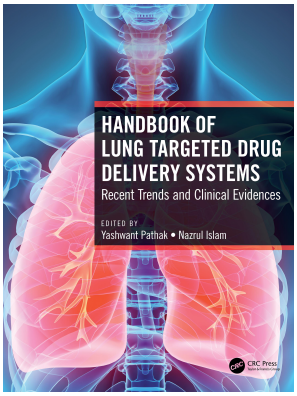

Handbook of Lung Targeted Drug Delivery Systems Recent Trends and Clinical Evidences

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Nanomedicine for the Management of Pulmonary Disorders

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Nanomedicine for the Management of Pulmonary Disorders

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18.1 Introduction

The National Nanotechnology Initiative defines nanotechnology as the control and understanding of matter at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications. The emerging field of nanotechnology includes a wide variety of sciences and is fundamentally based on nano-sized engineered substances with all the physicochemical properties of that specific element in improvised form (1,2). The miniaturization of systems differs greatly from its bulk-sized counterparts with many advantages, viz. new material properties, increased conductivity, enlarged surface area to volume ratio, decreased transport time of molecules, high linear flow rate and optical properties (3). Other than its extensive use in various fields of science and technology, one of the most revolutionary applications of nanotechnology is nanomedicine (4). Since the early 2000s, nanomedicine has emerged as a fascinating interdisciplinary field of research based on the use of nanoscale materials or devices for diagnosis and drug delivery as well as therapeutic methods for the development of nanopharmaceuticals. Driven by the massive progress in the nanomedicine field, solutions to clinical problems have emerged in the form of novel drugs and reformulation of already existing drugs with improved biopharmaceutical features, increased efficacy and minimized drug toxicity (5). Although nanomedicine is presently in its nascent stage of development, various nanotechnology-based pharmaceutical products have already hit the market. Ranging from quantum dots for molecular imaging and diagnostics (6) to therapy using nanocarriers and integrated medical nanosystems (7), nanomedical developments may perform multifaceted repair actions at the cellular level inside the body. Moreover, the permeability of nanoparticles (NPs) through cell membranes, including the blood–brain barrier (8), make them easily accessible to the cells and translocation via blood and lymph (9), thus making them an attractive tool for drug-delivery treatment and nutraceuticals. Applied nanotechnology can be harnessed for generating new concepts for dealing with challenging diseases like cancer, diabetes, lung and cardiovascular problems, inflammatory or infectious diseases, and neurological disorders.

Respiratory diseases, ranging from self-limiting ailments from the common cold to life-threatening chronic inflammatory lung diseases, have imposed an immense worldwide health burden, making them the most common lethal neoplasm in the world. Moreover, respiratory diseases make up 5 of the 30 most common causes of death and the numbers are likely to increase in the near future. The World Health Organisation (WHO) estimates that by the year 2030, the potentially fatal respiratory diseases, viz. tuberculosis (TB), pneumonia, chronic obstructive pulmonary disorder (COPD) and lung cancer, will account for about one in five deaths worldwide. It is noteworthy to mention that prevention, control and cure of these diseases and promotion of respiratory health must be a top priority in global decision-making in the health sector.

In recent years, nanomedicine has gained immense popularity for the targeted delivery of therapeutic and diagnostic compounds to the lung (10), mostly because it is non-invasive nature and bypasses first-pass metabolism (11). But the delivery of inhaled medicines to the lungs has proven to be highly inefficient due to large depositional losses and aerosols with a size range of about 40 nm, which lack effective deposition in the lungs and are exhaled out (12). Also, nanomedicine delivery to lungs can be challenging because of low predetermined lung–site deposition efficiencies. Again, the effectiveness of pulmonary delivery of protein drugs is affected by biobarriers like macrophages, mucus, ciliated cells and proteases in the epithelium of lungs, thus reducing their overall bioavailability, and also by the barrier between alveolar air and capillary blood (2). Furthermore, hypersecretion of mucus is the most common symptom in pulmonary disorders, which is a primary obstacle to circumvent targeted delivery of drugs. Therefore, direct drug delivery to the required lung sites has become an enticing research area. The primary advantage of NPs is their ability to evade clearance by macrophages, thereby entering the respiratory epithelium more easily than larger-sized particles. Among the various drug delivery systems available for pulmonary application, the use of biodegradable polymeric NPs represents several advantages for the treatment of respiratory diseases (13). Nanomedicines, like liposomes, dendrimers, micelles, nanotubes and nanocarriers, present the possibility to increase bioavailability and favor intracellular penetration of specific drugs into the lung tissue

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(14,15). In recent years, a number of respiratory diseases like COPD, TB, cystic fibrosis, asthma, cancer, etc. have been approached using NPs, since nanocarrier systems can be administered to the airways easily. Local delivery of medications to the pulmonary system represent an unmet medical need and is highly desirable where conventional therapy proves to be ineffective in maintaining the desired drug concentration in the blood plasma for a prolonged period (16).

In this chapter, we will summarize and discuss recent trends and developments in nanotechnology for the detection and therapy of various pulmonary disorders, focusing on NPs.

18.2 Physicochemical Characterization for Nanoparticle-Based Systems

The in vivo distribution and behavior of nanometric drug carriers depend mostly on the physicochemical characteristics. Thus, it is necessary to categorize suitable and robust techniques that can be used for this purpose. Again, the characterization of conventional nanomedicines is based on the assessment of physicochemical properties such as molecular weight, composition, identity, purity, solubility and stability. Routine techniques applied for characterization of conventional pharmaceuticals can be used for the characterization of nanomedicines (17), however, numerous specific characteristics of nanomaterials, viz. size, surface composition/energy/charge and shape are crucially important and need to be well investigated. Addressed below are brief descriptions of some of the most commonly used methods to evaluate the specific physicochemical properties of nanomaterials, and a tabular representation has been given along with their advantages (Table 18.1).

18.2.1 Dynamic Light Scattering

Dynamic light scattering (DLS), also known as photon correlation spectroscopy or quasi-elastic light scattering, is one of the most popular light scattering techniques which allows particle sizing (<1 nm diameter) in solutions and the performance of size-distribution studies. DLS is typically used for studing the stability of formulations according to time and/or variations of temperature, for identifying the presence of aggregates in formulations prepared by different procedures, and for rapid determination of the particle size of monodisperse samples (18). Characteristic applications are emulsions, micelles, polymers, colloids, proteins and nanoparticles. The basic principle of DLS is that the sample is illuminated by a laser beam and the fluctuations of the scattered light are detected at a known scattering angle, θ, by a fast photon detector.

Simple DLS instruments that measure at a fixed angle can determine the mean particle size in a limited size range. More elaborate multi-angle instruments can determine the full particle size distribution. From a microscopic point of view, the particles scatter the light and thereby imprint information about their motion. Analysis of the fluctuation of the scattered light thus yields information about the particles. Nanomaterials tend to aggregate in water, changing their size and surface properties, thereby leading to different interactions with the water molecules that surround the nanoparticles, which is a major drawback of DLS (19). As a result, the size obtained from DLS may be overestimated and size distribution may be altered due to environmental dependence.

18.2.2 X-ray Diffraction

X-ray diffraction (XRD) is a primary tool for analyzing the tertiary structures of crystal or polycrystal structures at the atomic scale in the range of 1–100 nm (20) and crystallinity mapping can be done along with DSC. For analyzing lipid-based formulations, both techniques can be used simultaneously. X-ray diffractograms of nanomaterials provide information from phase composition to crystallite size and from lattice strain to crystallographic orientation (21). In XRD, the sample is exposed to a collimated beams of X-rays, with detection of the type and intensity of scattering by stacked

TABLE 18.1

Commonly Used Analytical Techniques for Evaluation of the Physicochemical Characteristics of Nanomaterials

Characterization Technique	Physicochemical Characteristics Analyzed	Advantages
Dynamic light scattering (DLS)	Particle size and hydrodynamic size distribution	Wide time range, cheap and simple experimental setup
X-ray diffraction	Size, degree and orientation, phase, and chemical composition of crystalline materials	Least expensive, convenient and the best method for phase analysis
Scanning electron microscopy (SEM)	Size and size distribution, structure/shape, stability, identification of elemental composition, shape aggregation	Generates detailed three-dimensional and topographical images in digital form; fast and requires minimal preparation actions
Transmission electron microscopy (TEM)	Particle size, size distribution, structure/shape, stability, shape heterogeneity, aggregation, dispersion	Offers very powerful resolution and magnification, radiation resistant, good signal-to-noise ratio, simple sample preparation on grid
Zeta potential	Surface charge, surface chemistry and reactivity	Highest resolution as compared to other methods, small sample volume, rapid measurement
Ultraviolet-visible spectroscopy (UV-Vis)	Particle size and size distribution	High sensitivity, linearity over wide concentration ranges, small sample volume required

parallel crystalline atomic planes of the examined specimen, according to Bragg's law: $2d \sin\theta = n\lambda$, where n is an integer, λ is the wavelength, θ is the scattering angle, and d is the interplanar distance (22). XRD is non-contact and non-destructive, which makes it ideal for in situ studies and characterization of polymer-layered silicate nanocomposites.

18.2.3 Scanning Electron Microscopy

Scanning electron microscopy (SEM) uses beams of accelerated electrons and electromagnetic lenses for imaging, in contrast to light microscopy that uses visible light and glass lenses, with the main improvements including greater depth of field and higher magnification (>100,000×) (23). Consequently, the incident electron beam is scanned in a raster pattern across the surface of the sample, and the emitted electrons are detected by an electron detector for each position in the scanned area. Among these emissions, detection of the secondary electrons is the most common mode in SEM and can achieve resolution smaller than 1 nm (24). The sample electron emission can include either elastic or inelastic scattering events. This electron–sample interaction is used to extrapolate information about the exterior of the particles, including their morphology, orientation and composition. The size, distribution and shape of nanomaterials can be directly acquired from SEM, however, it can only be used for certain biological materials, due to degradation caused by the electron beam. Additionally, while scanned by an electron beam, nonconductive biomolecule samples may acquire charge and inadequately deflect the electron beam, leading to imaging artifacts (25). Therefore, coating an ultrathin layer of electrically conducting material onto the biomolecules is often required for sample preparation.

