

Nanocrystals based pulmonary inhalation delivery system: advance and challenge

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ABSTRACT

Pulmonary inhalation administration is an ideal approach to locally treat lung disease and to achieve systemic administration for other diseases. However, the complex nature of the structural characteristics of the lungs often results in the difficulty in the development of lung inhalation preparations. Nanocrystals technology provides a potential formulation strategy for the pulmonary delivery of poorly soluble drugs, owing to the decreased particle size of drug, which is a potential approach to overcome the physiological barrier existing in the lungs and significantly increased bioavailability of drugs. The pulmonary inhalation administration has attracted considerable attentions in recent years. This review discusses the barriers for pulmonary drug delivery and the recent advance of the nanocrystals in pulmonary inhalation delivery. The presence of nanocrystals opens up new prospects for the development of novel pulmonary delivery system. The particle size control, physical instability, potential cytotoxicity, and clearance mechanism of inhaled nanocrystals based formulations are the major considerations in formulation development.

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1. Introduction

Pulmonary inhalation administration not only is an ideal way to be used to locally treat lung disease, such as asthma, cystic fibrosis, chronic obstructive pulmonary disease, bronchitis, and lung cancer (Patton & Byron 2007; Mansour et al., 2009), but also to achieve systemic administration for other diseases, owing to the tremendous surface area (70–140 m² in the adult human lung) of the lung and fast transport of actives across the respiratory epithelium (relatively high blood flow up to 5000 mL/min) (Yang et al., 2014; Elsayed & Shalash 2018). Furthermore, it can bypass the liver's first pass effect and possess low enzymatic activity, thereby reducing the dosage and side effects of medication, with respect to the other administration, such as oral administration or injection administration (Gonda 2006; de Kruijf & Ehrhardt 2017). Unfortunately, pulmonary formulations were strictly restricted by the physiological characteristics of the lungs, such as branching structure, mucociliary, and macrophages (Ling et al., 2014), as well as certain properties of the drugs like particle size and solubility (Loira-Pastoriza et al., 2014).

Nanotechnology has a huge advantage in pulmonary delivery for poorly soluble drug, which could overcome the numerous biological and physical barriers of the pulmonary,

improve drug solubility, and prolong retention time in lung of drug to enhance bioavailability in the lungs (Sung et al., 2007; Roa et al., 2011; Muralidharan et al., 2015; Elsayed & AbouGhaly 2016; Mangal et al., 2017). The Arikayce[®] inhalation suspension as a liposome nanomedicine was approved by the FDA for the treatment of *Mycobacterium avium* complex lung disease (Shirley, 2019), which encouraged the development of more nanoparticles (NPs) based formulations for pulmonary drug delivery. It was well known that nanoformulations for pulmonary administration are classified into nanocarriers (liposomes, solid lipid NPs, nanostructured lipid carrier, micelles, and polymeric NPs) and polymer–drug conjugates (dendrimers and PEGylated NPs) (Xing et al., 2020). Nanocrystals as a free carrier-nanotechnology have obtained increasing attention for pulmonary administration of poorly soluble drugs owing to their improved dissolution rate and saturation solubility of poorly soluble drug, biological properties, and low toxicity (Gao et al., 2012; Pawar et al., 2014; Thakkar et al., 2017).

However, the complex physiological structures or barriers and clearance mechanisms in the respiratory tract could eliminate the foreign particles and hinder the effective delivery of drug (Yang et al., 2008). Moreover, the surface

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properties of NPs could also significantly influence their fate (adsorption or clearance) in the lung (Hu et al., 2013; Liu et al., 2020). Therefore, in this review, the physiological structure of lung and various clearance pathways of NPs in the lung are presented systematically. The advantages, advanced technologies for preparation of nanocrystal based pulmonary delivery system, as well as the applications of nanocrystals in pulmonary delivery system were highlighted. It is expected to provide reference for promoting the pulmonary administration of poorly soluble drugs.

The lungs are the organs that contact with the exchange of air between the organism and the outside world. They are divided into two main regions: the conducting airway region and the respiratory region. The airway is a continuous branch from the bronchi to the lungs and consists mainly of bronchi, bronchioles, and terminal bronchioles. As the bronchi continue to branch, the diameter of the tubes becomes smaller, the tube wall becomes thinner, and the structure of the tube wall changes gradually. The annular smooth muscles of the bronchi contract or relax under splanchnic nerves innervation and it is responsible for the regulation of airflow passage into the alveoli. This is the site where the lung tissue completes gas exchange consisting of respiratory bronchioles, alveolar ducts, lung sacs, and alveoli. The respiratory bronchiole is the transitional pipes between the pulmonary airway and the respiratory site. Each respiratory bronchiole branch is divided into 2–3 alveolar ducts. The alveolar sacs are the common opening of several alveoli and are connected to the alveolar ducts. The gut is the main site for the digestion and absorption of nutrients.

Alveoli, the terminal part of the bronchial tree, are the main site for the gas exchange. There are approximately 300–500 million alveoli in each lung (Yue et al., 2018).

The lung is composed of a single layer of epithelial cells with a large surface area (greater than $100\mu\text{m}$) and thin epithelium ($0.2\text{--}1\mu\text{m}$). Adequate blood supply allows the drug to be quickly absorbed in the lungs (Patton & Byron 2007), which is more attractive than systemic drug delivery. In addition, the relatively low enzyme activity in the lungs can avoid the damage caused by the acid–base of the gastrointestinal tract during oral administration, and help maintain a high bioavailability (Yang et al., 2010), noninvasive lung delivery pathway can also improve patient compliance (Gonda 2006; Mansour et al., 2009).

2. The barriers to lung inhalation administration

2.1. Mucociliary clearance

The absorption of drugs in the lungs is influenced by multiple barriers. Due to the different anatomical structures of lungs, the clearance mechanisms of drugs in different regions may be different as well (Figure 1). Mucus clearance, which can help to capture the foreign dust and microorganisms, then removes them from the lungs. Therefore, the mucus clearance is the primary drug clearance mechanism for conducting airway region. The conducting airway region consists mainly of ciliated epithelial cells and goblet cell that together form the bilayer mucin periciliary layer (O' Donnell & Smyth 2011). Notably, the upper is a gel-forming mucins that form a viscous mucus layer and the submucous layer is ciliated sol layer with low viscosity, and the cilia are covered with a viscoelastic mucus (Button et al., 2012). The elastic mucus is usually comprised of hydrogel (95%), mucin (2%), glycoprotein, lipid, and salt, which are secreted by the goblet cell and the submucosal glands (Sanders et al., 2009). The

