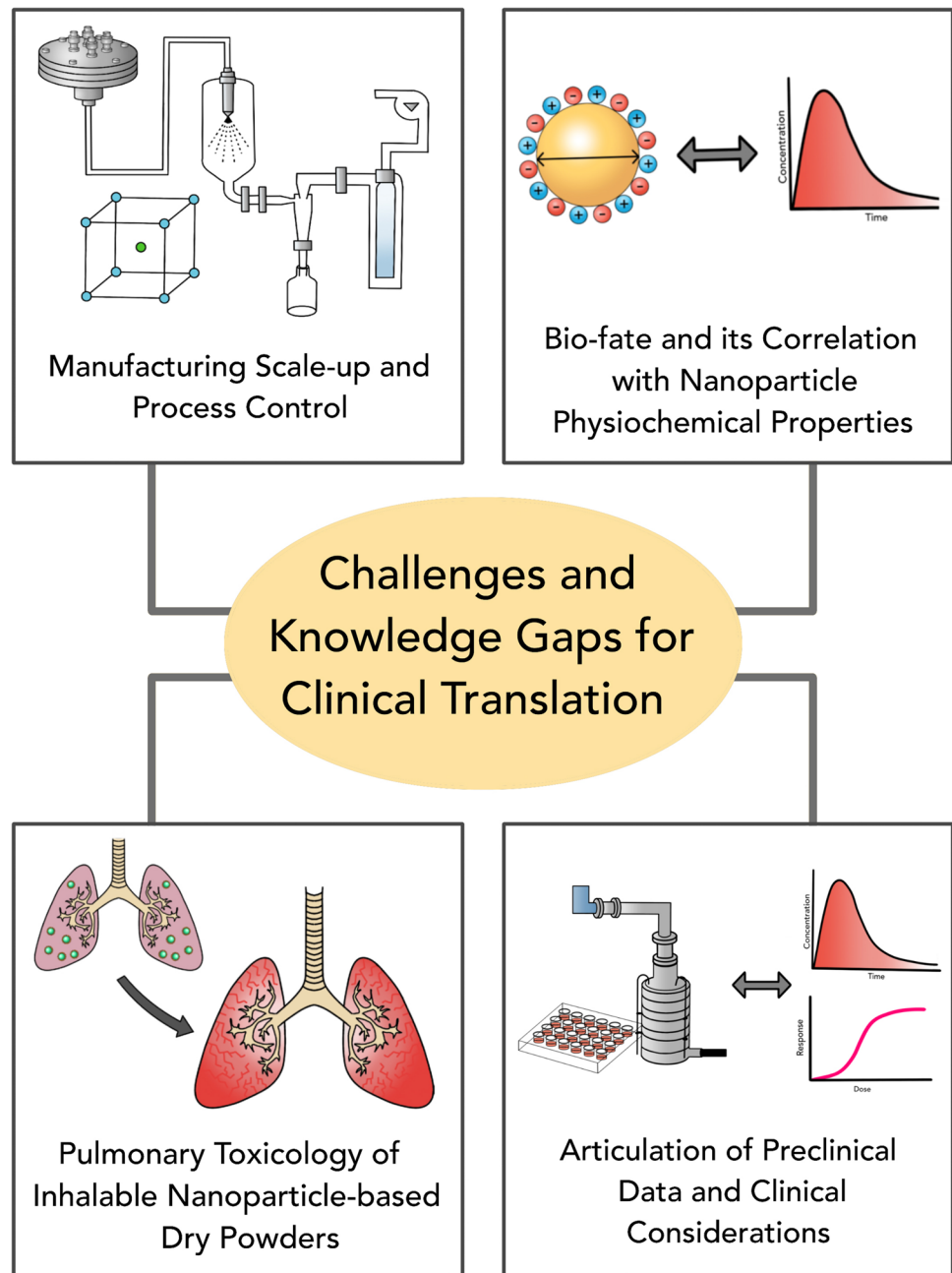
breast cancer cells compared to those with particle size of 50 nm and 90 nm [126], and Weng *et al.* reported that 40 nm and 150 nm cholecalciferol-loaded nanoparticles showed greater lung deposition compared to those with size between 60 and 125 nm [127]. Similarly, there remains a lack of understanding on the effect of nanoparticle surface charge on the bio-fate of inhaled nanoparticles. While studies have demonstrated that cationic nanoparticles may induce superior cell uptake into lung epithelial cells compared to their anionic and neutral counterparts [128], others have shown that neutral nanoparticles can more effectively penetrate the mucus layer [129]. More