18.2.4 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is one of the most frequently used techniques for characterizing nanomaterials at spatial scales ranging from <1 to 100 nm and up to the micrometer level, making it effective for novel applications. In TEM, a beam of electrons passes through an ultrathin sample specimen and the crystalline sample interacts with the electron beam by the process of diffraction generating an image that can be magnified/focused by an objective lens. Apart from particle size measurement, TEM images can be used to evaluate whether good dispersion has been achieved or whether agglomeration is present in the system. One important criterion of TEM is that the samples must be prepared as a thin foil (not more than 1 μm in diameter) for the electron beams to penetrate. Again, the samples must be held at liquid nitrogen temperatures post embedding to withstand the high vacuum inside the instrument. TEM is mostly used for metallurgy and biological sciences (26–28).

18.2.5 Zeta Potential Measurements

Zeta potential measurements are essential for predicting the stability of particles that can be measured using light-scattering techniques. In an ionic solution, the surface of a charged particle is surrounded by an electrical double layer of of a thin liquid layer named the Stern layer and an outer diffuse layer consisting of loosely associated ions known as the diffuse layer. In an ionic solution, an electrical double layer surrounds the surface of a charged particle (29). Given the tangential motion driven by an external force or Brownian motion of the charged particle, the movement of the charged particle shears ions migrating with the charge particle in the diffuse layer from ions staying with the bulk dispersant outside the layer. The zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle, determined by evaluation of the velocity of the charged species moving toward the electrode, in the presence of an external electric field across the sample solution. The higher the zeta potential, the higher is the repulsion between the particles and zeta potential with a value of ± 30 mV usually chosen to infer particle stability (30). Electrophoresis light scattering (ELS) also known as laser Doppler microelectrophoresis, is currently used for zeta-potential determination. It is worth noting that zeta potential is a property sensitive to environmental changes, including alteration of pH and ionic strength (31).

18.3 Criteria of Nano-formulations Intended for Pulmonary Delivery

Large surface area of approximately 70–140 m^2 coupled with the property of fast absorption owing to an efficient vascularization system makes adult human lungs an ideal candidate as a drug delivery system for various therapeutic agents. Moreover, the thin blood–alveolar barrier, rapid onset of action, high therapeutic ratio, lower administered dose, increased selectivity and bypassing of first pass metabolism in the lungs proved to be an added advantage for pulmonary targeted drug development (32–34). A majority of the pulmonary delivery systems found in the market basically fall under three categories: dry powder inhalation (DPI), metered dose inhalators (MDI) and nebulizers. However, most of the marketed pulmonary targeted products are short-acting, where drug concentration peaks initially, followed by prompt decline, and may lead to adverse systemic effects. Moreover, patients require inhaling several times a day to get the desired effect, which affects patient compliance (33). To overcome all these shortcomings, controlled release drug delivery was developed, but this system comes with formidable airway clearance mechanisms such as mucociliary clearance, alveolar or macrophage clearance, systemic absorption and metabolic degradation. To overcome these shortcomings, different formulations come into play, of which nano-formulations are the most prominent.

Different forms of nano-formulations are used for delivery in the pulmonary system, of which solid lipid nanoparticles (SLN) are well-known. Particle size of the drug delivery system plays an important role in deciding the targeted site, and depending upon the particle size, the administered drug gets deposited in various regions of the respiratory system. Upper airways, smaller airways and bronchioles and alveoli

are the three regions where administered particles get deposited depending upon their sizes. Inertial impaction, gravitational sedimentation and Brownian diffusion are the three widely accepted mechanisms of drug deposition which depends upon the particle size of the drug delivered (32). Apart from the drug delivery system, breathing parameters, specific disease types and cellular aspects of the transportation of pulmonary drugs are some of the vital parameters needing to be taken care of during development of drugs for pulmonary routes. Apart from all those criteria, choice of patient specific inhalation device in nano-based drug delivery for pulmonary administration is also of paramount importance. Thus, this section of the book chapter will focus on various aspects such as nano-formulation types, particle size, site and mechanism of deposition, cellular aspects of the transportation of pulmonary drug and inhaler types in specific disease conditions that play a crucial role in the case of nanoparticles intended for pulmonary delivery

18.3.1 Pulmonary Directed Nano-Drug Delivery Formulations

Till date various types of nano-formulations, viz. liposomes, micelles, SLN, dendrimers and polymers, are being developed to be used for drug delivery. Liposomes are basically lipid bilayer fabricated from cholesterol and natural nontoxic phospholipids. Their hydrophobic as well as hydrophilic characters and high stability make them unique among other nano-formulation types and are being utilized as carriers for some delicate drugs such as genetic materials and vaccines as well as steroids (35,36). SLN are an alternative to polymeric NPS, having solid lipid as a matrix material for drug delivery (37). Lipid analogous to endogenous lipids and phosoholipids like cholesterol, which are well tolerated by the body, are utilized for fabrication of SLN. Mobility of drug in solid lipid is low in comparison to liquid oils, so SLN are regarded as an ideal formulation to achieve controlled drug release (38).

Micelles are made from amphiphilic copolymers where the hydrophobic blocks of the copolymers form the micelle core, whereas the hydrophilic blocks are present in the outer core, thus enabling drugs to travel through hydrophilic channels, allowing the drugs to stay in the systemic circulation thus delaying the excretion rate (39,40). Apart from a wide range of drug carriers, dendrimers are developed to be a carrier for genetic materials acting as nonviral vectors. Amine groups found in dendrimers condense the nucleic acid into NPs by ionic interaction and guard the nucleic acid from enzymatic degradation (41). Lipid-based NPs like liposomes have their demerits, too. They lack encapsulation efficiency; leakage of water-soluble drugs occurs in the presence of blood components and they have storage stability issues. But polymeric NPs are devoid of such issues whereas they possess potential controlled release properties equipped with increased stability of drugs (42). Again, nanospheres and nano capsules are two types of widely fabricated polymeric NPs. A drug candidate is uniformly distributed in a matrix system in the case of nanospheres, whereas drugs are concentrated to a cavity having a suitable polymeric membrane in the case of nano capsules (43).

Incorporation and adsorption are the two methods by which drugs are loaded into polymeric NPs, but incorporation gives maximum drug loading as compared to the latter (44). Drugs administration via pulmonary route is a challenging task, owing to the unique physicochemical, physiological, biochemical as well as anatomical parameters of the respiratory tract. Deviation of agglomerated particles from their path due to the humid environment of the respiratory tract, abrupt pulmonary clearance in the form of mucociliary or alveolar clearance of the aerosolized drug, as well as particle accession by macrophages are various challenges that need to be dealt with while designing drugs for pulmonary administration (22,45). However, lipid-based NPs are advantageous over other polymeric based counterparts in pulmonary drug delivery due to the lipophilic environment of the respiratory system. Alveolar fluids and mucus are mainly composed of phospholipids, cholesterol as well as surface proteins that reduce the surface tension required for proper functioning of gas exchanges and removal of foreign particles by cilia of the epithelial cells (32).

Out of all lipid-based NPs, SLN holds promise to be the best formulation in terms of acceptability in the respiratory system. SLN are mainly made up of phospholipids and triglycerides, both endogenous to respiratory system, due to which there is high probability of acceptance and less toxicity (46). SLN nebulization in mice yields no pro-inflammatory cytokines or chemokines, which is an indication of little or no toxicity upon administration of SLN (47).

18.3.2 Particle Size and Materials

The site of deposition of a drug depends upon its particle size. Sizes above 5 μm deposit in the oropharyngeal region, sizes from 1–5 μm in the smaller airways as well as in the bronchial area, and sizes less than 0.5 μm deposit in the peripheral alveoli. Basically, NPS deposits in the peripheral alveolar region through the sedimentation mechanism, owing to their particle sizes. Many times, NPs become aggregated after releasing from the aerosol system and form micrometer size ranges, settle in the bronchial region due to increasing mass and thereby the desired effect is achieved (32). SLN, being a favorable NPs formulation, has recently grabbed attention worldwide due to their higher success rate during nebulization. Because SLN is a colloidal formulation and due to its nano-size, it is an ideal candidate for lymphatic interstitium penetration (48). When SLN was inhaled by a group of adult Wistar rats, the study showed significant uptake of a radio-labeled SLN into the lymphatics (49). A similar study in a model of non small cell lung cancer (NSCLC) in human alveolar adenocarcinoma epithelial A549 cells showed promising results of an erlotinib (ETB)-loaded SLN based formulation of dry powder inhaler (ETB-SLN DPI) having size below 4 μm; 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor (MTT) assay and 4',6-Diamidino-2-Phenylindole (DAPI) staining revealed enhanced cytotoxicity compared to free ETB (50).

Lipid based nano-formulation offers the best compatibility with the pulmonary delivery system compared to other material-based nano-formulations due to the enhanced dissolution rate, uniform drug distribution and because it is analogous to endogenous

surfactant found in the entire respiratory system (51). Cholesterol, lecithin and phospholipids are different types of pulmonary surfactants which are utilized synthetically in the fabrication of SLN.

18.3.3 Particle Size and Deposition in Various Regions of the Respiratory System

The respiratory system is subdivided into upper airways, smaller airways and bronchioles, and finally peripheral alveoli in terms of particle deposition. The pattern in which the particles are deposited in various regions of the lungs follows three widely accepted mechanisms: inertial impaction, gravitational sedimentation and Brownian diffusion. The deposition mechanism in various region of the respiratory system has been depicted in Figure 18.1. Deposition of particle size greater than 5 μm follows the mechanism of impaction. Dry powder inhalation (DPI) and metered dose inhalators (MDI) whose particle sizes are above 5 μm undergo the mechanism of impaction. On release of the particles from the inhalers, the high-speed particles collide in the respiratory wall with high impact under centrifugal force and get deposited in the oropharynx region. The mechanism of sedimentation mainly follows the law of gravitation where particle sizes ranging from 1–5 μm and having a sufficiently large mass are deposited in the smaller airways and bronchioles. Particle sizes less than 0.5 μm are deposited in the peripheral alveoli with the help of Brownian movement of the lung surfactant and smaller particles exhaled out from the system (32,45).