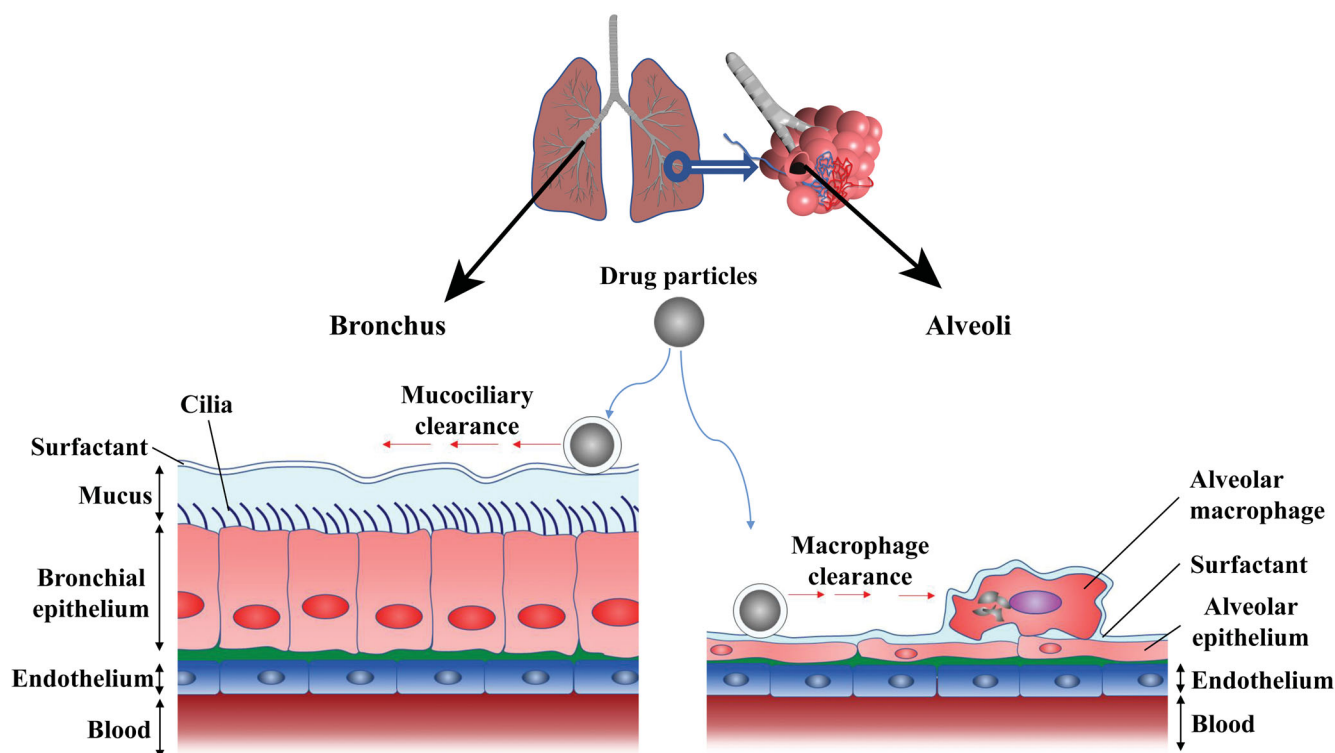


Figure 1. The structure of lung and pulmonary mucus barrier.

thickness of the mucus in the trachea is 10–30 μm , and 2–5 μm in the bronchus. The mucin is the main functional component of elastic mucus with the concentration range of mucin between 1 and 5%. Mucin producing the mucus has astringency and adhesion characteristics, thereby adhering foreign objects such as drugs (Bansil & Turner 2006; O'Donnell & Smyth 2011). Mucin is a hydrophilic linear peptide chain consisting of 8–169 amino acids of repeated proline, threonine, and serine, which is called as the PTS (proline, threonine, and serine) domain. The hydrophobic region composed of cysteine is called as the non-PTS region. Mucin monomers associate with the several other monomers via cysteine bridges in aqueous media, forming a mucin fiber mesh-like structure with variable porosity of 50–1800 nm with the different spacings diameters (Araújo et al., 2018). However, when the drugs cannot cross the mesh-like structure, the drugs are wrapped by viscous mucus, the swinging cilia may push the mucus which wrapped with drugs to the pharynx (Button et al., 2012). It enables the drugs to be swallowed or coughed out. With the help of the mucociliary clearance mechanism, the defense system of the lungs can remove foreign bodies and captured particles in the mucus within 15 minutes to two hours (Mansour et al., 2009). It has been found that the tracheal mucociliary clearance rate of healthy young people was about 4–20 mm/min (García-Díaz et al., 2018).

2.2. Phagocytosis by pulmonary macrophages

Pulmonary macrophages are one of the most common cells in the lung which are derived mainly from mononuclear cells produced by bone marrow and exist in four subgroups, and are widely distributed in pulmonary blood vessels, alveoli, interstitial, etc.

Pulmonary macrophages can play an important role in normal physiological process of the lungs and the occurrence and development of diseases process. It is generally in a microenvironment with relatively high oxygen partial pressure and has frequent contact with foreign bodies, and has physiological characteristics such as adhesion, deformation, migration, and phagocytosis (Praphakar et al., 2018). After the recognition procedure of the foreign bodies by the macrophages, the foreign bodies could be engulfed by macrophages and migrate to the respiratory tract and the foreign bodies were washed out by the effect of mucus. The phagocytosis of pulmonary macrophages is primarily associated with the size of phagocytic particles, the optimal particles size ranges for endocytosis was 1–3 μm . The particles in this range will be eliminated by macrophages. Due to active endocytosis of lung macrophages, the drug absorption after pulmonary administration is hindered, especially for macromolecular protein. Moreover, the phagocytosis of pulmonary macrophages results in the decrease of efficacy and concentration of drug. The lysosomes and other enzyme systems possessed by lung macrophages can lead to inactivation of macromolecular protein by degradation (Lu et al., 2018). The alveolar macrophages are the main drug clearance mechanism in the respiratory region of the lung (Liu et al., 2020).

The alveolar macrophages account for more than 90% of the airway immune cells (Lee et al., 2015), and there are 8–12 alveolar macrophages in each alveolar. The inhaled microorganisms or foreign bodies can be readily engulfed by alveolar macrophages and then transported to phagosomes, thereby triggering microorganisms or foreign bodies degradation (Alblas et al., 1981). It was reported that in order to achieve the same hypoglycemic effect as subcutaneous insulin, the drug concentration by pulmonary administration must be increased by 7–8 times, because more than 1/3 of insulin is cleared by pulmonary macrophages (Praphakar et al., 2018).

2.3. Effects of pulmonary surfactant

The pulmonary surfactant (PS) is synthesized and secreted by alveolar type II epithelial cells, and forms a surfactant membrane at the respiratory gas–liquid interface (Johnson et al., 2006; Button et al., 2012). Its main function is to reduce the surface tension in gas–liquid interface, and maintain the alveolar morphological stability and protect the alveolar epithelial cells, and PS is also involved in defensive function of lung host (Perez-Gil & Weaver 2010). The composition of PS is particularly complex, including 90% lipid and 10% protein (Arick et al., 2015). Among the various lipids found in PS, the dipalmitoyl lecithin (DPPC) is the most abundant component, together with several other phospholipids, accounting for 80% of lung surfactant lipids (Yu & Possmayer 2003). In contrast, cholesterol, phosphatidylinositol (PI), phosphatidylglycerol (PG), and acidic phospholipids were composed of 20% of lipids in PS (Wüstneck et al., 2005). PS is also involved in the process of drug clearance, and the proteins in PS can promote the adhesion and aggregation of drugs, making it easier to be cleared by mucosal cilia or recognized by macrophages and monocytes for phagocytosis (Ordonez et al., 2017). The proteins found in PS have four different forms according to previously literature reports (Goerke, 1998), the surface active proteins SP-B and SP-C are small hydrophobic proteins, which played an important role in maintaining and regulating the physiological function of PS (Ding et al., 2001; Whitsett & Weaver 2002). The SP-A and SP-D are large hydrophilic glycoproteins that can enhance the tendency and phagocytosis of macrophages. When the fungal infection occurs in the lung, SP-A and SP-D bind to fungal ligands in a Ca^{2+} dependent manner and regulate or promote the secretion of cytokines in order to enhance macrophage phagocytosis (Ordonez et al., 2017).

