**Review article**

# Inhaled nano- and microparticles for drug delivery

Ibrahim M. El-Sherbiny[1],*, Nancy M. El-Baz[1], Magdi H. Yacoub[2]

[1]Zewail City of Science and Technology, Center for Materials Science, 6th October City, 12588 Giza, Egypt
[2]Harefield Heart Science Centre, National Heart and Lung Institute, Imperial College, London, UK

*Email: ielsherbiny@zewailcity.edu.eg

## ABSTRACT

The 21st century has seen a paradigm shift to inhaled therapy, for both systemic and local drug delivery, due to the lung's favourable properties of a large surface area and high permeability. Pulmonary drug delivery possesses many advantages, including non-invasive route of administration, low metabolic activity, control environment for systemic absorption and avoids first bypass metabolism. However, because the lung is one of the major ports of entry, it has multiple clearance mechanisms, which prevent foreign particles from entering the body. Although these clearance mechanisms maintain the sterility of the lung, clearance mechanisms can also act as barriers to the therapeutic effectiveness of inhaled drugs. This effectiveness is also influenced by the deposition site and delivered dose. Particulate-based drug delivery systems have emerged as an innovative and promising alternative to conventional inhaled drugs to circumvent pulmonary clearance mechanisms and provide enhanced therapeutic efficiency and controlled drug release. The principle of multiple pulmonary clearance mechanisms is reviewed, including mucociliary, alveolar macrophages, absorptive, and metabolic degradation. This review also discusses the current approaches and formulations developed to achieve optimal pulmonary drug delivery systems.

*Keywords:* pulmonary delivery, pulmonary clearance mechanisms, bioavailability, particulate-based drug delivery system, liposomes, solid lipid nanoparticles, micelles, polymeric micro/nanoparticles

## INTRODUCTION

Inhalation therapy has a long and rich history in the treatment of different respiratory diseases using various natural inhalation remedies such as leaves from plants, vapors from aromatic plants, balsams, and myrrh. Demand for developing tailor-made inhalable drug formulations increased, which, coupled with advances in biology and engineering, have lead to a more optimized therapeutic efficiency[1].

The pulmonary route of administration has gained a great deal of attention since the early 1990s as an alternative to the parenteral route. At the beginning of 19th century, liquid nebulizers had been developed and used as a legitimate inhalable pharmaceutical therapy. Examples of nebulizer drugs that had been developed and investigated at this time were adrenaline, porcine insulin, penicillin and steroids.

The invention of liquid nebulizers paved the way for the development of various types of inhaler devices. In 1956, the pressured metered dose inhaler (pMDI) was introduced and became the main therapy for asthma. Despite advances in drug formulation technology, the majority of the inhalable aerosol therapeutics suffer from limitations such as short half-life, and low bioavailability, resulting in the necessity of increasingly frequent dosing. Consequently, there was an urgent need for an effective inhalation therapy which could overcome these limitations and provide sustained therapeutic effect. To formulate an effective inhalation therapy, the anatomy of the respiratory system, lung deposition mechanisms and lung defense mechanisms should be fully understood[2,4].

The respiratory system is divided into two main parts (Figure 1): the upper respiratory tract consisting of nose, nasal cavity and pharynx and the lower respiratory tract consisting of larynx, trachea, bronchi, alveoli, and lungs. Lungs are responsible for gas exchange throughout the body. Healthy lungs inhale about 1 pint of air about 12-15 times every minute. The lungs are composed of five lobes; right lung contains three lobes, while the left lung contains two lobes. The interior of the lung is comprised of bronchi, alveoli, blood vessels and lymph nodes. The bronchi are divided into bronchioles which branch in the lung, forming passageways for air, and terminate with the alveoli, which is responsible for gas exchange[5,6].
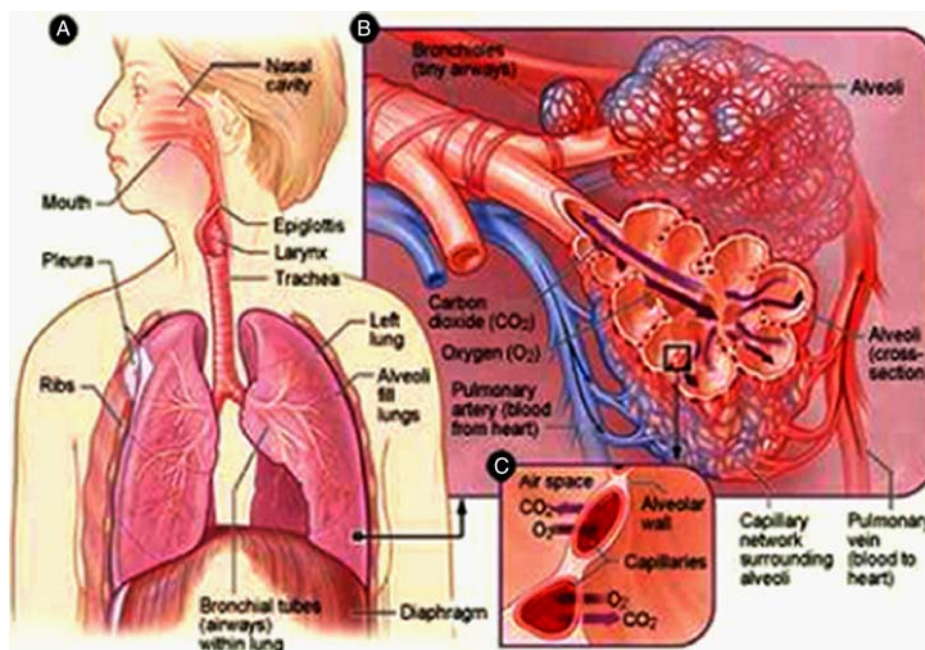


**Figure 1. Anatomy of respiratory system, A) shows different structures of the respiratory system. B) shows airways, alveoli, blood vessels and capillaries. C) shows gas exchange between alveoli and capillaries[8].**

There are over 300 million alveoli in the lung, and each alveolus is lined with pulmonary capillaries forming a huge network comprising over 280 billion capillaries, which provide a huge surface area of about 70 m² available as a blood gas barrier. The alveolar gas exchange mainly occurred at the interface consisting of alveolar epithelium, endothelium and interstitial cell layers, where the distance between the capillaries and alveolar is very small, about 0.5 $\mu$m, and thus facilitating gas exchange via diffusion.

The alveoli are coated with a layer of fluids and mucus, mainly composed of phospholipids and surface proteins, which reduce the surface tension and are important for the proper functioning of gas exchange. The lower respiratory passages are lined by a thin layer of connective tissue, surrounded with different cells such as fibroblasts, nerves, macrophages and lymph vessels. Due to the uniqueness of lung anatomy, it becomes a very appealing target for drug delivery of pulmonary and lymphatic system, due to its large absorptive surface area, large absorptive mucosal membrane, and high vascularity, forming a non-invasive route of drug administration[6,8].

## CHALLENGES FOR PULMONARY DRUG DELIVERY

The vast majority of current inhalation therapies suffer from a short half-life and low drug bioavailability at the targeted site, resulting in a suboptimal therapeutic effect and severe side effects. The short half-life and low drug bioavailability of inhaled drugs are due to three main clearance mechanisms (1) pulmonary clearance includes mucociliary clearance and alveolar macrophages, (2) enzymatic degradation, and (3) rapid systemic adsorption[8,10].