18.3.4 Peptide Based Nano-formulation for Transportation of Pulmonary Drugs

Two major cell types are found in pulmonary epithelium: Type I and Type II pneumocytes. Type I pneumocytes cover ~95% of all the alveolar epithelial surface, whereas Type II pneumocytes cover only 5%. Type I pneumocytes have endocytotic vesicles

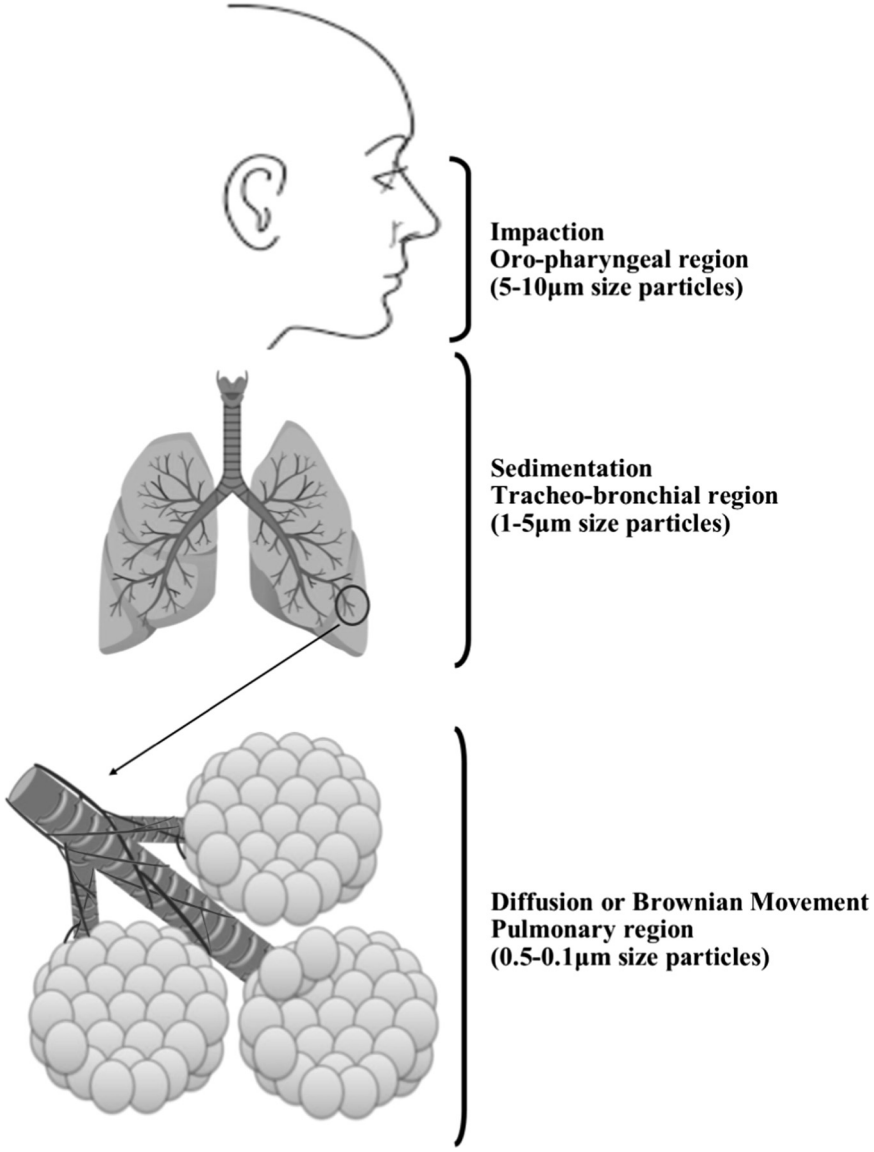

FIGURE 18.1 The Deposition Mechanism in Various Region of the Respiratory System Along with Varying Particle Size

which may function as a carrier of large proteins (34,52), and Type II pneumocytes produce surfactant protein and differentiate into Type I cells after injury in the epithelial barrier (53,54). Peptide transporter (Pept) 1 and Pept2 are two low-affinity and high-capacity transporters, mainly expressed in the small intestine, are also found in human lung tissue where Pept2 is abundantly found in Type-II cells responsible for oligopeptide transport as reported in a study involving rat lung epithelial cells (55). So, with the help of a Pept2-like cell transporter, recently many new technologies like peptide conjugation and peptidomimetic strategies have been developed. Peptide based NPs for pulmonary drug delivery is relatively a new and emerging field for systemic as well as local treatment in the lungs. Peptides are preferred for nonviral vector therapy, especially for their cell penetrating capacity (56). Recently, a peptide-based NP conjugate study demonstrated promising results in lung cancer (57). In the study intratrachealstudy, intra-tracheal (i.t.) administration of a dimerized transactivator of transcription (TAT) peptide (dTAT)-based nonviral gene delivery technique attenuated an acutely growing mouse Lewis lung carcinoma allografts in mouse lungs. The dTAT-based nonviral gene delivery technique contains an apoptosis-inducer gene, angiotensin II type 2 receptor (AT2R) alongwith along with a complex of Ca2+ (dTATpAT2R-Ca2þ).

18.3.5 Choice of Inhalation Device Depending Upon Diseased Condition

A successful pulmonary administration of aerosolized drugs relies on three factors: drug formulation, inhalation device and the disease condition the patient is suffering from. Often the administered drug in the form of an aerosol does not reach the desired site due to diseased condition or improper administration of drug arising from the inhaler. There are four different types of inhalers found in the market, viz., metered dose inhalers (MDIs), DPIs, soft mist inhalers (SMIs) and nebulizers. The aerodynamic particle size distribution of almost all the inhalers falls within the range of 1–5 μm. Drug administration via MDIs and DPIs as well as SMIs require inspiratory flow from the patient. But in severe conditions of diseases like asthma, COPD and pulmonary hypertension, owing to a congested condition, the patient is unable to inspire the aerosolized drug, leading to deposition of drug in the upper respiratory tract and resulting in inadequate effect at the site of action (58). This condition worsens in the case of infant, elderly and comatose patients where drug cannot be administered with the help of the above-mentioned inhalers. In such cases, a nebulizer comes to the rescue. A repertoire of nebulizer designs is found in the market, i.e. jet nebulizer, breath enhanced nebulizer, manual actuated nebulizer, breath actuated nebulizer, ultrasonic nebulizer and vibrating mesh nebulizer. Performance of a nebulizer is highly dependent on its specific design. A recently concluded study identified the vibrating mesh nebulizer as an efficient design in terms of inhaled and residual dose when tested in a spontaneous breathing lung model as compared to other contemporary designs. Many recent studies have reported the potential of NPs when administered via nebulizers. In two recently concluded studies, the researchers reported the potential of SLN loaded sildenafil citrate (SC) in pulmonary hypertension. In both the studies, the researchers developed a nano-formulation of SLN loaded SC and studied its efficacy in an in vivo model of the Wistar rat by nebulizing with a jet nebulizer. The researchers found the colloidal stability or the drug entrapment was intact with minimum occurrence of bleeding in lung parenchyma (59,60). These studies indicated that nebulizers are the most preferred inhalation devices over other devices nowadays when it comes to the administration of nano-formulations. Administration of nano-formulation in the pulmonary route is a challenging task, due to various challenges the formulation has to go through. Particles must be of specific sizes for the desired site of deposition in the respiratory system where nano-sized particles generally get deposited in the alveolar region.

Again, lipid-based nano-formulation is the widely accepted nano-formulation for pulmonary administration, of which SLN is a promising lipid-based nano-formulation due to the presence of physiological components in its composition. Peptide based nano-formulations are a relatively new technology that may be used for pulmonary administration due to their cell penetrating capacity and transportation of oligopeptides. The inhalation device also plays a crucial role in transporting drug to its desired site. Among all inhalation devices, the nebulizer has the advantage in administering drug to elderly, infant and comatose patients without any inspiration support from the patients. Most recently, researchers are utilizing jet and vibrating mesh nebulizers to nebulize their fabricated nano-formulations to the desired site. Although there is much hype surrounding the lipid-based drug delivery for pulmonary administration, there is paucity of information about the exact mechanism of transportation of drugs across pulmonary cells and these formulations have yet to testify clinically. Apart from the above discussed criteria of NPS intended for pulmonary delivery, there are other aspects, such as nanotoxicology, flow rate and volume administered as well as acceptability of these formulations in humans, etc., these must be addressed thoroughly before possible clinical trials. Some of the widely used nanocarriers and their advantages for pulmonary drug delivery are listed in Table 18.2.

18.4 Pulmonary Infectious Diseases and Nanomedicinal Approach

Worldwide, pulmonary diseases are among the primary causes of mortality and morbidity, however, a few nanomedicine based life-prolonging therapies and targeted drug deliveries have been developed for these maladies. With the revolution of nanotechnology many therapeutic strategies are being investigated for drug delivery to the respiratory system which includes small molecules, protein and peptide drugs, or siRNA targeted to specific genes. Most of these studies were generally focused on localized application of drugs via nanocarriers for obtaining the best possible therapeutic outcomes for chronic lung diseases (61). Lipid-based carrier systems, including liposomes (mostly lung surfactants and synthetic lipids) and nanoliposomes, are the most promising encapsulation technologies employed in the fast developing field of nanotechnology for controlled drug delivery to the lungs (11). Since a variety of nanocarriers have been

TABLE 18.2

Widely Used Nanocarriers for Pulmonary Drugs

Nanocarrier	Size	Advantages
Polymeric nanoparticles	10–1000 nm	Nontoxic, non-inflammatory, and non-immunogenic
Liposomal nano-carriers	0.5 μm–100 nm	Selective targeting to tumor tissue, enhanced pharmacokinetic effect and therapeutic index, decreased toxicity
Solid lipid nano-carriers	50–1000 nm	High drug loading and stability, incorporation of both hydrophilic and hydrophobic drugs, nontoxic, minimization of organic solvent use
Submicron emulsions	10–500 nm	High kinetic stability and solubilizing capacity, tiny globule size
Dendrimers		Polyvalent, self-assembling, electrostatic interactions, chemically stable, less cytotoxic and highly soluble
Nanocrystals	less than 500 nm	Enhanced solubility, zero-dimensional
Inorganic nanoparticles	3–6 nm	Nontoxic, hydrophilic, biocompatible and highly stable

investigated that can be transferred easily to the airways for the delivery of macromolecules to the respiratory tract, the ability of a nano-structured system may be approached for treating a number of respiratory diseases including COPD, genetic disorders affecting the airways and infectious diseases including TB and cancer.