3. Aerodynamic requirements for lung inhalation

The aerodynamic characteristics of inhalable microparticles are the main external factors affecting the pulmonary inhalation of drugs. The size distribution of the inhalable particles is usually expressed by aerodynamic diameter, which varies according to the shape, size and density of the objects. The aerodynamic diameter of inhalable particles determines whether they can be deposited in the lungs. There are three main mechanisms for its deposition in the lung: inertial

collision, gravity sedimentation, and Brownian diffusion. Inertial collision is a collision of particles with a mass median aerodynamic diameter (MMAD) greater than $5\ \mu\text{m}$ on the surface of the oropharynx and upper respiratory tract due to inertia effect. Gravity sedimentation is main mechanism of deposition on the surface of trachea, bronchus and alveoli. When the MMAD of particles is in the range of $1\text{--}5\ \mu\text{m}$, gravity sedimentation can occur. But when the particle MMAD is less than $0.5\ \mu\text{m}$, the sedimentation effect is not as effective as Brownian's diffusion. The particles with MMAD less than or equal to about $0.5\ \mu\text{m}$ are mainly deposited in small airways and alveoli owing to Brownian's diffusion. Meanwhile, the deposition of particles is affected by the action of breath holding which can facilitate deposition (Rogueda & Traini 2007; Wei et al., 2018). In order to successfully deposit in the deep lung, the particles must be small enough to avoid deposition in the upper respiratory tract due to inertial collision, and the particles must be large enough in order to avoid being exhaled during exhalation. The particles with MMAD less than $0.5\ \mu\text{m}$ would be discharged with the air-flow through Brownian's motion, usually up to 80%. Therefore, in order to obtain effective lung deposition, the MMAD of particles should be in the range of $1\text{--}5\ \mu\text{m}$.

4. Advantages of nanocrystals technology for lung inhalation of poorly soluble drug

In recent years, nanocrystals have become an intense focus of research of nanotechnology. Drug nanocrystals were generally pure drug particles with a particle size of $1\text{--}1000\ \text{nm}$, which can be stabilized by surfactants or stabilizers without the need of carrier materials. Drug nanocrystals can enhance the adhesion to biological membranes, increase the saturated solubility and dissolution of the drug and improve the bioavailability by virtue of its large specific surface area. The nanocrystals technology can be used as intermediate preparation technology, which is extensively employed in various routes of drug delivery (such as oral, intravenous, pulmonary, percutaneous, and ocular administration, etc.). Compared with other nanotechnology, nanocrystals technology has unique advantages in pulmonary drug delivery, which brings hope for the treatment of various lung diseases. Its main features are as follows:

4.1. High drug loading and good safety

Nanocrystals have high drug loading compared with other carrier based NPs. With the improvement of high drug loading and bioavailability, drugs nanocrystals can improve drug safety by reducing the dosage. The preparation of poorly water-soluble drug solutions requires a large number of surfactants or organic solvents to dissolve them. However, these surfactants and organic solvents have certain adverse effects on cells.

Nanocrystal formulations could solve these problems because they are usually prepared without using large amounts of organic solvents, thereby reducing co-solvent interference and potential toxicological effects in the body.

Moreover, the properties of nanocrystals help to increase the saturated solubility of the drug, have a higher drug loading capacity, without the need for higher concentrations of surfactants, and show better bioavailability compared with other nanoformulations (Müller et al., 2011; Kannan et al., 2013). Nanocrystals minimize the excessive use of harmful non-aqueous solvents, while the increase of drug loading and bioavailability reduces the dose and enhances the safety of lung administration. Rundfeldt et al. have found that itraconazole nanocrystals suspension is used in inhaled treatment of cystic fibrosis patients with allergic bronchopulmonary aspergillosis. Its long-lasting lung tissue concentration is much higher than the lowest inhibitory concentration of *Aspergillus* strain, so that patients only need to be administered once a day to reduce systemic exposure (Rundfeldt et al., 2013).

4.2. Enhanced mucus-penetrating ability

After the drug enters the lung, it is easy to be removed by mucociliary. At the same time, high viscoelasticity and adhesive mucus effectively prevent the delivery of nano drugs to the patient's lungs. It is found that rod-shaped nanocrystals can enhance the diffusion of drugs in mucus, and rod-shaped NPs have higher diffusion rate than spherical NPs in biological mucus medium. Wang et al. have shown that the aspect ratio of nanorods and their adhesion to host media play a key role in the hopping diffusion behavior of nanorods. In viscous polymer solution and gels, hopping diffusion makes the diffusion rate of nanorods higher than that of spherical NPs (Wang et al., 2018). Costabile et al. studied C109 nanocrystals with rod-shaped structure. C109 is an effective but poorly soluble filamentous temperature-sensitive protein Z (FtsZ) inhibitor, which has a good inhibitory effect on the central softening of the high-risk pathogen in patients with cystic fibrosis. The results show that C109 nanocrystals can smoothly penetrate the mucus of artificial cystic fibrosis for diffusion and exert therapeutic effect (Costabile et al., 2020).

4.3. Double drug release in the lungs

Nanocrystal drugs show immediate and prolonged release behaviors after topical administration. The high surface area of nanocrystals increases the saturation solubility and dissolution of the drug, which helps to obtain an initial high drug concentration to achieve absorption and trigger action (Gao et al., 2008). The high surface area of drug nanocrystals also helps to increase their interaction with biofilms and extend their residence time on the biofilm. In addition, the longer the residence time of these drug particles in the lungs, the longer the drug release will be (Ali et al., 2011).

4.4. Reduced macrophage clearance

The optimal particle size for macrophage endocytosis is $1\text{--}3\ \mu\text{m}$, while the particle size of nanocrystals greatly reduces the possibility of macrophage clearance.

Simultaneous studies have shown that NPs could minimize the clearance of macrophages (Oberdörster et al., 1994, 2005), and inhalation of drug nanocrystals can lead to deep lung deposition and smaller airway penetration, resulting in more uniform drug distribution, higher drug deposition rate, and more precise drug efficacy (Zheng & Bosch 1997).

5. Production of nanocrystals based pulmonary inhalation system

Nanocrystals based pulmonary inhalation delivery systems are a research hotspot for new drug delivery technologies. Due to the characteristics and advantages of nanocrystal technology, it has been widely used in pulmonary inhalation drug delivery systems for poorly soluble drugs, such as nano-suspension-based aerosol, dry powder inhaler, the inhalable nanocomposite particle, and so on.

5.1. Production method of drug nanocrystals

The production methods of drug nanocrystals are mainly divided into two categories: bottom-up technology and top-down technology, including micro-precipitation method, media milling method, high pressure homogenization method, precipitation-homogenization combinative technology, etc. (Figure 2).

5.1.1. Bottom-up technology

Bottom-up technique, also known as 'precipitation', is a method in which the drug is completely dissolved in one solvent and then added to another non-solvent. The main approaches for the preparation of nanocrystals are micro-precipitation and supercritical fluid (Sinha et al., 2013; Yue et al., 2018). The key of this technique is to control the crystal structure of the drug to avoid agglomeration and to control

the particle size in the nanometer range. Controlling the crystal structure of the drug to avoid agglomeration and the particle size in the nanometer range are the key factors of this technique. Advantages of this technique include: easy preparation, lower costs, and preparation done in steps. However, its poor reproducibility makes it difficult to scale up, and it is easy to residual the organic solvent because of the need for organic solvents in the preparation process. Thus, this method was not suitable for the drug that is insoluble in water and non-aqueous solvents (Du et al., 2015). Xiong et al. prepared resveratrol nanocrystal suspensions with an average particle size of 222.54 ± 1.66 nm and a polydispersity coefficient of 0.125 ± 0.035 using this technique (Xiong et al., 2020).

5.1.2. Top-down technology

Top-down techniques, also known as 'dispersion', in which the raw material comprises of larger solid particles than the resulting NPs and mechanical processes are the fundamental mechanism leading to particle size reduction. This method mainly includes media milling, high-pressure homogenization, and microfluidization method (Malamatari et al., 2018; Yue et al., 2018). Currently, the top-down technology is the major route of the research and development of listed products (Liu et al., 2018). The advantage of the top-down approaches is easy preparation.