### 1- Pulmonary clearance mechanisms

The primary function of the respiratory defense mechanism is to prevent foreign particles from entering the respiratory system and to maintain it healthy and sterile. Similar to foreign particles, when aerosol particles are administrated, the respiratory system eliminates the aerosol particles to avoid their interaction with the lung cells, leading to therapy failure. Clearance mechanism of inhaled particles is dependent on the deposition site within the lungs. For example, particles deposited in the tracheobronchial tree are rapidly eliminated by mucociliary escalator, while particles deposited in the lower alveolar region are cleared by macrophages[9].

### A- Mucociliary clearance

Mucociliary clearance is the primary defense mechanism in the upper respiratory tract. The upper airways are lined with epithelial cells consisting of two layers: ciliated cells and goblet (mucus-producing) cells, which both are known as a mucociliary escalator. The ciliated cells are covered with airway surface liquid (ASL) and is composed of two layers; mucus layer and periciliary layer (PCL). The PCL layer provides a desirable liquid environment which facilities the cilia displacement toward mucus clearance from the lung to mouth. The principle of mucociliary escalator includes entrapment of the foreign/inhalable particles in mucus layer before moving to the lower respiratory regions and then propelled along with mucus out of trachea either by coughing or swallowing[10,11]. The mucociliary escalator eliminates the majority of the inhaled particles of sizes greater than 6 $\mu$m. On the contrary, smaller particles escape the mucociliary escalator because they rapidly reach the epithelium and preferentially deposit in the alveolar region, where they dissolve or retain for long time span in the lungs. The mucociliary escalator provides an effective clearance owing to its ability to balance between the function of ciliated cells and mucus-producing cells. However, during lung infection or inflammation, the balance of mucociliary escalator disturbs resulting in accelerating clearance of the inhaled drug and in turn reduces its retention time and effectiveness[11,12].

### B- Alveolar macrophage clearance

Beside the mucociliary escalator clearance, in the deep lung, such as alveoli, there is another powerful clearance mechanism - the alveolar macrophages. The alveolar macrophages are phagocytic cells derived from monocytes and are present in large numbers in the lungs. Each alveolus is typically cleaned by 12-14 alveolar macrophages to keep it free from any foreign particles. Owing to the presence of alveolar macrophages, the half-life of inhalable drugs within the alveoli cannot exceed a few hours, which in turn results in an increasing dose frequency. It was also reported that alveolar macrophages engulf particles of sizes ranged between 1.5-3 $\mu$m[9]. This size-discriminating property has been used as a basis for formulating inhalable drugs, which can escape the alveolar macrophages and provide a controlled drug release in the deep lung. Although the alveolar macrophage clearance seems to be understood, the exact mechanism behind particle uptake, transport, and clearance in the alveolar epithelium is still unknown[12–14].

### 2- Enzymatic degradation

Inhaled drugs are also highly susceptible to enzymatic degradation in the lung, resulting in a suboptimal therapy. The primary detoxification enzyme in the lung are the cytochrome P450 (CYP)

families, which provide a line of defense against ingested or inhaled xenobiotics. There are several CYP isoforms expressed in the lungs[15], which are able to degrade a broad spectrum of chemically-different inhalable drugs, pollutant, toxicants, etc. Several inhaled drugs such as budesonide, ciclesonide, salmeterol, and theophylline are enzymatically degraded in the lung. In addition, peptide/proteins drugs such as insulin are highly vulnerable to peptidase and proteases enzymes present in lung. Although the lung has a low metabolic activity, compared to other organs such as the liver, enzymatic degradation significantly influences a drug's bioavailability at the lung, and hence should be carefully assessed during drug formulation[5,12].

### 3- Rapid systemic absorption

Another significant challenge facing inhalation therapeutics is their rapid systemic absorption from the lung. The rapid systemic absorption is ascribed to the lung's large surface area, good epithelial permeability and high vascularity, as well as the highly dispersed nature of therapeutic aerosols. The optimal absorption of inhalable aerosol relies on their site of actions, either locally or systemically. Therefore, to achieve an ideal local effect, an inhaled drug must be absorbed and terminated in the lung, whilst any systemic absorption results in a rapid elimination of the drug and adverse side effects.

On the other hand, the systemic effect is achieved when the inhaled drug is systemically absorbed from the lung into blood stream. The air-to-blood transfer of inhalable drugs often starts with the interaction between the drug and the surfactant after deposition on the mucosa of tracheobronchial airways or alveolar region. This interaction is greatly influenced by the drug's nature, and determines whether the drug is absorbed or eliminated. For instance, the contact between peptide drugs and lung surfactant causes particle aggregations, which in turn compromise their dissolution and accelerate their clearance via alveolar macrophage[12] (Figure 2).

On the contrary, the contact between small lipophilic drugs (i.e. glucocorticosteroids) and lung surfactant improves their solubility and increases their rate and extent of absorption[13,14]. Immediately below the lung surfactant layer, there is a 0.01-10 $\mu$m thick lining layer where the drug can diffuse to the epithelium, followed by interstitum and eventually diffuse to blood stream[16]. The mechanisms of systemic drug absorption include passive and active transport mechanisms such as paracellular or transcellular transport pore formation, vesicular transport and lymphatic drainage. The mechanisms of drug absorption across the epithelium are highly dependent on the drug nature, molecular weight and the targeted site[5,17].

Studies reported that small lipophilic and hydrophobic drugs are comprehensively absorbed within 1-2 minutes from the lung into the systemic circulation via passive diffusion, while small hydrophilic drugs are absorbed within 65 minutes, through the tight junction. Studies also found that hydrophilic and highly cationic small molecules exhibit a prolonged absorption[5,11,17−20]. Beyond these facts, the systemic adsorption of some drugs such as peptide drugs is still unclear. However, some speculated mechanisms stated that the absorption of peptide drugs take place either via transcytosis through caveoli or paracellularly through the tight junctions. The majority of researchers suggest that absorption through the tight junction is the predominant mechanism for peptide drugs. Consequently, these findings claim that drug absorption site and mechanism should certainly be considered while designing inhalation therapy in order to achieve an optimum lung-tissue retention and permeability[6,9,19−22].
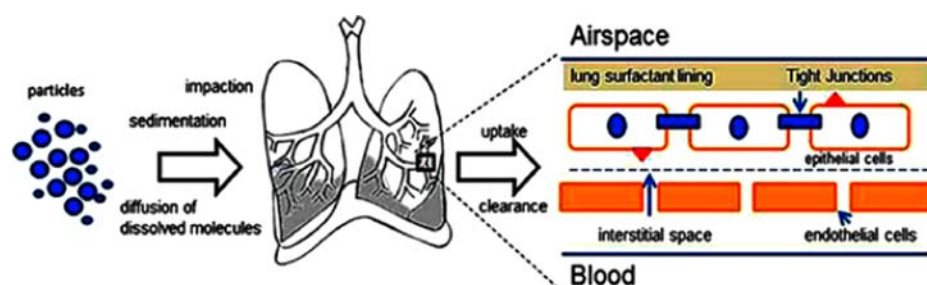


**Figure 2.** The deposition mechanism and uptake of particles in the lungs along with different cell types[6].

### SIGNIFICANCE OF PARTICULATE-BASED PULMONARY DRUG DELIVERY

As a result of the strong pulmonary clearance mechanisms and rapid systemic absorption, inhaled drugs exhibit low bioavailability at the lungs. The low drug bioavailability at the lung represents the

main obstacle toward formulating inhaled drugs with high therapeutic efficiency and sustained drug release. Since drug bioavailability at the targeted site (lung) is considered the key factor for optimal therapy, as it determines whether a drug causes a complete treatment or a partial treatment with high toxicity, several approaches have been developed to overcome the rapid drug absorption and prolong its half-life[5]. Among different approaches, particulate-based drug delivery systems which rely on using carriers, which encapsulate the inhaled drug, seem to be advantageous for pulmonary delivery over other approaches. The advantages of particulate-based drug delivery include (1) protect the drug from enzymatic degradation, (2) evade pulmonary clearance, (3) slow the drug absorption, (4) deliver the drug to targeted site at the lungs, (5) provide a controlled drug release, (6) reduce dose frequency, (7) maximize the therapeutic efficiency, and (8) minimize adverse side effects.