studies are needed to establish the correlation between nanoparticle physicochemical properties (including individual and interactive effects) and their biological fates and therefore the "optimal" nanoparticle size and surface charge for maximized therapeutic effect.

Pulmonary Toxicology of Inhalable Nanoparticle-Based Dry Powders

Toxicity has been cited as another major hurdle in bench-to-bedside translation of nanomedicines, especially since ultrafine inorganic particles (e.g., titanium dioxide, gold

nanoparticles, etc.) have well-established toxicological effects when inhaled, influenced by their physicochemical properties (e.g., particle size, surface charge, etc.) [130]. However, such results may not be translatable to organic nanoparticles which are the mainstay of inhalable nanoparticle-based dry powders. Nevertheless, novel polymers or lipids are sometimes used to fabricate these organic nanoparticles without thorough evaluation of its biocompatibility and biodegradability when delivered by oral inhalation, significantly hindering their clinical translation. Similarly, various polymers (e.g., PVA, methylcellulose) have not been approved as excipients for oral inhalation despite their potential merits over FDA-approved excipients such as lactose and mannitol as protectants in nanoagglomerate microparticle formulations. While *in vitro* cellular studies have generally demonstrated low cytotoxicity of inhalable nanoparticle-based dry powders in human pulmonary cell lines, limited *in vivo* studies investigating the safety of inhalable nanoparticle-based dry powders (especially under chronic exposure) are available, despite the fact that most of the dry powder formulations reported are intended for the treatment of chronic respiratory conditions. As such, research efforts should be dedicated to proper characterization of toxicological profiles of nanoparticle-based dry powder formulations (at both *in vitro* and *in vivo* levels) and effects of organic nanoparticle properties (e.g., particle size) on their pulmonary toxicity to ensure their safety in clinical practice.

Articulation of Preclinical Data and Clinical Consideration

The prediction of human *in vivo* behavior of inhaled nanoparticle-loaded dry powders from preclinical data is critical to their successful clinical translation. As only limited clinical trials have been conducted to-date on inhalable nanoparticle-based powders, there is lack of *in vitro* and clinical data available for establishing a robust IVIVC, and data available mostly concern nanoparticle-carrier systems. Nevertheless, currently available data suggest a good correlation between *in vitro* cascade impactor testing and clinical lung deposition data. For example, Bhavna *et al.* reported a higher respiratory fraction of nano-sized salbutamol-lactose blend over micronized salbutamol-lactose blend (45.2±5.2% *vs.* 31.3±3.1%) as measured with ACI, which corresponded to a greater deposition of salbutamol in the lungs (64.1±3.7% *vs.* 28.3±5.2%) as measured using scintigraphy [131]. A similar trend was reported by Kumar *et al.* for an inhalable edetate calcium sodium (Ca-Na$_2$EDTA) nanoparticle formulation compared to its micronized counterpart [17].