Top-down approaches are simple, easy to industrialize and reproducible processes, easy preparation, easily adaptable to industrialized production and re-use. However, the large number of cycles required to reach the desired drug particle size and the potential for aggregation of drug particles over time result in poor physical stability. Huang et al. prepared harmine nanocrystal suspension with an average particle size of 179.21 ± 0.01 nm and the zeta potentials were

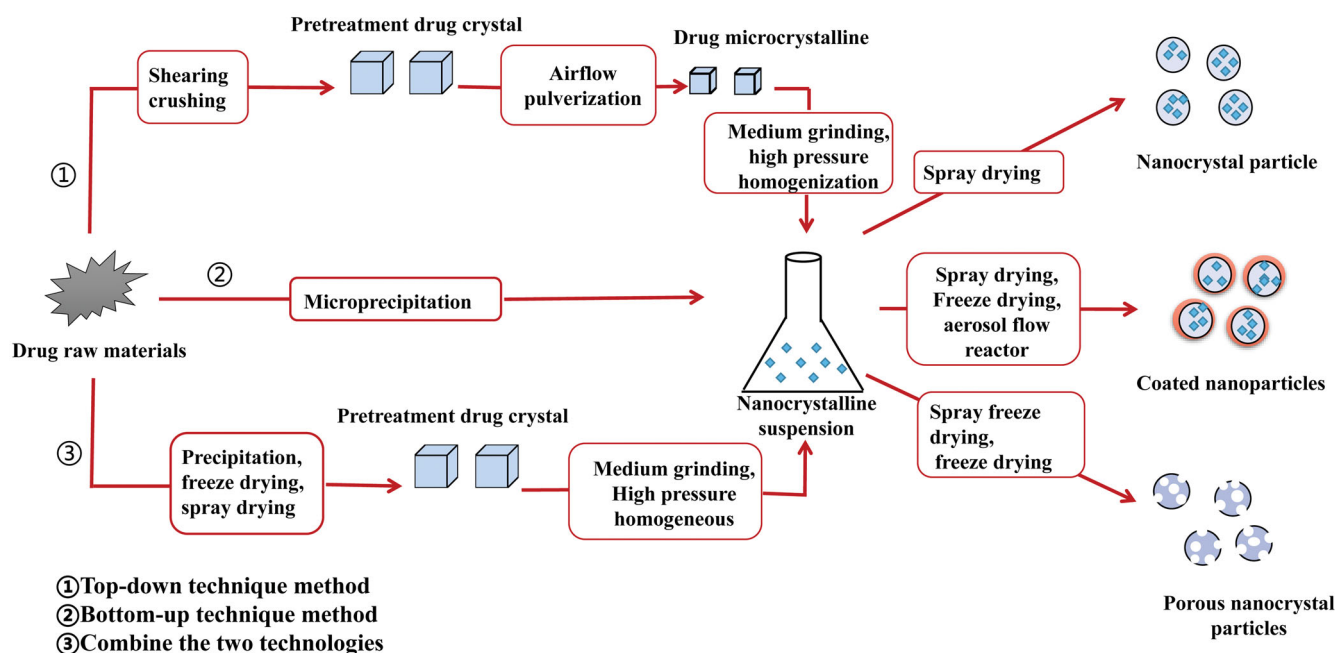


Figure 2. Preparation methods of nanocrystals based pulmonary inhalation delivery system.

all between -41.4 ± 1.08 mV, which indicated that the HARNs had good stability (Huang et al., 2021) (Table 1).

5.1.3. Combination method

In the process of nanocrystals preparation, it is difficult to achieve the requirements of uniform particle size and good stability by a single preparation technique. Therefore, combination technologies have also been developed that integrate a pretreatment step with a subsequent high energy step, like high pressure homogenization (Jermain et al., 2018). The combination of bottom-up and top-down technologies can make full use of their respective advantages to improve drug nanosizing efficiency and narrow particle size distribution to prepare safe and effective formulations. However, the high production cost and the complex

production processes of the combined techniques limit their industrialization. Tao et al. (2016) prepared the resveratrol nanocrystal suspensions by combination technology and obtained the resveratrol nanocrystals with an average particle size of 192 nm, which was much smaller than the average particle size of 569 nm prepared by high-pressure homogenization.

5.2. Production of nanocrystals based inhalable nanocomposite particles

The preparation process of inhalable nanocrystals based delivery system mainly includes two main steps: drug nanosizing and solidification. Solidification methods for converting nanosuspensions into inhalable nanocomposite particles

Table 1. Examples of nanocrystal preparation.

Drug	Disease	Method of preparation	Stabilizer (concentration)	Average particle size (nm)	Performance
Curcumin (Pelikh et al., 2021)	Atopic dermatitis, psoriasis, and androgenetic alopecia	Bead milling	TPGS (<i>D</i> - α -tocopherol polyethylene glycol 1000 succinate) (1.0% (w/w))	290	Nanocrystals were efficiently taken up by the hair follicles and more permeable.
Andrographolide (Basu et al., 2020)	Hepatoprotective	Solvent-diffusion followed by homogenization	Tween 80 (1%)	630 \pm 12	Aqueous solubility increased nearly fivefold. More significant liver protection effect.
Quercetin (Manca et al., 2020)	Skin disorders	Wet media milling	P188, Tween 80	326–474	<i>In vitro</i> studies showed the high biocompatibility of nanosuspensions and their ability to counteract the oxidative effect of hydrogen peroxide on cells.
Ginkgolide B (Liu et al., 2020)	Parkinson's disease	Antisolvent precipitation	Hydroxypropyl methylcellulose (HPMC) E5 (0.5 mg/mL)	83.48 \pm 1.77	C_{max} of the GB-NCs up to 13-fold higher than unformulated GB, AUC_{0-t} increased fivefold.
Resveratrol (Xiong et al., 2020)	Parkinson's disease	Antisolvent precipitation	HPMC (1.8 mg/mL)	222.54 \pm 1.66	No significant toxic effects on zebrafish embryos and larvae, exhibited more favorable pharmacokinetics than pure RES.
Lutein (Chang et al., 2018)	Prophylaxis of cardiovascular	Anti-solvent precipitation –ultrasonication	Soy phosphatidylcholine (0.08%)	110.7	The C_{max} and $AUC_{0-24 h}$ of lutein nanocrystals after oral administration in rats was 3.24 and 2.28 times higher than those of lutein suspension.
Glycyrrhetic acid (Lei et al., 2016)	Chronic hepatitis	Anti-solvent precipitation –ultrasonication	TPGS (0.5%)	220	The bioavailability of GA nanocrystals in rats was 4.3-fold higher than that of the coarse GA after oral administration.
Pramipexole (Li et al., 2018)	Parkinson's disease	Wet media milling	PVP K30 (2.4%)	352.07 \pm 21.29	<i>In vitro</i> permeation studies through rabbit ear skin indicated that the cumulative permeation amount of pramipexole from nanocrystals in 24 h was 2.75 times more than that from coarse suspension.
Meloxicam (Yu et al., 2018)	Rheumatoid arthritis and osteoarthritis	Nanoprecipitation technique	Poloxamer 407/Tween 80 (80/20, w/w) (0.1%)	175 \pm 4	2.58- and 4.4-fold increase in $AUC_{0 \rightarrow 24 h}$ was achieved by nanocrystals comparing with solution and suspension.
Baicalin (Wei et al., 2018)	Anti-oxidant; anti-inflammatory	High-pressure homogenization	TPGS 10% (w/w)	189.21 \pm 0.36	The transdermal flux of BCA-NC-gel with 1% HLA were 20.65-fold higher ($p < .01$) than that of coarse BCA-gel.

include spray-drying technology, freeze-drying technology, spray-freeze drying technology, and so on.