To achieve such inhalation therapeutic formula, several factors such as aerodynamic diameters, shape and surface properties of carriers should be tailored and optimized. Researchers from multidisciplinary fields such as chemistry, biology, toxicology, and biomaterials science heavily studied and investigated these factors in an attempt to identify the optimal parameters for developing an effective particulate-based pulmonary drug delivery system[5,6,21–23].

**FACTORS INFLUENCING PULMONARY DRUG DEPOSITION AND BIOAVAILABILITY**

**1-  Particle size**

In an attempt to evade both clearance mechanisms and simultaneously provide an effective therapy, several studies were conducted to identify the factors affecting drug bioavailability and deposition. It was found that modulating the particle size of an aerosol could deliver the drug to its targeted site and evade pulmonary clearance. Aerosols, inhalable suspensions composed of solid or liquid particles in a gas, are a form of particulate matter (PM). PM is referred to chemically heterogeneous disperse liquid droplets or solid particles, within the micro-scale (Figure 3). The size of PM in an aerosol can range from 0.001 to more than 100 microns. The aerosol particles can be divided into (1) ultrafine particles (less than 0.1 μm), (2) fine particles (0.1-2 μm), and (3) coarse particles (greater than 2 μm)[5].

The size of PM in aerosols plays a pivotal role in determining their deposition site and mechanism at the respiratory system. The mechanisms of respiratory deposition are classified based on particles sizes into different mechanisms, namely diffusion, sedimentation, impaction, and interception[5,9].

Particles with sizes smaller than 0.5 μm are deposited in the alveoli by diffusion mechanism. Diffusion is defined as the movement of particles from a region of high concentration to a region of low concentration owing to Brownian motion. Brownian motion is a random wiggling motion of particles due to the constant bombardment of air molecules. However, the majority of the particles are exhaled owing to their small size (<0.5 μm)[5,22–24].

Sedimentation is another deposition mechanism, which plays a significant role in the setting out particles of aerodynamic size between 1-5 μm in the smaller airways of bronchioles and alveoli where
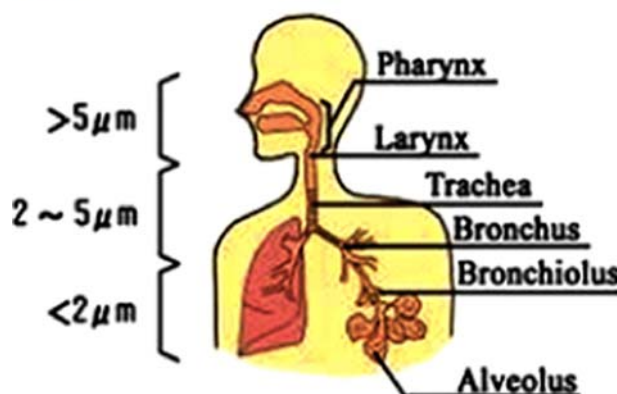


**Figure 3. Particle size determines the particles deposition site at the lung. Adapted from reference [79].**

the airflow is low. Sedimentation is governed by gravitational forces, particle velocity and aerodynamic size. Moreover, sedimentation is influenced by breathing pattern, where slow breathing provides a long time span for particles to slowly deposit. It was also speculated that sedimentation is main deposition mechanism of hygroscopic particles, particles which grow in size after passing through warm and humid air passages. For example, nanoparticles released from the aerosol bypass the warm humid airways aggregating and forming particles of micro-sizes, which allow their deposit in the bronchiolar region[24,25].

Particles with sizes greater than 5 $\mu$m are deposited at the bronchial regions by impaction. Impaction is highly dependent on aerodynamic diameter and mass. Impaction is the common deposition mechanism for dry powder inhalation (DPI) and metered dose inhalators (MDI)[5].

Interception is based on the physical size and shape of particles contacts the airway surface. In contrast to impaction, particles deposited by interception did not deviate from their air streamlines[26]. Interception commonly occurred at the small airways or when the air streamlines near to an airway wall. Interception is responsible for fiber deposition at the smallest airways owing to their small aerodynamic diameters relative to their sizes[6,24].

Despite the tunable size of inhaled particles, which permits particles to deposit at the targeted site in the respiratory system, lung clearance mechanisms represent the main obstacle facing inhalation therapy. It was also revealed that the particle's aerodynamic diameter, a diameter of sphere with density of 1 g/cm$^3$ with settling velocity similar to irregular particles, is the key factor, which can be manipulated to circumvent mucociliary and macrophage clearance mechanisms. The main challenge facing inhaled therapeutics is the mucociliary clearance and alveoli clearance, which exaggerate during lung diseases resulting in therapy failure. Studies reported that the particles with aerodynamic sizes between 1-5 $\mu$m could escape the mucociliary clearance and deposit in the lower airways. This aerodynamic size range is currently employed in many commercial inhalation aerosols commonly used for the treatment of asthma, such as terbutaline and salbutmol aerosols with aerodynamic diameter of 1.8-2.8 $\mu$m. Usami et al. recently formulated mono-dispersed aerosols, which have proven great preferential deposition ability in the deep lung. Although particles of aerodynamic sizes between 1-5 $\mu$m are able to escape the mucociliary clearance and deposit in the deeper lungs, it was found that they are rapidly eliminated by alveoli macrophage[27,28].

A particulate-based drug delivery system is a promising alternative approach, which depends on modulating the aerodynamic diameter of drug carriers to circumvent the alveoli macrophage and simultaneously deliver and release the drug into the deep lungs. One successful example of particulate-based drug delivery system includes the use of large porous microparticles (LPPs) with size greater than 5 $\mu$m, but with a density of about 0.1 g/L or less. The LPPs showed an appealing ability to escape macrophage uptake and deposit homogeneously at the deep lungs. Nanoparticles (NPs) have also been used as a drug carriers for overcoming mucociliary clearance and alveolar macrophages. Studies showed that NPs can deposit at the lining fluid and escape both mucociliary clearance and alveolar macrophages[29,30]. Although NPs seems to be advantageous for pulmonary delivery, their small sizes mean they are predominantly exhaled after inhalation. In order to overcome such an issue, a new particulate-based pulmonary drug delivery system has emerged based on encapsulating drug-loaded NPs in microparticles, known as "Trojan" particles. Trojan particles have shown a great ability in delivering and releasing NPs in the peripheral airways, hence evading the pulmonary clearance and offering a sustained drug release. Trojan particles are typically produced by spray drying of NPs, followed by their assembly into hollow microparticles of low density ($<$ 0.1 g/L)[31]. Although increasing the sizes of microparticles decreases their susceptibility to the alveolar macrophages clearance, this solution is restricted because the increase of microparticles' sizes reduces the delivered respirable fraction.