The major challenge in establishing IVIVC for inhalable nanoparticle-based powders is developing preclinical models that accurately reflect the fate of nanoparticle-based powders after oral inhalation, i.e., deposition of powders within the lungs, redispersion of nanoparticles in lung lining fluid (see subsection "Redispersibility and Stability of Redispersed Primary Nanoparticles"), and subsequent absorption and clearance of nanoparticles from the respiratory tract. For lung deposition, cascade impactors are known to have at best modest correlation with *in vivo* lung deposition data due to differences in "mouth-throat" geometry and inhalation profiles [132]. IVIVC could be improved by employing realistic "mouth-throats" connected to cascade impactors or 3D-printed lung models and simulated breathing profiles [117, 133], yet the selection of suitable anatomical "mouth-throats" and representative inhalation profiles remains to be controversial. Alternatively, *in silico* modeling by computational fluid dynamics (CFD) is a trendy strategy in predicting *in vivo* deposition, though they are more technically demanding compared to *in vitro* methods [134].

For conventional orally inhaled formulations, the absorption and clearance of dissolved drug molecules are assessed *in vitro* by cell cultures to establish correlation with pharmacokinetic or pharmacodynamic data [135]. However, the study of absorption and clearance of redispersed drug-loaded nanoparticles within the respiratory tract is far more complicated compared to conventional orally inhaled formulations by virtue of their different (or mixed) cellular uptake and clearance mechanisms and the co-presence of drug-loaded nanoparticles and free drugs within the lung lining fluid and cellular environment. Furthermore, animal models may overestimate therapeutic merits of nanoparticles due to interspecies differences in physiology, the enhanced permeability and retention (EPR) effect being the most notable example [136]. It is hard to assess the enhancement in pharmacokinetics or pharmacodynamics of inhaled drug-loaded nanoparticles relative to a conventional inhaled formulation and thereby predict a suitable *in vivo* dose. With advances in tissue engineering and 3D printing, 3D-bioprinted lung models may serve as a practical tool for *in vitro* evaluation of inhaled nanoparticle-based dry powders by mimicking the complexity of the human lung physiology and patient's conditions [135].

Future Perspectives and Conclusions

There is a gradual shift from small molecules to biologics in new drug development due to the high specificity of biologics. Based on the recent success of mRNA vaccines in COVID-19 prophylaxis, the development of inhalable formulations for treatment of respiratory conditions has become an area of active research [10]. Nanoparticle-based formulations play an important role in pulmonary delivery of biologics as they can protect biologics from enzymatic degradation and offer additional therapeutic benefits. For example, encapsulating nucleic acid therapeutics (e.g., siRNA) in nanoparticles has been shown to enhance their cellular uptake and transfection efficiency [72]. Recent

research has led to the successful development of inhalable nanoparticle-based dry powders for biologics that could effectively retain the integrity and activity of the encapsulated biologic (Table I). However, it is found that the nano-embedded microparticle strategy is most often employed for these purposes. Combined with their low encapsulation efficiency and drug loading within nanoparticles, inhalation of a large quantity of powder would be required to deliver optimal therapeutic doses which results in high costs and low treatment compliance. There is few, if any, literature available, regarding the adoption of nano-agglomerate microparticles for delivery of biologics, and it could be a propitious option to deliver nano-encapsulated biologics with the least amount of dry powder necessary and a lower requirement of inspiratory flow rates.

In conclusion, the emergence of novel respiratory infections (e.g., COVID-19) and rising prevalence of chronic respiratory conditions have sparked considerable interests in the development of new therapeutics. Formulating therapeutic agents as inhalable nanoparticle-based dry powders is a niche for effective drug delivery into the diseased lungs, combining the merits of pulmonary drug delivery and nanotechnology. Despite precedent successful approval of inhalable nanoparticle nebulized formulations and research efforts dedicated to their formulation development and particle engineering, there still exists knowledge gaps required to be filled for successful clinical translation. This review not only summarizes the current state-of-the-art fabrication of inhalable nanoparticle-based dry powders, but also provides directions to pave the path toward their successful clinical translation. With a suitable biorelevant characterization and comprehensive understanding on the correlation between the physicochemical properties and *in vitro* performance of inhaled nanoparticles with their clinical response, it is anticipated that the use of inhalable nanoparticle-based dry powders in clinical practice will soon no longer be a far-fetched fantasy but a reality.

Declarations

Conflict of Interest The authors declare no competing interests.

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