5.2.1. Spray-drying

Spray-drying is a single-step process that converts a liquid (solution, suspension, or emulsion) directly into dry particles (Alhajj et al., 2021). The main principle of spray-drying is the atomization of the liquid feedstock into fine droplets through the nozzle, and evaporate the solvent through the hot drying gas (Raula et al., 2013). During the spray-drying process, the particle properties (e.g. particle size distribution, shape, residual humidity, and density) can be controlled by adjusting the formulation and process parameters (e.g. feed rate and inlet temperature). The spray-drying technology has become the basic particle engineering technology for the development of inhalable preparations. Also, from the industrial perspective, due to its faster and more cost-effective than freeze-drying, spray-drying is more popular for inhalable preparations. Therefore, spray-drying of nanosuspensions can be used as a platform for pulmonary administration of poorly water-soluble drugs. Pomázi et al. (2013) developed meloxicam inhalable nanocrystal aggregates embedded in mannitol and other excipients (polymer and L-leucine) by applying high-pressure homogenization and spray-drying technology. It was found that due to the presence of the amino acid L-leucine, the adhesion between particles was reduced and the aerosolization of nanocrystals-aggregates was enhanced (Sou et al., 2011). The nanocrystal-aggregates of meloxicam are crystalline and exhibit enhanced *in vitro* nebulization (FPF >53%, MMAD <3.52 μm) and solubility properties. The itraconazole nanocrystals-aggregates could be an effective strategy for the treatment of pulmonary aspergillosis (Rundfeldt et al., 2013).

5.2.2. Freeze-drying

The freeze-drying (lyophilization) is a classical process for removing water from high value products (such as antibiotics, enzymes, vaccines, etc.). In order not to cause excessive damage to the active ingredients, the addition of water or other suitable aqueous diluents before freeze-drying increased the storage stability and restorability of drugs (Jakubowska & Lulek 2021).

The process of freeze-drying is to first freeze the liquid solution or suspension under atmospheric pressure and then heat it under vacuum in order to remove ice crystals by sublimation. Finally, the high-porosity powder with low moisture content was obtained. The freeze-drying process consists of three steps: freezing, primary drying, and secondary drying (Siow et al., 2016). Freeze-drying and spray-drying are most commonly used techniques for solidification of nanosuspension as they are easily applied in practice, their scale up production and high industrial acceptability. In order to form inhalable microparticles (NP-agglomerates), El-Gendy et al. (2009) used freeze-drying to solidify the nanosuspension, which was obtained through a controlled flocculation process (protocol under the name NanoClustersTM, owned by Savara Pharmaceuticals, Austin, TX). Budesonide nanocrystals-

agglomerates prepared by this method showed enhanced dissolution rate when compared with the raw drug, with an MMAD of $2.1 \pm 1.8 \mu\text{m}$ (mean standard deviation) in terms of aerosolization, which is aided by the penetration of the particles deeper into the lungs.

5.2.3. Spray-freeze-drying

The spray-freeze-drying is an important alternative technology for spray-drying, which has been already used for the preparation of thermolabile inhalable particles, especially proteins (Rouse et al., 2007). During the spray-freeze drying process, the liquid is fed and atomized by a nozzle, and the atomized droplets are rapidly frozen in liquid nitrogen to remove the freezing solvent (water) by sublimation and obtain a powdered product. Compared with spray-drying, the spray-freeze-drying is a low-temperature drying process that produces porous particles (Yue et al., 2018). Voriconazole powder prepared by spray-freeze-drying exhibited porous and spherical structure, and displayed crystalline characteristics (Liao et al., 2019). However, the spray-freeze-drying particles are partially composed of amorphous solids with a large specific surface area, and this state is hygroscopic which can lead to changes in physicochemical properties by humidity when the particles are stored over the long-term or under high humidity conditions such as in the lungs (Tanaka et al., 2020). Cocrystal technique has been used to solve such problems (Duggirala et al., 2016; Al-Obaidi et al., 2021).

5.2.4. Aerosol flow reactor

The aerosol flow reactor patented by Teicos Pharma (Espoo, Finland) has been used for the solidification of nanosuspensions. It is a simple and efficient one-step continuous process that produces particles directly in the desirable particle size range. In detail, the drug solution was atomized by ultrasonic to create droplets, then the droplets suspended in the carrier gas were passed (usually N_2) through a heated tubular laminar flow reactor where the evaporation occurs and finally the particles are collected (Torvela et al., 2011). Studies have found that changing the temperature of the reactor tube had an effect on the particle morphology. Specifically, the particle size increases significantly with increasing reactor temperatures of 160°C due to formation of hollow NPs. This situation has reversed at the temperature above 160°C , further increase in temperature results in decreasing particle size and shows smooth surfaces attributed to the collapse of the hollow structure of the NPs (Eerikäinen et al., 2003). Laaksonen et al. (2011) prepared indomethacin nanosuspensions by wet-bead milling method using Poloxamer 188 as stabilizer. The nanocrystals diluted with aqueous solution of mannitol and leucine were granulated by the aerosol flow reactor. In this way, the fast-dissolving composite microparticles of indomethacin nanocrystals embedded in a mannitol matrix with a leucine-rich coating layer were produced.

6. Application of nanocrystals based pulmonary inhalation system

6.1. Inhalable nanocrystals based aerosol

The inhalable nanocrystals based aerosol (INA) produces aerosol through an inhaler or aerosol inhaler that delivers the drug nanocrystals to the lungs after inhalation administration, as they are highly fine particles, whose distribution is more evenly on the surface of the lungs. The main advantages of INA are that they do not require propellants, are easy to produce, and compounds are not limited by solubility (Chiang et al., 2009), making them more acceptable to patients. Kraft et al. prepared budesonide INA for the treatment of bronchial asthma and tested its pharmacokinetic parameters *in vivo*. These results showed that there was no significant difference in AUC between INA and commercially inhaled budesonide suspension (Pulmicort Respules), but the C_{\max} was 1212 pg/mL and 662 pg/mL, and the T_{\max} was 8.4 min and 14.4 min, respectively, indicating that the INA was more effective (Kraft et al., 2004). Irene et al. prepared coenzyme Q10 INA with an average particle size lower than 100 nm, which overcomes the disadvantage of poor water solubility and has good atomization performance (Rossi et al., 2018).

6.2. Inhalable nanocrystals based composite microparticle (INCM)

Nanocrystals based nanocomposite particle is composed of 'primary particles' and 'secondary particles'. The 'primary particles' are inhalable composite microparticles loaded with nanocrystals particles, which are easily deposited in the lungs and reduce the phagocytosis of function pulmonary macrophages. The nanocrystals as secondary particles are dispersed by rehydration after inhalation. The INCM is composed of biodegradable matrix that is rapidly soluble in lung fluid and releases nanocrystals immediately (Luo et al., 2020). INCM is a promising drug pulmonary delivery system for poorly soluble drug, which can not only overcome the disadvantage of low deposition rate of nanocrystals in the deep lung, but also improve the lung deposition of drug nanocrystals owing to excellent aerodynamic requirements of inhalable microparticles (Abd Elwakil et al. 2018). Pomazi et al. developed INCM using mannitol and other excipients (polymers and L-leucine) as matrix formers, and found that the presence of amino acids L-leucine reduced the adhesion between INCM and thus enhanced the aerosol of INCM (Sou et al., 2011). The results showed that meloxicam INCM was crystalline with a fine particle content greater than 53% and the aerodynamics particle size was less than 3.52 μm . Ninety percent of meloxicam could be dissolved and released within 5 min, indicating its enhanced aerosolization and dissolution performance *in vitro* (Pomázi et al., 2013). Chen et al. (2021) reported a design of novel inhalable nanocrystals embedded microparticles for pulmonary delivery of breviscapine (BVC). The TPGS modified BVC nanocrystals (BVC-NC@TPGS) were fabricated by high pressure homogenization, and further converted into nanocrystals-embedded microparticles (BVC-

NEP) via spray-drying. BVC-NEP with 10% TPGS as stabilizer and 200% Mannitol (MN) as matrix formers presented the highest FPF value and most excellent flowability as well as redispersibility. The *in vivo* pharmacokinetic results illustrated that the $AUC_{(0-\infty)}$ of the inhalable BVC-NEP/MN was 6.29 times ($p < .05$) as high as that of the coarse BVC, and not significantly from that of BVC injection (Table 2).