In an attempt to overcome this challenge, a more recent particulate-based pulmonary drug delivery system has been developed by El-Sherbiny et al., relying on smart stimuli-responsive microparticles as carriers for drug-loaded NPs[32,33]. These smart microparticles are swellable, with sizes between 1-5 $\mu$m in the dry state, while in moisture - such as in the warm and humid airways - they absorb water and swell, therefore they can preferentially deposit in the deep lung and escape the macrophage clearance. For instance, El-Sherbiny et al. (Figure 4) developed smart microparticles composed of PEG grafted onto chitosan crosslinked hydrogel and combined with Pluronic F-108 for pulmonary delivery. In-vitro studies showed that the microparticles swell in a controllable manner forming larger particles, which cannot be engulfed by macrophage cells[32]. El-Sherbiny et al. also formulated sodium alginate-based
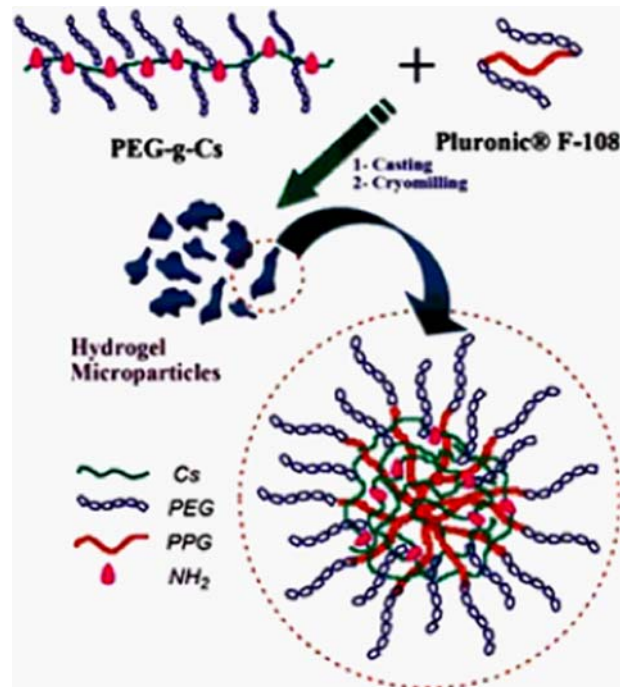
**Figure 4.** Schematic illustration of the smart swellable microparticles[32].

microspheres encapsulating hybrid PEG grafted onto N-phthaloylchitosan and chitosan/hyaluronic acid nanoparticles for deep pulmonary drug delivery. The characterization studies revealed that nanoparticles are well-dispersed and completely encapsulated within the swellable microcarriers[33].

## 2- Particle shape and orientation

In addition to particle size, it was reported that particle shape also influences the alveolar macrophage clearance. Champion and Mitragotri developed non-opsonized and opsonized geometrically IgG anisotropic polystyrene (PS) particles of various sizes and shapes, such as spheres, rectangular disks, elliptical disks and oblate ellipsoids. The in-vitro study showed that the shape and orientation of particles significantly influences the phagocytosis of both non-opsonized and opsonized particles.
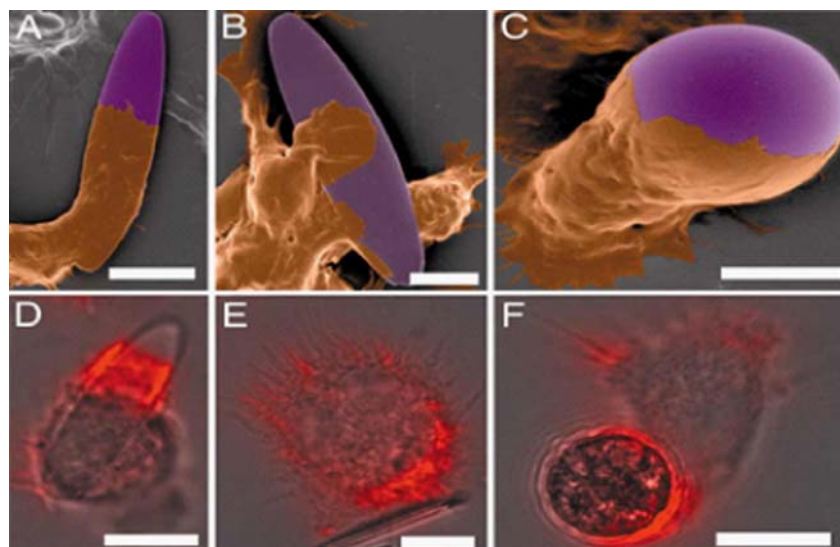


**Figure 5.** Scanning electron micrographs (A-C) and actins staining (D-F) explain the effect of particle shape on macrophages uptake[34].

Scanning electron microscope (SEM) results demonstrated that macrophages exhibit orientation bias, where phagocytosis occurs in less than 6 minutes when macrophages attached to the major axis of elliptical disks. On the contrary, macrophages attached to the minor axis or flat surface of elliptical disks, cannot engulf the particles even after 2 hrs. Similar results have been reported to other shapes including rectangular disks and oblate ellipsoids, but it was not the case for spheres because they are rapidly engulfed by the macrophages regardless the attachment point[34] (Figure 5). The same research group conducted another in-vitro study to evaluate the effect of worm-like particles on macrophages uptake using time-lapse video microscopy and fluorescence microscopy. Spherical and worm-like particles were prepared and tested on rat macrophage cell line. The in-vitro study confirmed that worm-like particles exhibit negligible phagocytosis compared to spherical particles due to low curvature region of worm-like particles. Macrophages engulf the worm-like particles only when attached to the major axis, which exhibits high curvatures, while attachment anywhere along the particles' length inhibits the macrophage engulfment due to the low curvature[35].

### 3- Stealth characteristics
In order to avoid rapid drug absorption, degradation, and evade the pulmonary clearance as well as simultaneously prolong inhalable drug's half-life at the lung, inhaled drugs with stealth characteristics were developed. Stealth characteristics are achieved by attaching or coating the drugs with a stealth material. The stealth material forms a hydration layer over the drug which prevents biofouling and immune recognition[36]. For example, hyaluronic acid (HA), a mucoadhesive polysaccharides present in lung, is combined with inhaled drugs. HA-conjugated drug suppresses the phagocytosis and provides a prolonged drug effect at the lung[37]. Surendra Kumar *et al.* in-vivo evaluated the release of insulin from inhaled HA-based dry powder. The inhaled HA-coated insulin-loaded microparticles demonstrated a prolonged mean residence time and half-life compared to unmodified inhaled insulin[38]. Evora *et al.* also found that peroxidase-loaded PLGA microspheres coated with lipid dipalmitoylphosphatidyl-choline (DPPC) reduces the macrophage uptake of protein peroxidase[39]. Recently, polyethylene glycol (PEG) is being widely used as a coating of micro/nanocarriers for different drug delivery purposes. PEG provides stealth characteristics by forming a hydration layer over the NPs that sterically prevents biofouling and phagocytosis[40]. Niven *et al.* found that PEGylated rhG-CSF possesses a prolonged pulmonary effect and are nontoxic. Nektar Company also uses PEGylated particulate-based drug delivery system to develop long acting inhaled insulin[41,42].

### TYPES OF PARTICULATE-BASED PULMONARY DRUG DELIVERY SYSTEMS
Particulate-based pulmonary drug delivery systems offer great opportunities to formulate local and systemic targeted therapy for various diseases such as respiratory diseases, diabetes, and cancer therapy. Several types of carriers have been used for formulating various particulate-based pulmonary drug delivery systems attempting to optimize drug loading, residence half-life, drug release, toxicity and simultaneously overcome the multiple lung clearance mechanisms, enzymatic degradation, and rapid systemic absorption. Therefore, the selection of drug carriers is of significant importance, but several other factors such as physicochemical properties of the drug, the used inhaled device, targeted site, diseases status, the nature and safety of the carrier should also be considered[24,43].