18.4.1 Asthma

According to reports of the WHO, more than 300 million individuals globally suffer from asthma (62) and its socioeconomic burden exceeds the sum of human adenovirus/acquired immune deficiency syndrome (HIV/AIDS) and TB. Little has been done in the field of asthma therapy during the last 20 years, and with the exception of a very few drugs like tiotropium, indacaterol and omalizumab, no revolutionary changes have occurred (63). Treatment with corticosteroids and the combinations of steroids with bronchodilators is the first-line control strategy for asthma (64) that can manage asthma symptoms on a day-to-day basis but it has severe adverse effects, including hypertension, cataracts, osteoporosis in elderly patients and stunted growth in children (65). Novel therapeutic agents with effective routes of administration and prolonged action may be an alternative to corticosteroids for management of asthma. PEGylated poly(amidoamine) (PAMAM) dendrimer, a typical dendrimer, has been widely studied and applied (66) which can perk up drug solubility and increase lung accumulation capacity for insoluble drugs like beclomethasone dipropionate (BDP) (67). Recently, bilirubin-based nanoparticles (BRNPSs) composed of freely water dispersible, entirely PEGylated bilirubin were developed based on the report that asthmatic symptoms are rapidly relieved during jaundice, indicating a positive role of augmented bilirubin levels in serum (68). The effects of BRNPSs on T-helper-2-type (Th2) immune responses were investigated both in vivo and in vitro showing potent anti-inflammatory effects in an *A. oryzae* protease allergen-induced asthma model. It has been reported that nanocarrier-encapsulated steroids achieved better and long-term therapeutic effects in airway inflammation sites as compared with that of free steroids. Again, a study reported that in the lungs, liposomes delayed the retention of salbutamol sulfate maintaining an effective drug concentration for more than 10 hours, thereby achieving significantly higher efficacy than the free drug solution (69). Moreover, the selection and construction of apposite nanocarriers are cutting-edge topics in the gene therapy of asthma. According to a study reported by Kumar et al., chitosan-IFN-γ pDNA NPS (CIN) can significantly reduce airway hyper-responsiveness and lung histopathology scoring in BALB/c mice with ovalbumin (OVA) induced allergic asthma (70).

Since angiogenesis is a feature of asthmatic inflammatory responses, anti-angiogenesis nano-therapy could offer a new therapeutic approach to this serious disease. Recently, the results of study done by Lanza et al. indicated that lipase labile phospholipid prodrug forms of fumagillin (Fum-PD) or docetaxel (Dxtl-PD) incorporated into lipid-based αvβ3-integrin targeted micelles for drug delivery ameliorated asthma in the house dust-mite-triggered Brown Norway rat model (71). Again, SLN formulation of curcumin was capable of overcoming its poor solubility and rapid metabolization, which was the major drawback of curcumin affecting its clinical efficacy as a potent anti-asthmatic. In another recent study, it was reported that curcumin loaded in SLNs suppressed airway hyper-responsiveness and infiltration of inflammatory cells by significantly inhibiting the expression of Th2 cytokines in a murine model of OVA induced asthma (72). Furthermore, the bioavailability and efficacy of andrographolide (AG), a labdane diterpene lactone was improved over free drug by encapsulating it in PLGA nanoparticles, thereby, resulting in a promising therapeutic agent against OVA induced asthma in mice (73).

18.4.2 COPD

COPD, the culmination of chronic bronchitis, small airways disease and emphysema (existing separately or in combination) is associated with structural changes and inflammatory response contributing to bronchial hyper-responsiveness and reduced lung function. Again, increased number of T-lymphocytes, particularly CD8+ T cells, macrophages and neutrophils is a characteristic of COPD (5). COPD is considered to be among the greatest global health hazards in terms of mortality and morbidity, and approximately 210 million people in the world are currently estimated to suffer from COPD, with a large disease burden in developing countries (74) and it is estimated that COPD will be ranked the third cause of death by 2020. Moreover, it has been reported as the second leading cause of disability and third leading cause of mortality in the United States (US) (75). Unfortunately, a

suitable model demonstrating the role of nanomedicine in COPD is lacking. Additionally, COPD is basically steroid insensitive and is therefore unresponsive to traditional inhaled anti-inflammatory steroid therapies (1). There are few reports where lipospheres and proliposomes have been used as drugs for dry powder inhalation aerosol delivery (76). Various preclinical studies with murine models have proven nanoparticle-based therapy to show promising results against lung inflammation (77). Morever, inhaled NPs, owing to their small size, can easily traverse to distal airspaces, where they are deposited exclusively by diffusion (78). Properly designed nanoparticles, like methotrexate-loaded albumin NP and doxorubicin-loaded SLN, are specialized for the purpose of pulmonary delivery and have proven to be highly distributed in the lungs. Polyethylene glycol (PEG) is a polymer that can rapidly navigate through the mucus barrier (79). Therefore, if NPs are adequately coated with such muco-alert polymers, it can easily gain access to the underlying epithelia. Furthermore, special emphasis has been put on the inhibition of neutrophil infiltration, the hallmark feature in the pathogenesis of COPD.

8081 In COPD, the common pathogenic mechanisms is found to be progressive inflammatory response involving a significant increase in number of neutrophils and macrophages. In a recent study, neutrophil-targeted polylactide-coglycolide (PLGA)-PEG NPs (PNPs) for ibuprofen delivery has been demonstrated as an effective drug-delivery nanosystem for treating COPD (80). In this study, PNPs is found to exert its ability to control neutrophil mediated proteas anti-protease imbalance in Pa-LPS/cigarette-smoke induced COPD. Since cytokines and chemokines possess a critical role in the orchestration of COPD, therefore, the highly selective monoclonal antibodies are valuable treatment options for COPD. Clinical trials of anti-interleukin (IL)-8 antibodies abgenix, anti-tumor necrosis factor (TNF)-α antibody infliximab has been conducted, however the outcome was not totally fruitful in improving lung function (82). Thus, the use of nano delivery system (NDS) may allow administration by inhalation, increasing local concentrations and limiting possible side effects. Another such monoclonal antibody is cetuximab, which could be a valuable option for the treatment of COPD.

Again, alveolar macrophages, the most abundant, versatile cells in the respiratory tract, play a major role in the pathogenesis of COPD as a key source of mediators (83). A recent study with biocompatible PEGylated dextran-coated magnetic iron oxide NPs has been reported to specifically target one subpopulation of macrophages in the lung of a COPD animal model by maintaining a balance in the number of M1 and M2 macrophages (84). Lipid NPs, viz. SLN and nanostructured lipid carriers (NLC), result in a longer dosing interval and better patient compliance and have been investigated as a possibility to improve therapy of COPD.

18.4.3 Tuberculosis

TB is another global public health problem due to its high prevalence in many countries, and is closely linked to socio-economic conditions, making it the second leading cause of death from an infectious disease after HIV. According to WHO fact sheets an estimated 1.7 million, 10.4 million and 1.6 million cases of death due to TB were recorded in the years 2009, 2016 and 2017, respectively (85–87). Although TB is practically curable in 100% of the cases, a majority of cases occur in developing countries where diagnosing TB is difficult due to technical limitations and lack of resources.

The first-line drugs or multiple anti-tubercular drugs (ATDs) must be administered continuously for a minimum of 6 months, which leads to patient noncompliance with prescribed treatment regimens, treatment-related adverse effects and misuse, which may cause emergence of mult-drug-resistant TB (MDR-TB). Again, the options for treatment of MDR-TB are the second line ATDs which are comparatively more toxic, less effective and not easily available due to their high cost (88). Misuse or indiscriminate use of second-line ATDs further results in the development of extensively drug resistant TB (XDR-TB) (89). Moreover, the situation is worsened by the fact that many countries lack proper infrastructure to accurately diagnose MDR-TB and XDR-TB, which increases TB associated mortality. Besides these limitations, the current therapies available for TB have inadequate ability to penetrate granulomas and also have reduced effects on dormant bacilli (90). In this context, improvement of the therapeutic index of existing ATDs, by their encapsulation into NDs, should be emphasized.

Recent advances in nanotechnology have enabled the development of novel strategies and new diagnostic platforms for TB, which are aimed at more sensitive and faster pathogen detection and effective parenteral or mucosal TB vaccine delivery (91,92). As reviewed previously, inhalable nanocarriers like nanocrystals and NPs as well as aerosols offer a latent value in local and passive delivery of anti-TB therapy (93). Pulmonary delivery of vaccines may prove to be an effective alternative to intradermal (i.d.) or subcutaneous (s.c.) routes, since the lung is the primary site of infection for *Mycobacterium tuberculosis* (Mtb), the causative strain for TB (94). Drug carriers and radiopharmaceuticals like 99Tc-EMB, graphene oxide, etc., that are nontoxic to humans, can encapsulate potent ATDs to efficiently deliver in the target site (95). Furthermore, liposomes are attractive nanocarriers for their versatile and unique structures and are one of the successful drug delivery systems for increasing the effectiveness of antibiotics (96).