6.3. Inhalable nanocrystals based adhesive microparticles

Microparticles are susceptible to the clearance by mucus cilia, which affects the efficiency of inhalable drug particles. Inhalable nanocrystals based adhesive microparticles (INAM) can enhance adhesion to the mucus layer by loading the nanocrystals into inhalable polymer materials with the function of mucus adhesion, and avoid being cleared by mucus, thereby prolonging the retention time in the lungs. Liu et al. prepared ultrafine budesonide nanocrystals by wet-milling method and loaded them into hyaluronic acid particles by spray-drying method to prepare an inhalable budesonide INAM. Budesonide INAM hydrates the swelling after lung deposition and then release the drug (Figure 3). After pulmonary administration in rats, the pharmacokinetic results showed that the $AUC_{0-24 \text{ hr}}$, C_{\max} , T_{\max} , and $T_{1/2}$ of budesonide nanocrystals and INAM were 2.85 ± 1.57 and $0.54 \pm 0.37 \mu\text{g} \cdot \text{h} \cdot \text{mL}^{-1}$, 0.62 ± 0.56 and $1.15 \pm 0.60 \text{ h}$, 0.20 ± 0.04 and 0.63 ± 0.35 , 2.56 ± 8.39 and $0.98 \pm 1.11 \text{ h}$, respectively. These results indicated that a significant increase in the bio-availability of budesonide INAM. Meanwhile, the drug clearance rate of INAM was $167.62 \pm 68.34 \text{ mL} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$, which was much lower than the $1184.34 \pm 725.09 \text{ mL} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ of budesonide nanocrystals suspension (Figure 3) (Liu et al., 2018). This can be attributed to the adhesion of hyaluronic acid to mucus, which resists mucociliary clearance by anchoring polymer chains to mucociliary components, thus prolonging the retention time of active substances in the lungs (Rouse et al., 2007). Meanwhile, the highly viscous inhalable nanocrystals based adhesion particles decreased drug release rate and reduced systemic exposure.

6.4. Inhalable mucus-penetrating nanocrystals (IMN)

The treatment of many lung diseases requires that inhalable particles must be able to penetrate the lung mucus and reach the bronchial epithelial cells. Nanocrystals based mucus-penetrating particles are to functionalize the surface of nanocrystals, so that they can quickly penetrate the mucus barrier and be absorbed into the blood. Once the drug is administered to the lungs, undissolved particles may be rapidly cleared by the mucociliary or engulfed by alveolar macrophages. INMP will not be easily captured by the mucosal network structures, thus reducing the possibility of being cleared. Gabriella et al. reported that C109 mucus-penetrating nanocrystals (C109-IMN) modified by tocopherol polyethylene glycol 1000 succinate (TPGS) were prepared to improve the inhalation effect of C109 (an effective but insoluble FtsZ inhibitor) (Figure 4). The results showed that C109-

Table 2. Examples of nanocrystals pulmonary inhalation system.

Nanocrystals lung inhalation delivery system	Drug	Preparation methods	Effect of drug administration
Inhalable nanocrystals based aerosol	Budesonide (Kraft et al., 2004)	Nanometer precipitation method	Fast adsorption, less systemic exposure
	Itraconazole (Rundfeldt et al., 2013)	Wet ball mill method	
	Betamethasone dipropionate (Song et al., 2013)	High pressure homogenization method	
	Fluticasone (Chiang et al., 2009)	Wet ball mill method	
	Coenzyme Q10 (Rossi et al., 2018)	High pressure homogenization method	
Inhalable nanocrystals based composite microparticle	Beclomethasone (Ostrander et al., 1999)	Dielectric grinding method	Deep lung deposition rate and fast dissolution rate
	Meloxicam (Pomázi et al., 2013)	High pressure uniform method, spray drying method	
	Salbutamol sulfate (Bhavna et al., 2009)	Antisolvent precipitation method, spray drying method	
	Tranilast (Onoue et al., 2011)	Wet ball grinding, freeze drying	
	Carvedilol (Abdelbary et al., 2015)	Antisolvent precipitation ultrasonic method, freeze drying	
	Nicosamide (Costabile et al., 2015)	High pressure homogenization method, spray drying method	
	Budesonide + salbutamol sulphate (Raula et al., 2013)	Wet ball milling, aerosol flow reactor	
	Breviscapine (Chen et al., 2021)	homogenization method, spray drying	
	Indometacin (Laaksonen et al., 2011)	Wet ball milling, aerosol flow reactor	
	A ring spore element (Yamasaki et al., 2011)	Antisolvent precipitation method, spray drying method	
	Ciprofloxacin (Cipolla et al., 2016)	Freeze-thaw method	
Rifampicin (Mehanna et al., 2019)	Antisolvent precipitation ultrasonic method, spray drying method		
Inhalable nanocrystals based adhesive microparticles	Budesonide (Liu et al., 2018)	Wet ball grinding, spray drying	Prolonged lung retention and slow drug release
	Resveratrol (Liu et al., 2019)	High pressure homogenization method, spray drying method	
	Cinaciguat (Ni et al., 2017)	High pressure homogenization, spray drying	
Inhalable mucus-penetrating nanocrystals	C109 (Costabile et al., 2020)	Nano precipitation, freeze drying	Easily penetrate mucus and reduce drug clearance

IMN could rapidly penetrate the artificial cystic fibrosis mucus due to the presence of PEG shells modified by TPGS and the optimized aspect ratio (Figure 4) (Costabile et al., 2020). He et al. (2020) fabricated three different sized curcumin nanocrystals (CUR-NC) modified by poloxamer 188 for pulmonary delivery and studied the size effect on the mucus-penetrating process *in vitro*. The results demonstrated that a reduction in the crystal size of hydrophobic drugs could improve plasma concentrations and systemic absorption. The multiple particle tracking experiments revealed that CUR-NC with particle size of 246.16 ± 21.98 nm had larger mean squared displacement during diffusion in simulated mucus. Moreover, CUR-NC also possessed the enhanced cellular uptake and transport efficiency in Calu-3 cells (Figure 5). The improved penetration ability of small nanocrystals could be attributed to the increase in the mucus diffusion ability, cellular uptake and transport in lung epithelium cells.

7. Outlook

Nanocrystals have been widely used in the oral, topical and parenteral route of drug delivery. To date, more than 20 nanocrystal-based formulations are in the market and in clinical trials. Nanocrystals also provide an effective strategy for pulmonary delivery of poorly soluble drugs. Nanocrystal-based inhalation formulations can be applied to the administration of topical as well as systemic disease by immediate

and controlled release of the drugs, which can improve the dissolution rate and saturation solubility of poorly soluble drug and reduce the pulmonary clearance as well as enhance the adsorption of drug. These breakthroughs have inspired the researchers and industry to develop nanocrystal-based inhalation formulation. With the development of nanocrystals technologies for manufacturing drug inhalable formulations, we can predict many nanocrystal-based drug products on market in the near future.