### LIPOSOMES
Recently, liposomes have become a focus of research in pulmonary drug delivery applications owing to their potential advantages including biocompatibility, biodegradability, sustained drug release, and reduce local irritation. Liposomes are mainly produced from natural or synthetic phospholipids that have either no charge, or net positive/negative charge. The commonly used phospholipids are lecithins, phosphatidylethanolamines, sphingomyelins, phosphatidyl glycerol and phatidylserines[44]. The structure of liposomes consists of an aqueous volume surrounded by a single layer or bilayer of lipid and is capable of encapsulating either hydrophobic or hydrophilic drugs. Liposomal-based formulations can be prepared either as a liquid or in a dry powder form. The deposition site of liposomes and drug release rate are based on liposomes' composition and size, charge, drug/lipid ratio, and method of delivery. Therefore, liposomal-based aerosol formulations can be tailored to deliver the drug to a specific area within the lung by changing its composition or the preparation

method[45]. The first liposomal product in the market is the Alveofact®, which is used the treatment of respiratory distress syndrome[19].

The advances in the development of liposomal-based aerosol formulations with jet nebulizers has opened up new possibilities for effective use of aerosols in the treatment of various pulmonary diseases[5,46]. Studies revealed that liposomal-based aerosol formulations enhanced the drug stability and bioavailability, protected the drug from enzymatic degradation, prolonged the retention half-life, allowed intracellular delivery and over came pulmonary clearance[47]. Nieminen *et al. in-vivo* studied the absorption, drug release, and clearance of liposomes encapsulating cyclosporine A (CsA). Results obtained from this study demonstrated that the retention time of CsA-liposomes was 16.9-fold longer than free CsA in normal lung and 7.5-fold longer in inflamed lung[48]. Arppe *et al.* developed CsA encapsulated in liposomal for pulmonary delivery and compared its retention time with free CsA. It was reported that the retention time of free drug was 17 minutes, while liposomal aerosol was about 4.8 hrs[49]. In another study, formulation of CsA and paclitaxel with PEGylated liposomes provided evidence of an enhanced drug deposition and increased therapeutic index by sustaining the therapeutic action in the lung.

Poyner *et al.* conducted also comparative study between tobramycin-loaded PLA-microparticles and tobramycin-loaded liposomes administrated by endotracheal and intravenous delivery. The renal elimination of the intravenously administrated tobramycin-loaded PLA microparticles was higher than tobramycin-loaded liposomes after 6 and 24 hrs. On the other hand, endotracheal administrated tobramycin-loaded liposomes demonstrated pulmonary level 3-fold higher than free drug after 6 and 24 hrs[50]. Gibbons *et al.* encapsulated leukocyte protease inhibitors (rSLPI) in liposomal for pulmonary delivery to enhance the bioavailability of rSLPI. In fact, the study showed that the bioavailability of rSLPI-liposomes at the lung significantly increased and reached about 92.6%[51]. Shahiwala and Misra compared the bioavailability of intratracheally and orally administrated levonogestrel-liposomal formulation and free drug. The study showed that the intratracheally administrated levonogestrel-liposomal maintained the effective therapeutic concentration in plasma over 6-60 hrs, which can decrease the dose frequency and adverse side effects[52]. PEGylated liposomes have been developed and used for pulmonary drug delivery system to benefit from the stealth effect and achieve a prolonged circulation half-life. Nag *et al.* showed that PEGylated-liposomes exhibited a prolonged circulation time compared to uncoated-liposomes. The *in-vivo* study conducted on rabbits also showed that PEGylated liposomes with sizes of 275 nm exhibita prolonged circulation half-life[13,44,53].

## SOLID-LIPID NANOPARTICLES

Solid-lipid nanoparticles (SLN) are submicron-sized nanocarriers consisting of monolayer phospholipid shell and solid hydrophobic core. SLNs are widely used as carriers for many drugs for both local and systemic delivery[54]. For pulmonary delivery, several studies examined the potential of SLNs using human alveolar epithelial cell line (A549) and murine precision-cut lung slices (PCLS). The study showed that SLNs cause no signs of inflammation and thus confirming the suitability of SLNs as drug carriers for pulmonary delivery[55,56]. SLNs have been also used for pulmonary delivery of insulin and demonstrated high *in-vitro* and *in-vivo* stability of insulin and a prolonged therapeutic effect[57]. In another study, itraconazole (ITZ)-loaded SLNs colloidal dispersion showed a great suitability for nebulization with proper aerodynamic properties for upper and lower lung delivery. The bioavailability of itraconazole (ITZ)-loaded SLNs was increased by reducing the particle size, which increases permeation and dissolution rate[58]. The rate of drug encapsulation within SLNs also depends on many factors which should be considered during preparation such as (1) the drug solubility into lipids, (2) the miscibility of the drugs into lipids, (3) chemical and physical structures of the lipid-solid matrix, and (4) the polymorphous state of the lipids.

PEGylated SLNs have been developed and showed a great potency in enhancing the drug stability and bioavailability at the lung. Ligands-conjugated cationic SLNs have also been developed and *in-vitro* examined on alveolar macrophages. The study demonstrated that ligands-conjugated cationic SLNs exhibit an efficient DNA hybridization through ionic interaction and induce site-specific gene transfection on alveolar macrophages[46]. Other drugs such as prednisolone, diazepam and camptotecin have been encapsulated into SLNs for pulmonary delivery applications[59]. Torjan approach has been also applied for enhancing the pulmonary delivery of SLNs. Microparticles encapsulating thymopentin-loaded SLNs were prepared using co-spray-drying. The prepared microparticles containing drug-loaded SLNs were spherical and showed significant aerosolization efficiency.

The bioavailability and therapeutic effectiveness of the intratracheally administered microparticles containing drug-loaded SLNs found to be significantly higher than free drug[60,61].

## POLYMERIC MICRO/NANOPARTICLES

The polymeric microparticles are spheres with size ranged between 1-1000 $\mu$m, while nanoparticles are spheres with size ranging from 1-1000 nm. Polymeric micro/nanoparticles are mainly produced from natural or synthetic biocompatible and biodegradable polymers such as chitosan, poly(lactic-co-glycolic) acid, poly(lactic) acid, poly(butylcyanoacrylate), and poly(lactic-co-lysine graft lysine)[5,46,62]. The utilization of polymeric micro/nanoparticles offers potential advantages over other carriers such as liposomes, because of their higher stability, higher drug loading capacity, slower drug release and longer pharmacological activity of payloads. Moreover, the properties of polymeric micro/nanoparticles such as morphology, size, and porosity can be easily tailored to meet the requirement of pulmonary delivery[62,63].

Microparticles are frequently used for pulmonary delivery because they preferentially deposit in the deep lung and do not aggregate under shear force. Different drugs such as corticosteroids, insulin, and chemotherapeutics have been formulated within polymeric microparticles[63]. Insulin-PLGA micro-particles have been developed and showed a prolonged residence time extended from 6-48 hrs compared to free insulin[64]. Wang *et al.* developed and examined the effect of docetaxel (DTX)-loaded chitosan microspheres *in-vitro* and *in-vivo*. The prepared microspheres were mono-dispersed and spherical in shape with drug encapsulation efficiency about 88%. The *in-vitro* and *in-vivo* studies showed that DTX was sustainably released, which increases its bioavailability at the lung, while minimizing the systemic toxicity[65].

On the other hand, polymeric nanoparticles-based aerosols have been recently formulated and studied *in-vitro* and *in-vivo*[66]. For example, poly butylcyanoacrylate (PCL) nanoparticles are widely used for pulmonary delivery owing to its safety, biocompatibility and biodegradability. Effervescent dry powder containing ciprofloxacin-loaded PCL nanoparticles have been produced by spray drying. The prepared ciprofloxacin-NPs evolved with aerodynamic diameter of 2.71 $\mu$m and released 56% of ciprofloxacin. Due to the rapid exhalation of nanoparticles, Grenha *et al.* developed chitosan nanoparticles encapsulated into mannitol microspheres and *in-vitro* tested on respiratory epithelium cells. The *In-vitro* study showed that chitosan nanoparticles encapsulated microspheres have good aerodynamic properties, high drug loading efficiency and exhibit high biocompatibility, which confirm their potential for formulating an efficient pulmonary drug delivery system[67].