PEGylated, phosphatidylcholine, dimyristoylphosphatidylcholine (DMPC), DPPC, dimyristoylphosphatidylglycerol (DMPG) and distearoylphosphatidylcholine (DSPC)-based systems are some of the examples of liposomes having remarkable potential as direct drug delivery systems for frontline ATDs to lungs (97–99). However, one disadvantage of liposomes is that they are vulnerable to intestinal lipases, therefore oral route of administration cannot be performed (100). Moreover, the preferential accumulation of liposomes with ATDs after i.v administration occurs at the liver and spleen, which can be controlled by the addition of ligands to liposomes to accomplish lung specificity (101). In contrast to the potential problems and poor chemical stability (102) with liposomal and biodegradable polymer delivery systems, Clemens et al. reported mesoporous silica (MS) NPs as a promising delivery platform for ATDs in effectively killing intracellular Mtb as compared to that of free drug. In an attempt to incorporate ATDs in controlled release proniosome derived niosomes, high encapsulation efficiency was obtained and might prove to be adantageous to solve the problem of MDR-TB (103).

18.4.4 Lung Cancer

Worldwide, lung cancer is the second most common cancer in both men and women and is the leading cause of 23% of total cancer deaths, with a dismal 5-year survival rate of only 15% (104). Lung cancer is manifested in one of two forms: small cell lung carcinoma (SCLC), which represents 13% of the total lung cancer cases, and non small cell lung carcinoma (NSCLC), which represents 80–85% and is considered significantly more aggressive (105). Further, NSCLC is categorized as epidermoid, large cell, bronchoalveolar, adenocarcinoma, and squamous cell carcinoma (104). Presently, chemotherapy, radiotherapy and surgery are the available therapies for lung cancer treatment. However, multi-drug resistance to cytotoxic agents, nonspecific toxicity to healthy cells, deficiency in early-stage diagnostics, lack of therapeutic efficiency and intratumoral genetic heterogeneity (106) paved the path for the need for efficient and less toxic treatment alternatives. It is noteworthy to mention that for effective drug targeting, parameters like physicochemical characteristics of the chemotherapeutics, carrier properties and type of the inhalation device, the tumor type and location as well as targeting strategy must be considered. Active targeting via NP-based ligand or monoclonal antibodies may hold a stronger potential than passive targeting based on the well-known enhanced permeability and retention (EPR) effect. Active targeting strategies are advantageous as this ideally allows for cell-specific detection and killing of both primary tumor cells and metastatically spread circulating cancer cells (107). Furthermore, the main route of application in recent preclinical studies is based on I.V. delivery of NPs (108) which remained with partial success due to poor site-specific drug availability (109). Therefore, methods to improve tumor-targeted delivery of chemotherapeutics that will result in increased drug, improved pharmacological properties and least toxicity to normal tissues retain their precedence in cancer therapy.

NPs can optimize the biodistribution profile, enhance transport properties and target the drug to the tumor regions, which increases therapeutic efficiency and reduces nonspecific toxicity of anti-cancer drugs (110). Recently, the US Food and Drug Administration (FDA) approved an albumin-bound formulation (Abraxane®) of cremophor EL and paclitaxel for NSCLC in combination with carboplatin, in an attempt to reduce the toxicity of cremophor EL and to improve the overall efficacy of paclitaxel (111). Furthermore, nanotechnology-based therapeutic delivery with pulmonary/inhalational route of administration has received much attention in the recent decade for lung cancer treatment. The inhalational delivery of doxorubicin (DOX)-loaded NPSs in a cancer-bearing mouse model (BALB/c model) (112) and 5-fluorouracil (5-FU) NPS produced by a supercritical antisolvent process in lung cancer were successfully established (113). Again, numerous proteins have been targeted by small interfering ribonucleic acid (siRNA) embedded into nonviral delivery systems in cancer pathologies based on cell cycle, proliferation, apoptosis and angiogenesis pathway studies. The encapsulation of hydrophilic drugs such as nucleic acids and proteins has been already developed for numerous formulation processes (114,115). Yet again, perfect sequence homology has been observed between the strands of endogen micro RNA (miRNA) and messenger RNA (mRNA), referred to as the RNAi concept. In a murine mouse model of lung cancer, a lipid/polymer nanoparticle delivering miRNA slowed tumor growth (116). This plethora of evidence confirms that polymeric NPs have been extensively explored using various highly used pulmonary drugs for the treatment of asthma, COPD, TB and lung cancer.

However, the biodegradability and toxicity of the polymers for long term use should be closely examined in the formulation of polymeric NPs for pulmonary delivery, to avoid accretion of polymer carriers following repeated doses.

18.5 Regulation and Guidelines for Marketing

Nanotechnology is viewed by the European Union (EU) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) as one of the major technological drivers of innovation (117). Since 1995, more than 50 nanopharmaceuticals have been approved by regulatory authorities, including liposomes, non-liposomal lipid formulations, peptides, PEGylated proteins, nanocrystals, aptamers, protein-drug conjugates, polymer-based nanoformulations, surfactant-based nanoformulations, virosomes and metal-based nanoformulations (118) and are presently available for clinical use (119). Among those, 18 are marketed nanopharmaceuticals and other products for imaging and diagnostic applications. Moreover, more than 70 nanomedical products are undergoing clinical trials.

The approval process for nanomedicines in humans is regulated by the FDA in the US (120). Both the FDA and the EU have already set up robust schemes for the approval and legislation of new nanopharmaceuticals and provide comprehensive pharmacological and toxicological profiles of novel nanopharmaceuticals in maintaining a balance between innovation and safety (121). Following the discovery or invention, the material undergoes a preclinical phase of testing, which usually involves animal studies for demonstration of efficacy, safety, toxicity profile and identification of appropriate dose ranges (122).

In June 2014, the FDA issued one draft and three final guidance documents for the industry regarding the use of nanotechnology in FDA-regulated products, including nano drugs (123). Data demonstrating the physicochemical properties, efficacy and toxicity of a nano drug are then compiled into an Investigational New Drug (IND) application for FDA review and approval. Moreover, researchers can send nanomaterials to the Nanotechnology Characterization Laboratory (NCL) to have them tested and validated according to a series of emerging protocols (124). Following FDA approval, clinical trials are undertaken with human volunteers to determine safety and efficacy. Further, the fourth phase of the clinical trial, i.e. post-marketing surveillance, is initiated at the request of the FDA or health care professionals to assess the risks associated with nano drugs. The entire process is estimated to cost $1 billion per new drug and take approximately 10–15 years. Some of the US FDA-approved nanomedicines for pulmonary disorders have been listed in Table 18.3.

TABLE 18.3

Nanomedicines for Pulmonary Disorders in Clinical Trials or US FDA-Approved (111,119,140, 141, 142)

Name	Description	Medical Indications	Current Status
Arikayce	Inhaled liposomal formulation of amikacin	Serious chronic lung infections	Marketed
BLP25	Liposome vaccine	Lung neoplasms non small cell lung carcinoma	Phase II completed
CRLX101	A drug–conjugate formulation of camptothecin and a cyclodextran-PEG polymer	Lung cancers (SCLC and NSCLC)	Phase 1 and 2 clinical trials ongoing
Curosurf	Phospholipid fraction from porcine lung	Respiratory distress syndrome	Marketed
Pulmaquin	Combination of liposomal and aqueous-phase ciprofloxacin	Cystic fibrosis (CF) or non-CF bronchiectasis	Completed company-sponsored phase 2 studies
SN-38	An active metabolite of the topoisomerase inhibitor irinotecan	Solid tumors, NSCLC	Two phase 1 trials have been completed
UMIN000014940	PLGA NP- pitavastatin	Pulmonary artery hypertension	Completed investigator-initiated phase I clinical trial

18.6 Safety Assessment of NPs

Although nanomedicines have undoubtedly shown potential for the treatment of chronic lung diseases, possible organ toxicity is a major concern in pulmonary medicine. With the rapid expansion of new applications of nanomedicine and use of nanotechnologies in the 21st century for innovative treatment strategies, a balance between therapeutic efficacy and safety of the NDS with the aim to increase the benefit-to-risk ratio is the need of the hour. With regard to toxicological response, it is noteworthy to mention that the lung is the primary and probably the most important target during inhalation of NPs and a secondary target after I.V. injection due to the high blood irrigation of this organ and the small space between alveolar epithelial cells and the blood capillaries. Furthermore, a wide range of NP-based strategies have been reported to cause respiratory disorders (2,82). Lung inflammation has been observed following the administration of carbon nanotubes via i.t. installation. However, the type of response, whether innate or inflammatory, varies between molecular and nano-sized forms (125), surface area and charge (126), long-term toxicity verses subacute and acute toxicity (127), exposure level (128), type of particles (biological or non-biological) used, etc. Again, the probabilty for adverse health effects may occur due to direct exposure to intentionally produced nanomaterials and/or byproducts allied with their applications. The major cellular responses caused by nanotoxicology in the lungs include release of reactive oxygen (ROS) and nitrogen species (RNS), proinflammatory/inflammation-associated proteins as well as injury of nuclear DNA (129).

Furthermore, functional disturbances could be observed after NP exposure, including airway hyper-reactivity (AHR), tissue injury and effects on existing pulmonary inflammation. Therefore, reliable and reproducible screening protocols are needed to understand the novel physicochemical properties of nanomaterials and their interactions at the nano/bio interface responsible for biological hazards (130).