8. Expert opinion

The presence of nanocrystals opens up new prospects for the development of novel pulmonary delivery system. The particle size control, physical instability, potential cytotoxicity, and clearance mechanism of inhaled nanocrystals based formulations are the major considerations in formulation development. To our knowledge, there are no marketed therapeutics related to nanocrystals-based drug inhalable formulations. To take full use of the advantages of nanocrystals for pulmonary drug delivery and develop commercially relevant products, extensive research efforts should be dedicated to the following research aspects in the future:

- Novel formulation strategy that enhances the physical stability of nanocrystals aerosols and dry powder

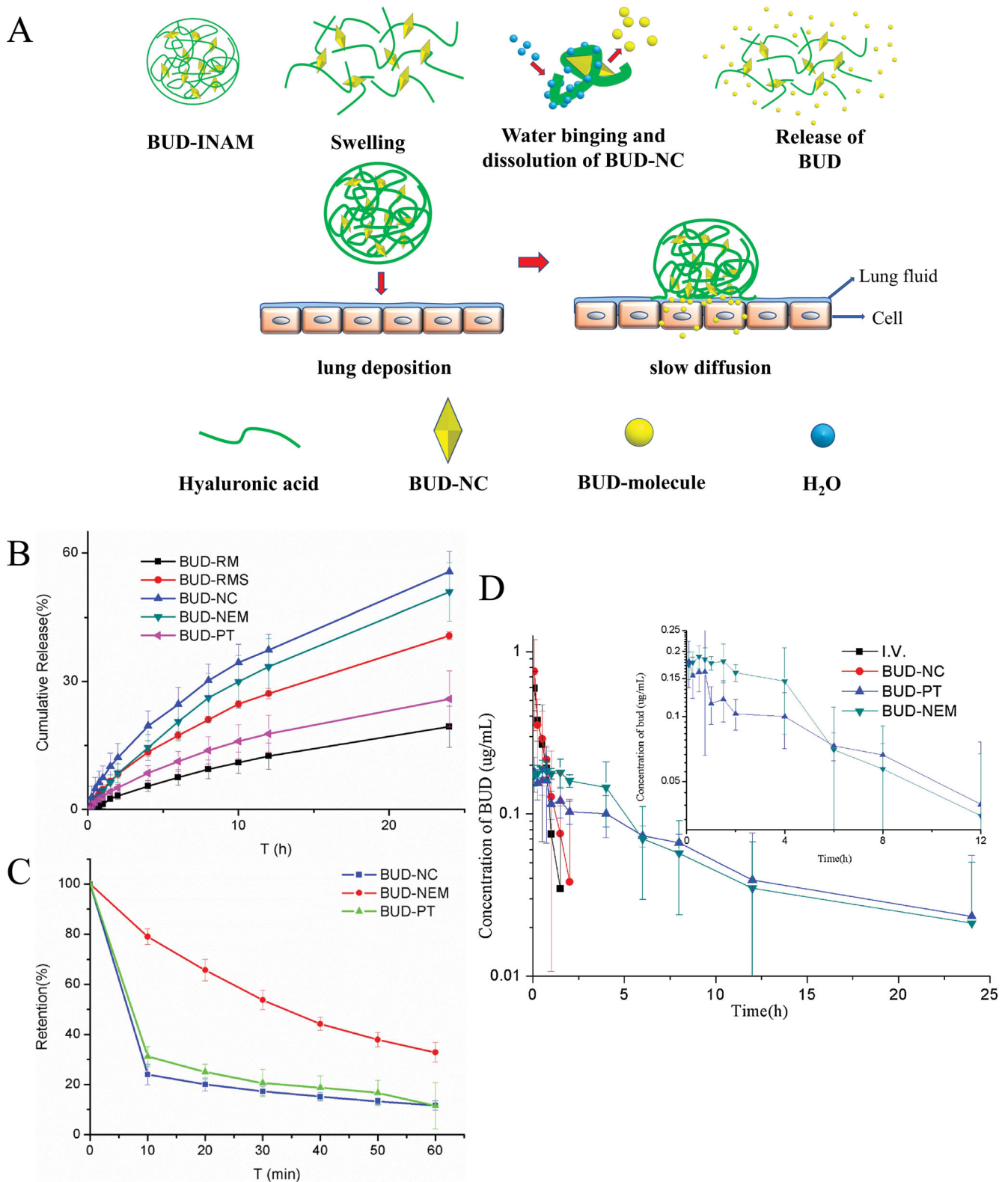
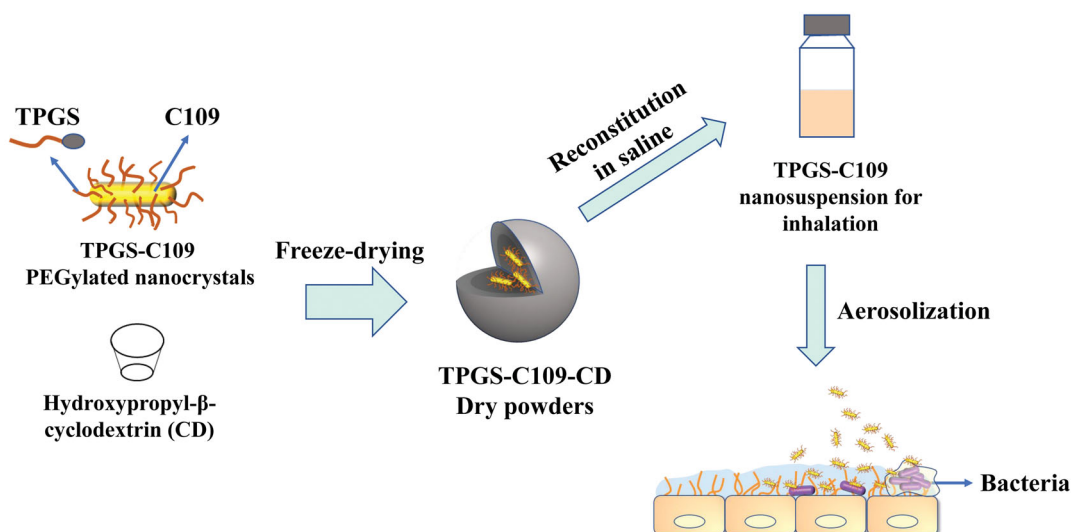


Figure 3. (A) Schematic diagram of BUD-INAM *in vitro* and *in vivo* release of BUD, (B) *in vitro* release profile, (C) retention on the porcine tracheal mucosa surface as a function of time, and (D) plasma concentration–time profiles. Adapted with permission from Liu et al. (2018).

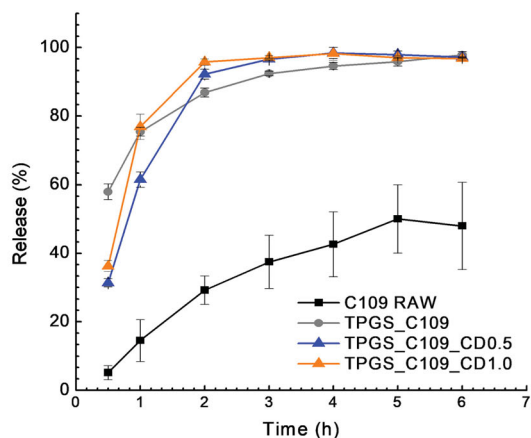
formulation, such as critical processing parameters and suitable matrix formers/carriers.

- The release rate of nanocrystals from carriers/matrix formers or the rate of de-agglomeration in nanocrystals embedded composite particles, and their correlation to the pulmonary pharmacokinetics after inhalation administration.
- The effects of particle size/morphology and surface chemistry on potential cytotoxicity and clearance rate of inhalable nanocrystals.