Studies showed that inhaled anticancer drugs can be efficiently delivered to lung parenchyma, which in turn selectively treat lung cancer and reduce the systemic toxicity. 5-flurouracil has been formulated into lipid-coated nanoparticles and showed a sustained drug release and potent anticancer activity against lung cancer. Recently, nano-in-macro (SIMANIM) particles have been developed and applied for pulmonary delivery of antibodies. These SIMANIM particles were prepared by spray drying of a double-emulsion containing IgG antibodies. The *in-vitro* release of antibodies at pH 2.5 was monitored for 35 days and the activity of the released antibodies was tested using gel electrophoresis and enzyme-linked immunosorbent assay. Both assays confirmed the stability and efficiency of the released antibodies even after 35 days[68].

## MICELLES

Micelles are aggregates of surfactant molecules dispersed in a liquid solution. Polymeric micelles, amphiphilic macromolecules self-assemble to core-shell nanostructure in aqueous solution, are the most common type of micelles used in drug delivery applications because of their better stability, higher tendency of micellization, slower dissolution rate, longer circulation half-life and non-toxicity. The polymeric micelles are similar to the structural and functional properties of biological transport systems such as virus and lipoproteins. The polymeric micelles offer many advantages in drug delivery because of their distinctive structure and physico-chemical properties.

Micelles are composed of a hydrophilic shell which allows them to escape the reticulo-endothelial system and therefore exhibit a prolonged their circulation half-life. The small sizes of micelles (100 – 400 nm) also facilities their cellular uptake[69,70]. The structure of polymeric micelles can also be chemically altered to design ideal delivery carriers that can improve drug stability, control drug release, and provide targeted drug delivery[71].

Jones and Leroux formulated inhaled beclomethasone dipropionate-loaded polymeric micelles within nano-sizes for the treatment of asthma and chromic pulmonary obstructive diseases. This study revealed that the prepared drug-loaded polymeric micelles were able to evade the alveolar macrophages due to their hydrophilic shells, and in turn provide sustained drug release. It was also reported that the drug-loaded polymeric micelles pass through the mucus layer, penetrate the epithelial cells, and eventually reach the inflamed site. This finding indicates that polymeric micelles could be ideal carriers for hydrophobic corticosteroids such as beclomethasone dipropionate, which are used for the treatment of chronic pulmonary obstructive diseases: the main reason behind the failure of chronic pulmonary obstructive therapy is the inability of drug to pass through the mucus layer associated with bronchial inflammatory diseases and reach the inflamed site[5]. Gaber *et al.* also developed beclomethasone dipropionate-loaded poly-(ethyleneoxide)-block-distearoylphosphatidyl-ethanolamine (mPEG-DSPE) polymeric micelles. The prepared drug-loaded mPEG-DSPE micelles showed high drug entrapment efficiency, sustained drug release and high therapeutic efficiency, which reflects the potency of polymeric micelles in delivering the hydrophobic corticosteroids to the inflamed site[5,72].

## CYCLODEXTRINS

Cyclodextrins (CDs) are cyclic polymers of $\alpha$-D-glycopyranose composed of cyclic oligosaccharides. There are three types of CDs; $\alpha$-, $\beta$- and $\gamma$-CDs, the $\beta$-CDs are commonly used for pharmaceutical applications because of their high loading capacity, high complexation efficiency and low production cost. CDs are extensively used as drug carriers for pulmonary delivery due to their unique structure, composed of an outer layer containing hydroxyl groups, and a lipophilic inner cavity, which allows the formation of an inclusion complex with the hydrophobic drugs. The formation of the inclusion complex results in enhancement of the aqueous solubility of the payload drug[46,73]. CDs can also behave as solubilizers in aerosol formulations of lipophilic water-insoluble drugs to decrease the drug irritation following pulmonary delivery[47,74]. Moreover, the CD complexes aid in enhancing the drug delivery through biological barriers without influencing their barrier function. These properties render CDs an ideal penetration enhancer for intranasal drug delivery[74].

An aerosol formulation of itraconazole (ITZ)-loaded on 2-hydroxy-propy-beta-cyclodextrins (HPbetaCDs) has been developed for pulmonary delivery. The pharmacokinetic profile of ITZ-HPbetaCDs was investigated in comparison with colloidal dispersion of ITZ-loaded nanoparticles. This study revealed that ITZ-HPbetaCDs exhibit faster systemic absorption across the lung epithelium compared to ITZ nanoparticles[75]. In another study, nebulized solution of voriconazole complexed-sulfobutyl ether-beta-CDs has been developed for targeted pulmonary drug delivery. It was found that voriconazole complexed-sulfobutyl ether-beta-CDs exhibit fast absorption and high bioavailability after pulmonary administration[76]. CDs are also used in combination with other carriers in order to enhance the drug encapsulation efficiency and provide a controlled drug release. Hydroxypropyl-beta-CDs have been employed for the development of large porous PLGA-loaded insulin. The prepared CD-based PLGA-loaded insulin demonstrated a high bioavailability and prolonged therapeutic effect compared to free insulin[77]. An inhalable dry powder of recombinant human growth hormones solubilized with dimethyl-beta-CD has also been formulated for systemic delivery. The *in-vivo* studies showed that the inhaled dry powder exhibits an excellent aerosol performance and rapid systemic absorption after pulmonary administration to the rat lung[78].

## CONCLUSION

The lung is a very promising target either for local or systemic therapies owing to its large surface area, lower metabolic activity, and the fact that it avoids the first pass metabolism. The main obstacles facing the development of successful pulmonary drug delivery are pulmonary clearance mechanisms, rapid systemic absorption, metabolic degradation and control over drug deposition site and rate. To circumvent these obstacles, particulate-based pulmonary systems were developed based on the use of different carriers such as liposomes, solid lipid nanoparticles, polymeric micro/nanoparticles, micelles, and cyclodextrins. The particulate-based pulmonary systems demonstrated a great potency in enhancing the drug bioavailability and therapeutic effectiveness at the deep lung. Ongoing research focusing on exploring and understanding the complexity of lung will help in identifying many matters such as the molecular basis of pulmonary diseases and the challenges facing pulmonary drug delivery. The knowledge and understanding of these matters could aid in formulating an efficient and safer

pulmonary delivery systems. Therefore, a lot of effort is still needed to minimize the clinical and technical gaps to enable translating these particulate-based pulmonary approaches into commercial pharmaceutical products.

**REFERENCES**

[1] Weers JG, Bell J, Chan H-K, Cipolla D, Dunbar C, Hickey AJ, Smith IJ. Pulmonary formulations: What remains to be done? *Journal of Aerosol Medicine and Pulmonary Drug Delivery*. 2010;23(S2):S-5−S-23.

[2] Courrier H, Butz N, Vandamme TF. Pulmonary drug delivery systems: Recent developments and prospects. *Critical Reviews in Therapeutic Drug Carrier Systems*. 2002;19(4-5).

[3] Ungaro F, d'Angelo I, Miro A, La Rotonda MI, Quaglia F. Engineered PLGA nano-and micro-carriers for pulmonary delivery: Challenges and promises. *Journal of Pharmacy and Pharmacology*. 2012;64(9):1217−1235.

[4] Marieb EN, Hoehn K. *Human anatomy & physiology*. Pearson Education; 2007.

[5] Smola M, Vandamme T, Sokolowski A. Nanocarriers as pulmonary drug delivery systems to treat and to diagnose respiratory and non respiratory diseases. *International Journal of Nanomedicine*. 2008;3(1):1.