But before proceeding with the nanomaterial toxicity testing, some of the discussion points become crucial to answer, such as which toxicological end points to screen for, the comprehensiveness of the screening effort, the correct balance of in vitro versus in vivo testing, the cost of the effort, whether current regulation, testing and classification protocols are suitable and who should be responsible for screening and safety assessment of nanomaterials (131). It is also a matter of concern that generally the toxicity of the whole formulation is analyzed before marketing a product, but results of the toxicity profile of NPs are often ignored. Consequently, a specific emphasis on the toxicity of the non-drug loaded particles, specially for the non-biodegradable and inorganic ones, should be given since biodegradable NPs becom degraded by metabolic pathways (118). From a safety perspective, the need to connect several major themes, viz. safety, regulation, research quality and innovation of current respiratory nanotoxicology is apparent, including:

a. Comprehensive nanoparticle characterization in the relevant delivery vehicle and physiological matrices (132).
b. Predictive in vitro studies with the self-propagating human lung cell lines A549 or BEAS-2B can help to predict the hazard potential of a series of ambient particles based on an established mechanistic pathway at the molecular, cellular, organismal and ecosystem levels, and an in vivo outcome (133). However, analysis of in vitro studies has to be cautiously performed since they are usually carried out at high doses.
c. Cell culture studies are the hallmark of nanotoxicological analysis; however, data obtained from in vitro experiments could be misleading for varied reasons as in the case of a report produced by Monteiro-Riviere et al. where the dye-based MTT assay produced invalid results with some NPs. Hence, more than a single assay may be performed when determining NP toxicity for risk assessment (134).
d. Nanomaterials may interfere with the read-out systems of commonly used assays for cell viability and/or mitochondrial function, generating false results. In spite of performing in vitro studies, the toxicological status of NPs should further be verified from in vivo experiments by examining molecular markers of oxidative stress and/or inflammation measured within the bronchoalveolar lavage fluid (BALF) and histology (135) in both diseased and healthy lungs.

e. The adverse health effects of particulate matter (PM) are measurable as exacerbations of respiratory disease and deaths as well as hospitalizations and deaths from respiratory and cardiovascular disease (136), inflammation being the common factor that binds these adverse effects.

f. Preclinical testing of novel nano-therapies for lung cancer with a detailed and stratified analysis in mice including, first, toxicological and pharmacokinetic analysis of the nanoengineered formulations in healthy animals, and second, theranostic application of the nanoparticles with simultaneous imaging of particle biodistribution, drug release and testing of the therapeutic efficacy in appropriate lung cancer models (137). The molecular heterogeneity and histopathological features of lung tumors need to be defined in detail to permit personalized approaches of nanomedical therapies. Comprehensive identification of differential receptor expression of tumor versus normal cells needs to be defined by the omics technologies that have fostered systems-based analysis of different tumors (138, 139).

g. Tailoring the surface of NPs by functionalization or tuning parameters like surface charge, spacer length, hydrophobicity, aggregation propensity, etc., is a scheduled process to improve the behavior of NPs for nanomedicine application. Moreover, to assess the role of surface charge on the toxicity and inflammogenicity of NPs in the lung environment, well-engineered, nontoxic and chemically stable model NPs are preferred (140).

18.7 Conclusion

It has become essential to bring material and pharmaceutical scientists together to find new avenues through multidisciplinary approaches that can speed up the development of new diagnostic and therapeutic solutions. Nanopharmaceuticals have massive potential to address the failures of traditional drugs that could not be effectively formulated due to factors such as poor water solubility or a lack of target specificity. Although studies on NPs for pulmonary application are still in an initial phase, studies performed so far suggest that NPs are an interesting choice in the systemic or local treatment of respiratory diseases. Again, some novel herbal NDs together with NPs are under exploration for establishment for operative delivery of phytomedicine resulting in reduced toxic total side effects and enhanced patient compliance.

REFERENCES

1. Thorley AJ and Tetley TD. New perspectives in nanomedicine. *Pharmacology & Therapeutics* 2013; 140(2):176–185.
2. Pison U, Welte T, Giersig M, et al. Nanomedicine for respiratory diseases. *European Journal of Pharmacology*. 2006; 533(1–3): 341–350.
3. Bhaskar S, Tian F, Stoeger T, et al. Multifunctional nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: Perspectives on tracking and neuroimaging. *Particle and Fibre Toxicology*. 2010; 7(1): 3.
4. Rosi NL and Mirkin CA. Nanostructures in biodiagnostics. *Chemical Reviews*. 2005; 105(4): 1547–1562.
5. Roy I and Vij N. Nanodelivery in airway diseases: Challenges and therapeutic applications. *Nanomedicine-Nanotechnology, Biology and Medicine*. 2010; 6(2): 237–244.
6. Akerman ME, Chan WC, Laakkonen P, et al. Nanocrystal targeting in vivo. *Proceedings of the National Academy of Sciences*. 2002; 99(20): 12617–12621.
7. Moghimi SM, Hunter AC, andMurray JC. Nanomedicine: Current status and future prospects. *FASEB Journal*. 2005; 19(3): 311–330.
8. Rao KS, Ghorpade A, and Labhasetwar V. Targeting anti-HIV drugs to the CNS. *Expert Opinion on Drug Delivery*. 2009; 6(8): 771–784.
9. Buzea C, Pacheco II, and Robbie K. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases*. 2007; 2(4): MR17–MR71.
10. Foldvari M and Elsabahy M. Nanotechnology enables superior medical therapies. *Current Drug Delivery*. 2011; 8(3): 225–226.
11. Mansour HM, Rhee YS, and Wu X. Nanomedicine in pulmonary delivery. *International Journal of Nanomedicine*. 2009; 4: 299–319.
12. Jaques PA and Kim CS. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. *Inhalation Toxicology*. 2000; 12(8): 715–731.
13. Sadikot RT, Kolanjiyil AV, Kleinstreuer C, et al. Nanomedicine for treatment of acute lung injury and acute respiratory distress syndrome. *Biomedicine Hub*. 2017; 2(2): 1–12.
14. Resnier P, Mottais A, Sibiril Y, et al. Challenges and successes using nanomedicines for aerosol delivery to the airways. *Current Gene Therapy*. 2016; 16(1): 34–46.
15. van Rijt SH, Bein T, and Meiners S. Medical nanoparticles for next generation drug delivery to the lungs. *European Respiratory Journal*. 2014; 44: 765–774.
16. Codrons V, Vanderbist F, Verbeeck RK, et al. Systemic delivery of parathyroid hormone (1–34) using inhalation dry powders in rats. *Journal of Pharmaceutical Sciences*. 2003; 92(5): 938–950.
17. Patri A, Dobrovolskaia M, Stern S, et al. Preclinical characterization of engineered nanoparticles intended for cancer therapeutics. In: Mansoor M. Amiji (ed.) Nanotechnology for Cancer Therapy. CRC Press. 2006; 105–138.
18. Brar SK and Verma M. Measurement of nanoparticles by light-scattering techniques. *Trends in Analytical Chemistry*. 2011; 30(1): 4–17.
19. Hall JB, Dobrovolskaia MA, Patri AK, et al. Characterization of nanoparticles for therapeutics. *Nanomedicine (London)*. 2007; 2(6): 789–803.
20. Sapsford KE, Tyner KM, Dair BJ, et al. Analyzing nanomaterial bioconjugates: A review of current and emerging purification and characterization techniques. *Analytical Chemistry*. 2011; 83: 4453–4488.
21. Joshi M, Bhattacharyya A, and Ali SW. Characterization techniques for nanotechnology applications in textiles. *Indian Journal of Fibre & Textile Research*. 2008; 33: 304–317.

22. Cantor CR and Schimmel PR. Techniques for the Study of Biological Structure and Function. W. H. Freeman, San Francisco, CA. 1980.
23. Manaia EB, Abuçafy MP, Chiari-Andréo BG, et al. Physicochemical characterization of drug nanocarriers. *International Journal of Nanomedicine*. 2017; 12: 4991.
24. Johal MS. Understanding Nanomaterials. CRC Press, Boca Raton, FL. 2011.
25. Lin PC, Lin S, Wang PC, et al. Techniques for physicochemical characterization of nanomaterials. *Biotechnology Advances*. 2014; 32(4): 711–726.
26. Anjum DH. Characterization of nanomaterials with transmission electron microscopy. *IOP Conference Series: Materials Science and Engineering*. 2016; 146(1): 012001.
27. Akbari B, Tavandashti MP, and Zandrahimi M. Particle size characterization of nanoparticles-A practical approach. *Iranian Journal of Materials Science and Engineering*. 2011; 8(2): 48–56.
28. Smith DJ. Characterization of nanomaterials using transmission electron microscopy. In: Nanocharacterisation. 2, 2015; 1–29.
29. Clogston J and Patri A. Zeta potential measurement. In: McNeil SE. (ed.) Characterization of Nanoparticles Intended for Drug Delivery. Humana Press. 2011; 63–70.
30. Mueller R. Solid lipid nanoparticles (SLN) for controlled drug delivery-A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*. 2000; 50: 161–177.
31. Xu R. Progress in nanoparticles characterization: Sizing and zeta potential measurement. *Particuology*. 2008; 6: 112–115.
32. Paranjpe M and Müller-Goymann C. Nanoparticle-mediated pulmonary drug delivery: A review. *International Journal of Molecular Science*. 2014; 15(4): 5852–5873.
33. Liang Z, Ni R, Zhou J, et al. Recent advances in controlled pulmonary drug delivery. *Drug Discovery Today*. 2015; 20(3): 380–389.
34. Groneberg DA, Witt C, Wagner U, et al. Fundamentals of pulmonary drug delivery. *Respiratory Medicine*. 2003; 97(4): 382–387.
35. Jeevanandam J, San Chan Y, Danquah MK. Nanoformulations of drugs: Recent developments, impact and challenges. *Biochimie*. 2016; 128: 99–112.
36. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: Classification, preparation, and applications. *Nanoscale Research Letters*. 2013;8 (1): 102.
37. Müller RH, Mäder 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.
38. Mehnert W and Mäder K. Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*. 2012; 64: 83–101.
39. Sen S, Han Y, Rehak P, et al. Computational studies of micellar and nanoparticle nanomedicines. *Chemical Society Reviews*. 2018; 47(11): 3849–3860.
40. Kwon GS, Yokoyama M, Okano T, et al. Biodistribution of micelle-forming polymer–drug conjugates. *Pharmaceutical Research*. 1993; 10(7): 970–974.
41. Eichman JD, Bielinska AU, Kukowska-Latallo JF, et al. The use of PAMAM dendrimers in the efficient transfer of genetic material into cells. *Pharmaceutical Science and Technology Today*. 2000; 3(7): 232–245.
42. Soppimath KS, Aminabhavi TM, Kulkarni AR, et al. Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release*. 2001; 70(1-2): 1–20.
43. Langer R. Biomaterials in drug delivery and tissue engineering: One laboratory's experience. *Accounts of Chemical Research*. 2000; 33(2): 94–101.
44. Ueda M, Iwara A, and Kreuter J. Influence of the preparation methods on the drug release behaviour of loperamide-loaded nanoparticles. *Journal of Microencapsulation*. 1998; 15(3): 361–372.
45. Cheng YS. Mechanisms of pharmaceutical aerosol deposition in the respiratory tract. *AAPS PharmSciTech*. 2014; 15(3): 630–640.
46. Beck-Broichsitter M, Ruppert C, Schmehl T, et al. Biophysical investigation of pulmonary surfactant surface properties upon contact with polymeric nanoparticles in vitro. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2011; 7(3): 341–350.
47. Nassimi M, Schleh C, Lauenstein HD, et al. 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.
48. Reddy LH, Sharma RK, Chuttani K, et al. Influence of administration route on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in Dalton's lymphoma tumor bearing mice. *Journal of Controlled Release*. 2005; 105(3): 185–198.
49. Videira MA, Botelho MF, Santos AC, et al. Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. *Journal of Drug Targeting*. 2002; 10(8): 607–613.
50. Bakhtiary Z, Barar J, Aghanejad A, et al. Microparticles containing erlotinib-loaded solid lipid nanoparticles for treatment of non-small cell lung cancer. *Drug Development and Industrial Pharmacy*. 2017; 43(8): 1244–1253.
51. Khadka P, Ro J, Kim H, et al. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian Journal of Pharmaceutical Sciences*. 2014; 9(6): 304–316.
52. Beck-Broichsitter M, Merkel OM, and Kissel T. Controlled pulmonary drug and gene delivery using polymeric nano-carriers. *Journal of Controlled Release*. 2012; 161(2): 214–224.
53. Groneberg DA, Eynott PR, Doring F, et al. Distribution and function of the peptide transporter PEPT2 in normal and cystic fibrosis human lung. *Thorax*. 2002; 57: 55–60.
54. Groneberg DA, Nickolaus M, Springer J, et al. Localization of the peptide transporter PEPT2 in the lung: Implications for pulmonary oligopeptide uptake. *American Journal of Pathology*. 2001; 158: 707–714.
55. Gukasyan HJ, Uchiyama T, Kim KJ, et al. Oligopeptide transport in rat lung alveolar epithelial cells is mediated by Pept2. *Pharmaceutical Research*. 2017; 34(12): 2488–2497.
56. Sebbage V. Cell-penetrating peptides and their therapeutic applications. *Bioscience Horizons*. 2009; 2(1): 64–72.