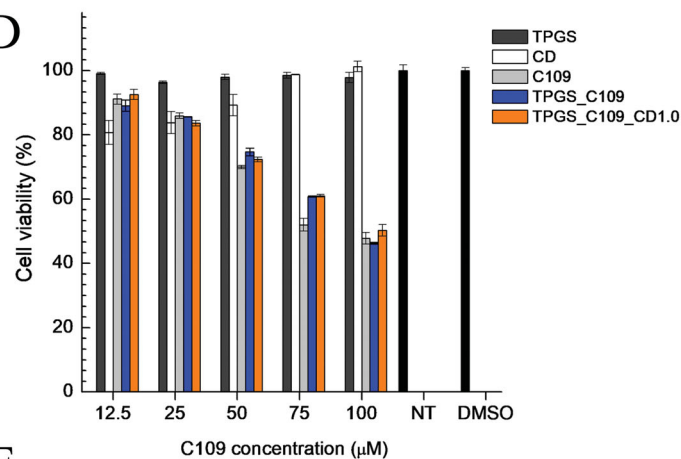
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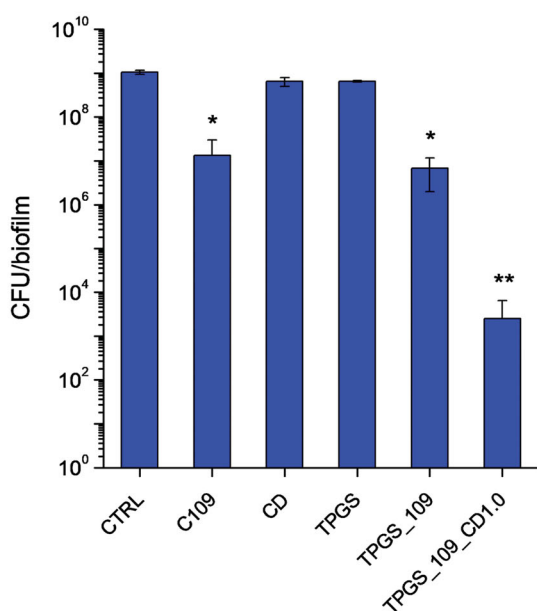
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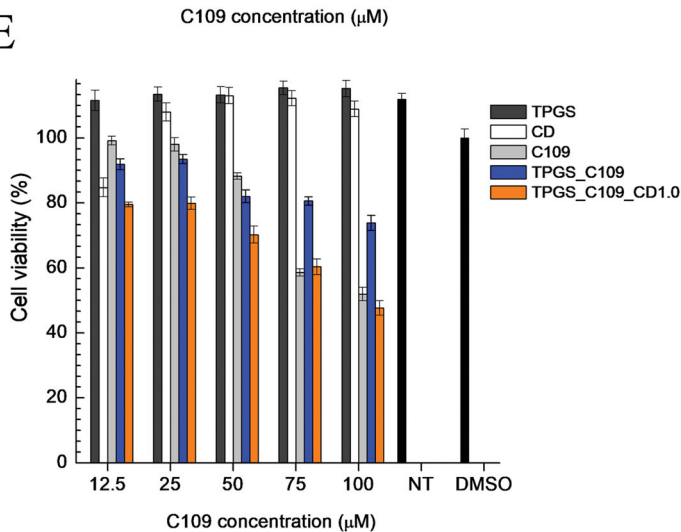


Figure 4. (A) Schematic diagram of PEGylated mucus-penetrating nanocrystals and lung treatment *in vivo*, (B) *in vitro* release profile, (C) inhibition ability *B. cenocepacia* J2315 biofilm, cytotoxicity of C109 formulations to wild type 16HBE (D) and CF (CFBE41o-) bronchial epithelial cells (E). Adapted with permission from Costabile et al. (2020).

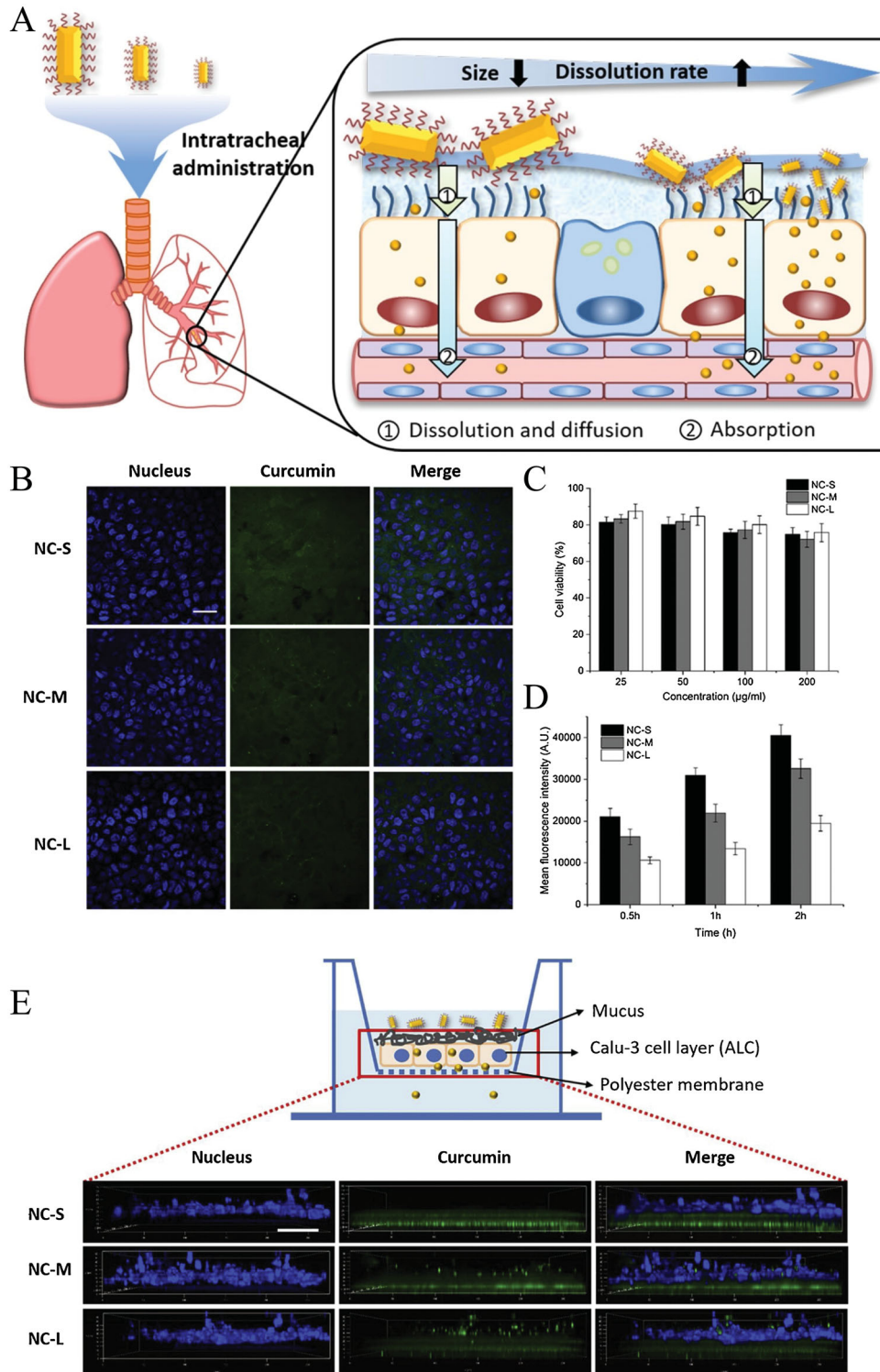


Figure 5. (A) Schematic illustration of curcumin nanocrystals on dissolution, airway mucosa penetration, lung tissue distribution, and absorption by pulmonary delivery. (B) Confocal microscopy images of Calu-3 cells after incubation with CUR-NCs for 2 h (the scale bar is 50 μm); (C) Calu-3 cell viability upon exposure to NC formulations at different drug concentrations; (D) mean fluorescence intensity of cells determined by flow cytometry ($n = 3$); (E) z-stack confocal images of the Calu-3 cell layer with the polyester membrane after transport of NCs formulations for 1 h (the scale bar in xy plane was 50 μm). Adapted with permission from He et al. (2020).

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