[6] Paranjpe M, Müller-Goymann CC. Nanoparticle-mediated pulmonary drug delivery: A review. *International Journal of Molecular Sciences*. 2014;15(4):5852−5873.

[7] McCorry LK. *Essentials of human physiology for pharmacy*. CRC Press; 2004.

[8] The Respiratory System-How the lung works. 2012 [2 July]:1-7; Available from: http://www.nhlbi.nih.gov/health/health-topics/topics/hlw/system.html

[9] El-Sherbiny IM, Villanueva DG, Herrera D, Smyth HD. Overcoming lung clearance mechanisms for controlled release drug delivery. *Controlled pulmonary drug delivery*. Springer; 2011:101−126.

[10] Patton JS, Brain JD, Davies LA, Fiegel J, Gumbleton M, Kim K-J, Sakagami M, Vanbever R, Ehrhardt C. The particle has landed—characterizing the fate of inhaled pharmaceuticals. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*. 2010;23(S2):S-71−S-87.

[11] Hogg J. Response of the lung to inhaled particles. *The Medical Journal of Australia*. 1985;142(13):675−678.

[12] Olsson B, Bondesson E, Borgström L, Edsbäcker S, Eirefelt S, Ekelund K, Ekelund K, Gustavsson L, Hegelund-Myrbäck T. Pulmonary drug metabolism, clearance, and absorption. *Controlled pulmonary drug delivery*. Springer; 2011:21−50.

[13] Oberdörster G. Lung clearance of inhaled insoluble and soluble particles. *Journal of Aerosol Medicine*. 1988;1(4):289−330.

[14] Parkinson A. *Biotransformation of xenobiotics*. New York: McGraw-Hill; 2001.

[15] Wiedmann T, Bhatia R, Wattenberg L. Drug solubilization in lung surfactant. *Journal of Controlled Release*. 2000;65(1):43−47.

[16] Patton JS. Mechanisms of macromolecule absorption by the lungs. *Advanced Drug Delivery Reviews*. 1996;19(1):3−36.

[17] Patton JS, Byron PR. Inhaling medicines: Delivering drugs to the body through the lungs. *Nature Reviews Drug Discovery*. 2007;6(1):67−74.

[18] Liao X, Wiedmann TS. Solubilization of cationic drugs in lung surfactant. *Pharmaceutical Research*. 2003;20(11):1858−1863.

[19] Mansour HM, Rhee Y-S, Wu X. Nanomedicine in pulmonary delivery. *International Journal of Nanomedicine*. 2009;4:299.

[20] Beck-Broichsitter M, Gauss J, Packhaeuser CB, Lahnstein K, Schmehl T, Seeger W, Kissel T, Gessler T. Pulmonary drug delivery with aerosolizable nanoparticles in an ex vivo lung model. *International Journal of Pharmaceutics*. 2009;367(1):169−178.

[21] Labiris N, Dolovich M. Pulmonary drug delivery. Part I: Physiological factors affecting therapeutic effectiveness of aerosolized medications. *British Journal of Clinical Pharmacology*. 2003;56(6):588−599.

[22] Davies C, Muir D. Deposition of inhaled particles in human lungs. *Nature* 1966;211:90−91.

[23] Jain KK. Drug delivery systems-an overview. *Drug delivery systems*. Springer; 2008:1−50.

[24] Thulasiramaraju TV, Tejeswar Kumar B, Nikilesh Babu M. Pulmonary drug delivery systems-an overview. *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*. 2013:16−34.

[25] Yang W, Peters JI, Williams III RO. Inhaled nanoparticles—a current review. *International Journal of Pharmaceutics*. 2008;356(1):239−247.

[26] Boucher R. Regulation of airway surface liquid volume by human airway epithelia. *Pfluegers Archiv*. 2003;445(4):495−498.

[27] Champion JA, Walker A, Mitragotri S. Role of particle size in phagocytosis of polymeric microspheres. *Pharmaceutical Research*. 2008;25(8):1815−1821.

[28] Usmani OS, Biddiscombe MF, Underwood SR, Barnes PJ. Characterization of the generation of radiolabeled monodisperse albuterol particles using the spinning-top aerosol generator. *Journal of Nuclear Medicine*. 2004;45(1):69−73.

[29] Edwards DA, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. *Journal of Applied Physiology*. 1998;85(2):379−385.

[30] Ehrhardt C, Fiegel J, Fuchs S, Abu-Dahab R, Schaefer U, Hanes J, Lehr CM. Drug absorption by the respiratory mucosa: Cell culture models and particulate drug carriers. *Journal of Aerosol Medicine*. 2002;15(2):131−139.

[31] Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards D. Trojan particles: Large porous carriers of nanoparticles for drug delivery. *Proceedings of the National Academy of Sciences*. 2002;99(19):12001−12005.

[32] El-Sherbiny IM, McGill S, Smyth HD. Swellable microparticles as carriers for sustained pulmonary drug delivery. *Journal of Pharmaceutical Sciences*. 2010;99(5):2343−2356.

[33] El-Sherbiny IM, Smyth HD. Biodegradable nano-micro carrier systems for sustained pulmonary drug delivery:(I) Self-assembled nanoparticles encapsulated in respirable/swellable semi-IPN microspheres. *International Journal of Pharmaceutics*. 2010;395(1):132−141.

[34] Champion JA, Mitragotri S. Role of target geometry in phagocytosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(13):4930–4934.

[35] Champion JA, Mitragotri S. Shape induced inhibition of phagocytosis of polymer particles. *Pharmaceutical Research*. 2009;26(1):244–249.

[36] Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. *Nature Reviews Drug Discovery*. 2003;2(3):214–221.

[37] Suzuki Y, Yamaguchi T. Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release. *Agents and Actions*. 1993;38(1):32–37.

[38] Surendrakumar K, Martyn G, Hodgers E, Jansen M, Blair J. Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs. *Journal of Controlled Release*. 2003;91(3):385–394.

[39] Evora C, Soriano I, Rogers RA, Shakesheff KM, Hanes J, Langer R. Relating the phagocytosis of microparticles by alveolar macrophages to surface chemistry: The effect of 1, 2-dipalmitoylphosphatidylcholine. *Journal of Controlled Release*. 1998;51(2):143–152.

[40] Hickey AJ. *Pharmaceutical inhalation aerosol technology*. CRC Press; 2003.

[41] Niven RW, Whitcomb KL, Shaner L, Ip AY, Kinstler OB. The pulmonary absorption of aerosolized and intratracheally instilled rhG-CSF and monoPEGylated rhG-CSF. *Pharmaceutical Research*. 1995;12(9):1343–1349.

[42] Meenach SA, Vogt FG, Anderson KW, Hilt JZ, McGarry RC, Mansour HM. Design, physicochemical characterization, and optimization of organic solution advanced spray-dried inhalable dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine poly (ethylene glycol)(DPPE-PEG) microparticles and nanoparticles for targeted respiratory nanomedicine delivery as dry powder inhalation aerosols. *International Journal of Nanomedicine*. 2013;8:275.

[43] Jaafar-Maalej C, Elaissari A, Fessi H. Lipid-based carriers: Manufacturing and applications for pulmonary route. *Expert Opinion on Drug Delivery*. 2012;9(9):1111–1127.

[44] Lasic DD. *Liposomes in gene delivery*. CRC press; 1997.

[45] Bai S, Gupta V, Ahsan F. Cationic liposomes as carriers for aerosolized formulations of an anionic drug: Safety and efficacy study. *European Journal of Pharmaceutical Sciences*. 2009;38(2):165–171.