57. Ishiguro S, Alhakamy NA, Uppalapati D, et al. Combined local pulmonary and systemic delivery of AT2R gene by modified TAT peptide nanoparticles attenuates both murine and human lung carcinoma xenografts in Mice. *Journal of Pharmaceutical Sciences*. 2017; 106(1): 385–394.
58. Prajapati S, Saha S, Kumar CD, et al. Nebulized drug delivery: An overview. *International Journal of Pharmaceutical Sciences and Research*. 2019; 10(8): 3575–3582.
59. Makled S, Nafee N, and Boraie N. Nebulized solid lipid nanoparticles for the potential treatment of pulmonary hypertension via targeted delivery of phosphodiesterase-5-inhibitor. *International Journal Pharmaceutics*. 2017; 517(1–2): 312–321.
60. Nafee N, Makled S, and Boraie N. Nanostructured lipid carriers versus solid lipid nanoparticles for the potential treatment of pulmonary hypertension via nebulization. *European Journal of Pharmaceutical Sciences*. 2018; 125: 151–162.
61. Upadhyay S, Ganguly K, and Palmberg L. Wonders of nanotechnology in the treatment for chronic lung diseases. *Journal of Nanomedicine and Nanotechnology*. 2015; 6(6): 1.
62. Yin LM, Jiang GH, Wang Y, et al. Use of serial analysis of gene expression to reveal the specific regulation of gene expression profile in asthmatic rats treated by acupuncture. *Journal of Biomedical Science*. 2009; 16(46): 1–13.
63. Szelenyi I. Nanomedicine: Evolutionary and revolutionary developments in the treatment of certain inflammatory diseases. *Inflammation Research*. 2012; 61(1): 1–9.
64. Tan LD, Bratt JM, Godor D, et al. Benralizumab: A unique IL-5 inhibitor for severe asthma. *Journal of Asthma and Allergy*. 2016; 9: 71–81.
65. Inapagolla R, Guru BR, Kurtoglu YE, et al. In vivo efficacy of dendrimer-methylprednisolone conjugate formulation for the treatment of lung inflammation. *International Journal of Pharmaceutics*. 2010; 399: 140–147.
66. Wang L, Feng M, Li Q, et al. Advances in nanotechnology and asthma. *Annals of Translational Medicine*. 2019; 7(8): 180.
67. Nasr M, Najlah M, D'Emanuele A, et al. PAMAM dendrimers as aerosol drug nanocarriers for pulmonary delivery via nebulization. *International Journal of Pharmaceutics*. 2014; 461: 242–250.
68. Kim DE, Lee Y, Kim M, et al. Bilirubin nanoparticles ameliorate allergic lung inflammation in a mouse model of asthma. *Biomaterials*. 2017; 140: 37–44.
69. Chen X, Huang W, Wong BC, et al. Liposomes prolong the therapeutic effect of anti-asthmatic medication via pulmonary delivery. *International Journal of Nanomedicine*. 2012; 7: 1139–1148.
70. Kumar M, Kong X, Behera AK, et al. Chitosan IFN-gamma-pDNA nanoparticle (CIN) therapy for allergic asthma. *Genetic Vaccines and Therapy*. 2003; 1: 3.
71. Lanza GM, Jenkins J, Schmieder AH, et al. Anti-angiogenic nanotherapy inhibits airway remodeling and hyper-responsiveness of dust mite triggered asthma in the Brown Norway rat. *Theranostics*. 2017; 7(2): 377.
72. Wang W, Chen T, Xu H, et al. Curcumin-loaded solid lipid nanoparticles enhanced anticancer efficiency in breast cancer. *Molecules*. 2018; 23(7): 1578.
73. Chakraborty S, Ehsan I, Mukherjee B, et al. Therapeutic potential of andrographolide-loaded nanoparticles on a murine asthma model. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2019;20:102006
74. Adeloye D, Chua S, Lee C, et al. Global Reference Health Epidemiology Group. Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. *Journal of Global Health*. 2015; 5(2): 020415.
75. Mercado N, Ito K, and Barnes PJ. Accelerated ageing of the lung in COPD: New concepts. *Thorax*. 2015; 70(5): 482–489.
76. Sadikot RT. The potential role of nanomedicine in lung diseases. *Medical Research Archives*. 2018; 6(5): 1–9.
77. Ghosh N, Singh B, and Chaudhury K. Recent advances in nanomedicine for respiratory diseases: A systematic review. *Anti-Cancer Drugs*. 2014; 18: 23.
78. Verbanck S and Paiva M. Gas mixing in the airways and airspaces. *Comprehensive Physiology*. 2011; 1(2): 809–834.
79. Lai SK, O'Hanlon DE, Harrold S, et al. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proceedings of the National Academy of Science*. 2007; 104(5): 1482–1487.
80. Vij N, Min T, Bodas M, et al. Neutrophil targeted nanodrug delivery system for chronic obstructive lung diseases. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2016; 12(8): 2415–2427.
81. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet*. 2004; 364(9435): 709–721.
82. Boland S, Guadagnini R, Baeza-Squiban A, et al. Nanoparticles used in medical applications for the lung: Hopes for nanomedicine and fears for nanotoxicity. *Journal of Physics: Conference Series*. 2011; 304: 012031.
83. Alber A, Howie SE, Wallace WA, and Hirani N. The role of macrophages in healing the wounded lung. *International Journal of Experimental Pathology*. 2012; 93(4): 243–251.
84. Al Faraj A, Shaik AS, Afzal S, Al Sayed B, and Halwani R. MR imaging and targeting of a specific alveolar macrophage subpopulation in LPS-induced COPD animal model using antibody-conjugated magnetic nanoparticles. *International Journal of Nanomedicine*. 2014; 9: 1491.
85. Ballester M, Nembrini C, Dhar N, et al. Nanoparticle conjugation and pulmonary delivery enhance the protective efficacy of Ag85B and CpG against tuberculosis. *Vaccine*. 2011; 29(40): 6959–6966.
86. Gordillo-Marroquín C, Gómez-Velasco A, Sánchez-Pérez H, et al. Magnetic nanoparticle-based biosensing assay quantitatively enhances acid-fast bacilli count in paucibacillary pulmonary tuberculosis. *Biosensors*. 2018; 8(4): 128.
87. Bothamley G. The tuberculosis network European trials group (TBNET): New directions in the management of tuberculosis. *Breathe*. 2017; 13(3): 65–71.
88. Costa A, Pinheiro M, Magalhães J, et al. The formulation of nanomedicines for treating tuberculosis. *Advanced Drug Delivery Reviews*. 2016; 102: 102–115.
89. Sun Z, Zhang J, Song H, et al. Concomitant increases in spectrum and level of drug resistance in Mycobacterium tuberculosis isolates. *International Journal of Tuberculosis and Lung Disease*. 2010; 14(11): 1436–1441.
90. Wallis RS and Hafner R. Advancing host-directed therapy for tuberculosis. *Nature Reviews Immunology*. 2015; 15(4): 255.