[46] Kaur G, Narang R, Rath G, Goyal AK. Advances in pulmonary delivery of nanoparticles. *Artificial Cells, Blood Substitutes and Biotechnology*. 2012;40(1-2):75–96.

[47] Schreier H, Gonzalez-Rothi RJ, Stecenko AA. Pulmonary delivery of liposomes. *Journal of Controlled Release*. 1993;24(1):209–223.

[48] Saari SM, Vidgren MT, Koskinen MO, Turjanmaa ViM, Waldrep JC, Nieminen MM. Regional lung deposition and clearance of 99mTc-labeled beclomethasone-DLPC liposomes in mild and severe asthma. *CHEST Journal*. 1998;113(6):1573–1579.

[49] Arppe J, Vidgren M, Waldrep J. Pulmonary pharmacokinetics of cyclosporin A liposomes. *International Journal of Pharmaceutics*. 1998;161(2):205–214.

[50] Poyner E, Alpar H, Almeida A, Gamble M, Brown M. A comparative study on the pulmonary delivery of tobramycin encapsulated into liposomes and PLA microspheres following intravenous and endotracheal delivery. *Journal of Controlled Release*. 1995;35(1):41–48.

[51] Gibbons AM, McElvaney NG, Taggart CC, Cryan S-A. Delivery of rSLPI in a liposomal carrier for inhalation provides protection against cathepsin L degradation. *Journal of Microencapsulation*. 2009;26(6):513–522.

[52] Shahiwala A, Misra A. Pulmonary absorption of liposomal levonorgestrel. *AAPS PharmSciTech*. 2004;5(1):96–100.

[53] Nag OK, Awasthi V. Surface engineering of liposomes for stealth behavior. *Pharmaceutics*. 2013;5(4):542–569.

[54] Beck-Broichsitter M, Ruppert C, Schmehl T, Guenther A, Betz T, Bakowsky U, Seeger W, Kissel T, Gessler T. Biophysical investigation of pulmonary surfactant surface properties upon contact with polymeric nanoparticles in vitro. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2011;7(3):341–350.

[55] Nassimi M, Schleh C, Lauenstein H, Hussein R, Hoymann H, Koch W, Pohlmann G, Krug N, Sewald K, Rittinghausen S, Braun A, Müller-Goymann C. A toxicological evaluation of inhaled solid lipid nanoparticles used as a potential drug delivery system for the lung. *European Journal of Pharmaceutics and Biopharmaceutics*. 2010;75(2):107–116.

[56] Nassimi M, Schleh C, Lauenstein H-D, Hussein R, Lübbers K, Pohlmann G, Switalla S, Sewald K, Müller M, Krug N, Müller-Goymann CC, Braun A. Low cytotoxicity of solid lipid nanoparticles in in vitro and ex vivo lung models. *Inhalation Toxicology*. 2009;21(S1):104–109.

[57] Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z. Solid lipid nanoparticles for pulmonary delivery of insulin. *International Journal of Pharmaceutics*. 2008;356(1):333–344.

[58] Yang W, Tam J, Miller DA, Zhou J, McConville JT, Johnston KP, Williams RO 3rd. High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers. *International Journal of Pharmaceutics*. 2008;361(1):177–188.

[59] MuÈller RH, MaÈder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*. 2000;50(1):161–177.

[60] Li Y-Z, Sun X, Gong T, Liu J, Zuo J, Zhang Z-R. Inhalable microparticles as carriers for pulmonary delivery of thymopentin-loaded solid lipid nanoparticles. *Pharmaceutical Research*. 2010;27(9):1977–1986.

[61] Weber S, Zimmer A, Pardeike J. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for pulmonary application: A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*. 2014;86(1):7–22.

[62] Illum L, Watts PJ. Polysaccharide microspheres for the pulmonary delivery of drugs. Google Patents 1997.

[63] Feng S-S, Chien S. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. *Chemical Engineering Science*. 2003;58(18):4087–4114.

[64] Williams III RO, Barron MK, José Alonso M, Remuñán-López C. Investigation of a pMDI system containing chitosan microspheres and P134a. *International Journal of Pharmaceutics*. 1998;174(1):209–222.

[65] Wang H, Xu Y, Zhou X. Docetaxel-loaded chitosan microspheres as a lung targeted drug delivery system: In vitro and in vivo evaluation. *International Journal of Molecular Sciences*. 2014;15(3):3519–3532.

[66] Doan T, Olivier J. Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization. *International Journal of Pharmaceutics*. 2009;382(1):61–66.

[67] Grenha A, Grainger CI, Dailey LA, Seijo B, Martin GP, Remuñán-López C, Forbes B. Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro. *European Journal of Pharmaceutical Sciences*. 2007;31(2):73–84.

[68] Kaye RS, Purewal TS, Alpar HO. Simultaneously manufactured nano-in-micro (SIMANIM) particles for dry-powder modified-release delivery of antibodies. *Journal of Pharmaceutical Sciences*. 2009;98(11):4055–4068.

[69] Marsh D, Bartucci R, Sportelli L. Lipid membranes with grafted polymers: Physicochemical aspects. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2003;1615(1):33–59.

[70] Davidsen J, Vermehren C, Frokjaer S, Mouritsen OG, Jørgensen K. Drug delivery by phospholipase A$_2$ degradable liposomes. *International Journal of Pharmaceutics*. 2001;214(1):67–69.

[71] Lavasanifar A, Samuel J, Kwon GS. Poly (ethylene oxide)-block-poly (l-amino acid) micelles for drug delivery. *Advanced Drug Delivery Reviews*. 2002;54(2):169–190.

[72] Gaber NN, Darwis Y, Peh K-K, Tan YT-F. Characterization of polymeric micelles for pulmonary delivery of beclomethasone dipropionate. *Journal of Nanoscience and Nanotechnology*. 2006;6(9-10):3095–3101.

[73] Duchêne D, Ponchel G, Wouessidjewe D. Cyclodextrins in targeting: Application to nanoparticles. *Advanced Drug Delivery Reviews*. 1999;36(1):29–40.

[74] Duchene D, Wouessidjewe D, Ponchel G. Cyclodextrins and carrier systems. *Journal of Controlled Release*. 1999;62(1):263–268.

[75] Yang W, Chow KT, Lang B, Wiederhold NP, Johnston KP, Williams III RO. In vitro characterization and pharmacokinetics in mice following pulmonary delivery of itraconazole as cyclodextrin solubilized solution. *European Journal of Pharmaceutical Sciences*. 2010;39(5):336–347.

[76] Tolman JA, Nelson NA, Son YJ, Bosselmann S, Wiederhold NP, Peters JI, McConville JT, Williams RO 3rd. Characterization and pharmacokinetic analysis of aerosolized aqueous voriconazole solution. *European Journal of Pharmaceutics and Biopharmaceutics*. 2009;72(1):199–205.

[77] Ungaro F, d'Emmanuele di Villa Bianca R, Giovino C, Miro A, Sorrentino R, Quaglia F, La Rotonda MI. Insulin-loaded PLGA/cyclodextrin large porous particles with improved aerosolization properties: In vivo deposition and hypoglycaemic activity after delivery to rat lungs. *Journal of Controlled Release*. 2009;135(1):25–34.

[78] Jalalipour M, Najafabadi AR, Gilani K, Esmaily H, Tajerzadeh H. Effect of dimethyl-β-cyclodextrin concentrations on the pulmonary delivery of recombinant human growth hormone dry powder in rats. *Journal of Pharmaceutical Sciences*. 2008;97(12):5176–5185.

[79] Makino K. Pulmonary drug delivery system. [Available from: http://www.tus.ac.jp/rist/lab/introduction/2research-centers/906.html