91. Gao L, He X, Ju L, et al. A label-free method for the detection of specific DNA sequencesusing gold nanoparticles bifunctionalized with a chemiluminescent reagent and a catalyst as signal reporters. *Analytical and Bioanalytical Chemistry*. 2016; 408(30): 8747–8754.
92. Hawkridge T and Mahomed H. Prospects for a new, safer and more effective TB vaccine. *Paediatric Respiratory Reviews*. 2011; 12(1): 46–51.
93. Mehanna MM, Mohyeldin SM, and Elgindy NA. Respirable nanocarriers as a promising strategy for antitubercular drug delivery. *Journal of Controlled Release*. 2014; 187: 183–197.
94. Källenius G, Pawlowski A, Brandtzaeg P, et al. Should a new tuberculosis vaccine be administered intranasally? *Tuberculosis (Edinburgh)*. 2007; 87(4): 257–266.
95. Saifullah B, Chrzastek A, Maitra A, et al. Novel antituberculosis nanodelivery formulation of ethambutol with graphene oxide. *Molecules*. 2017; 22(10): 1560.
96. Sosnik A, Carcaboso AM, Glisoni RJ, Moretton MA, and Chiappetta D. New old challenges in tuberculosis: Potentially effective nanotechnologies in drug delivery. *Advanced Drug Delivery Reviews*. 2010; 62(4–5): 547–559.
97. Anabousi S, Kleemann E, Bakowsky U, et al. Effect of PEGylation on the stability of liposomes during nebulisation and in lung surfactant. *Journal of Nanoscience and Nanotechnology*. 2006; 6(9–10): 3010–3016.
98. Finlay WH and Wong JP. Regional lung deposition of nebulized liposome-encapsulated ciprofloxacin. *International Journal of Pharmaceutics*. 1998;167(1–2): 121–127.
99. Pandey R and Khuller GK. Nanotechnology based drug delivery system(s) for the management of tuberculosis. *Indian Journal of Experimental Biology*. 2006; 44: 357–366.
100. Deol P, Khuller G, and Joshi K. Therapeutic efficacies of isoniazid and rifampin encapsulated in lung-specific stealth liposomes against Mycobacterium tuberculosis infection induced in mice. *Antimicrobial Agents and Chemotherapy*. 1997; 41(6): 1211–1214.
101. Foradada M, Pujol MD, Bermúdez J, and Estelrich J. Chemical degradation of liposomes by serum components detected by NMR. *Chemistry and Physics of Lipids*. 2000; 104(2): 133–148.
102. Clemens DL, Lee BY, Xue M, et al. Targeted intracellular delivery of antituberculosis drugs to Mycobacterium tuberculosis-infected macrophages via functionalized mesoporous silica nanoparticles. *Antimicrobial Agents and Chemotherapy*. 2012; 56(5): 2535–2545.
103. Jemal A, Bray F, Center MM, Ferlay J, Ward E, and Forman D. Global cancer statistics. *CA Cancer Journal for Clinicians*. 2011; 61(2): 69–90.
104. Gennatas S, Noble J, Stanway S, et al. Patterns of relapse in extrapulmonary small cell carcinoma: Retrospective analysis of outcomes from two cancer centres. *BMJ Open*. 2015; 5(1): e006440.
105. Burrell RA, McGranahan N, Bartek J, and Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. *Nature*. 2013; 501(7467): 338–345.
106. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, and Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology*. 2007; 2(12): 751–760.
107. Choi SH, Byeon HJ, Choi JS, et al. Inhalable self-assembled albumin nanoparticles for treating drug-resistant lung cancer. *Journal of Controlled Release*. 2015; 197: 199–207.
108. Yuan D, Lv Y, Yao Y, et al. Efficacy and safety of Abraxane in treatment of progressive and recurrent non-small cell lung cancer patients: A retrospective clinical study. *Thoracic Cancer*. 2012; 3(4): 341–347.
109. Akhter S, Ahmad I, Ahmad MZ, et al. Nanomedicines as cancer therapeutics: Current status. *Current Cancer Drug Targets*. 2013; 13(4): 362–378.
110. Landesman-Milo D, Ramishetti S, and Peer D. Nanomedicine as an emerging platform for metastatic lung cancer therapy. *Cancer and Metastasis Reviews*. 2015; 34(2): 291–301.
111. Roa WH, Azarmi S, Al-Hallak MH, et al. Inhalable nanoparticles, a non-invasive approach to treat lung cancer in a mouse model. *Journal of Controlled Release*. 2011; 150: 49–55.
112. Kalantarian P, Najafabadi AR, Haririan I, et al. Preparation of 5-fluorouracil nanoparticles by supercritical antisolvents for pulmonary delivery. *International Journal of Nanomedicine*. 2010; 5: 763–770.
113. Wu SY, Singhania A, Burgess M, Putral LN, Kirkpatrick C, Davies NM, et al. Systemic delivery of E6/7 siRNA using novel lipidic particles and its application with cisplatin in cervical cancer mouse models. *Gene Therapy*. 2011; 18: 14e22.
114. David S, Resnier P, Guillot A, Pitard B, Benoit JP, and Passirani C. siRNA LNCs e a novel platform of lipid nanocapsules for systemic siRNA administration. *European Journal of Pharmaceutics and Biopharmaceutics*. 2012; 81: 448e52.
115. Xia W, Wang J, Xu Y, Jiang F, and Xu, L. L-BLP25 as a peptide vaccine therapy in non-small cell lung cancer: A review. *Journal of Thoracic Disease*. 2014; 6(10): 1513.
116. Savolainen K, Backman U, Brouwer D, et al. Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations. Finnish Institute of Occupational Health. 2013.
117. Cockburn A, Bradford R, Buck N, et al. Approaches to the safety assessment of engineered nanomaterials (ENM) in food. *Food and Chemical Toxicology*. 2012; 50(6): 2224–2242.
118. Caster JM, Patel AN, Zhang T, and Wang A. Investigational nanomedicines in 2016: A review of nanotherapeutics currently undergoing clinical trials. *Wiley Interdisciplinary Reviews Nanomedicine and Nanobiotechnology*. 2017; 9(1): e1416.
119. Eifler AC and Thaxton CS. Nanoparticle therapeutics: FDA approval, clinical trials, regulatory pathways, and case study. In: Hurst SJ. (ed.) Biomedical Nanoetechnology. Humana Press, US. 2011; 325–338.
120. Kim J, Chankeshwara SV, Thielbeer F, et al. Surface charge determines the lung inflammogenicity: A study with polystyrene nanoparticles. *Nanotoxicology*. 2016; 10(1): 94–101.
121. Bobo D, Robinson KJ, Islam J, Thurecht KJ, and Corrie SR. Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to date. *Pharmaceutical Research*. 2016; 33(10): 2373–2387.

122. Wolfram J, Zhu M, Yang Y, et al. Safety of nanoparticles in medicine. *Current Drug Targets*. 2015;1 6(14): 1671–1681.
123. Ventola CL. Progress in nanomedicine: Approved and investigational nanodrugs. *Pharmacology & Therapeutics*. 2017; 42(12): 742.
124. Ferreira AJ, Cemlyn-Jones J, and Cordeiro CR. Nanoparticles, nanotechnology and pulmonary nanotoxicology. *Revista Portuguesa de Pneumologia (English Edition)*. 2013; 19(1): 28–37.
125. Vinardell MP. Toxicological highlight: In vitro cytotoxicity of nanoparticles in mammalian germ-line stem cell. *Toxicological Sciences*. 2005; 88(2): 285–286.
126. Chan VS. Nanomedicine: An unresolved regulatory issue. *Regulatory Toxicology and Pharmacology*. 2006; 46(3): 218–224.
127. Oberdörster G. Safety assessment for nanotechnology and nanomedicine: Concepts of nanotoxicology. *Journal of Internal Medicine*. 2010; 267(1): 89–105.
128. Muhlfeld C, Rothen-Rutishauser B, Blank F, Vanhecke D, Ochs M, and Gehr P. Interactions of nanoparticles with pulmonary structures and cellular responses. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2008; 294(5): L817–L829.
129. Nel AE, Mädler L, Velegol D, et al. Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials*. 2009; 8: 543–557.
130. Donaldson K, Borm P, Castranova V, and Gulumian M. The limits of testing particle-mediated oxidative stress in vitro in predicting diverse pathologies; Relevance for testing of nanoparticles. *Particle and Fibre Toxicology*. 2009; 6: 13.
131. Kumar A, Dailey LA, and Forbes B. Lost in translation: What is stopping inhaled nanomedicines from realizing their potential? *Therapeutic Delivery*. 2014; 5(7): 757–761.
132. Nel A, Xia T, Madler L, et al. Toxic potential of materials at the nanolevel. *Science*. 2006; 311: 622–627.
133. Monteiro-Riviere NA, Inman AO, and Zhang LW. Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line. *Toxicology and Applied Pharmacology*. 2009; 234(2): 222–235.
134. Maurer-Jones MA and Haynes CL. Toward correlation in in vivo and in vitro nanotoxicology studies. *The Journal of Law, Medicine & Ethics*. 2012; 40(4): 795–801.
135. Brook RD, Franklin B, Cascio W, et al. Air pollution and vascular disease. A statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation.* 2004; 109: 2655–2671.
136. Bölükbas DA and Meiners S. Lung cancer nanomedicine: Potentials and pitfalls. *Nanomedicine*. 2015; 10(21): 3203–3212.
137. Suzuki H, Aoki K, Chiba K, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nature Genetics*. 2015; 47(5): 505–511.
138. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015; 348(6230): 124–128.
139. Kim J, Chankeshwara SV, Thielbeer F, et al. Surface charge determines the lung inflammogenicity: A study with polystyrene nanoparticles. *Nanotoxicology*. 2016; 10(1): 94–101.
140. Explore 320,846 Research Studies in All 50 States and in 209 Countries. https://clinicaltrials.gov
141. https://www.medicines.org.uk/emc/product/6450/smpc
142. Nakamura K, Matsubara H, Akagi S, et al. Nanoparticle-mediated drug delivery system for pulmonary arterial hypertension. *Journal of Clinical Medicine*. 2017; 6(5): 48.