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Review Article

Evaluating the Controlled Release Properties of Inhaled Nanoparticles Using Isolated, Perfused, and Ventilated Lung Models

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Polymeric nanoparticles meet the increasing interest for inhalation therapy and hold great promise to improve controlled drug delivery to the lung. The synthesis of tailored polymeric materials and the improvement of nanoparticle preparation techniques facilitate new perspectives for the treatment of severe pulmonary diseases. The physicochemical properties of such drug delivery systems can be investigated using conventional analytical procedures. However, the assessment of the controlled drug release properties of polymeric nanoparticles in the lung remains a considerable challenge. In this context, the isolated lung technique is a promising tool to evaluate the drug release characteristics of nanoparticles intended for pulmonary application. It allows measurements of lung-specific effects on the drug-release properties of pulmonary delivery systems. *Ex vivo* models are thought to overcome the common obstacles of *in vitro* tests and offer more reliable drug release and distribution data that are closer to the *in vivo* situation.

1. Introduction

Pulmonary drug delivery has become a well-established approach in the treatment of respiratory diseases and offers several advantages over other routes of administration. Inhalation therapy enables the direct application of a drug to the respiratory tract. The "local" or "regional" deposition of the administered drug facilitates a targeted treatment of respiratory diseases avoiding high-dose exposures to the systemic circulation. With the direct delivery of therapeutic agents to the desired site of action, rapid onset of drug action, lower systemic exposure, and consequently, reduced side effects can be achieved. Site-specific or targeted delivery, therefore, would also enable a reduction in the necessary dose to be administered [1–4]. A significant disadvantage of inhalation therapy is the relatively short duration of drug action demanding multiple daily inhalation maneuvers, ranging up to 9 times a day [5]. Moreover, "conventional" inhalation therapy does not permit targeted cell-specific drug delivery or modified biological distribution of drugs, both at the organ and cellular level, and drug deposition in different lung areas is only poorly controllable [6–8].

Strategies for further advancements of inhalation therapy include the development of aerosolizable controlled release formulations with the aim to improve the drug effect, as well as the patient's convenience and compliance. A large number of carrier systems have been conceived and investigated as potential controlled drug delivery formulations to the lung [9–11]. In the recent years, nanomedicine has become an attractive concept for the controlled and targeted delivery of therapeutic and diagnostic compounds to the desired site of action. Nanotechnology opens new perspectives in the design of novel drug delivery vehicles that not only facilitate targeting of an organ, tissues, cells, or subcellular compartments, but also affect the duration and the intensity of the pharmacological effect [12–16]. In particular, nanoparticulate drug delivery systems enable the controlled delivery of the pharmacological agent to its site of action at a therapeutically optimal rate and dose regimen [17–19]. Among the various drug delivery systems considered for pulmonary application,

polymeric nanoparticles demonstrate several advantages for the treatment of respiratory diseases, for example prolonged drug release and cell-specific targeted drug delivery [20–24].

Numerous manufacturing techniques are known for the production of drug-loaded polymeric nanoparticles. The choice of the nanoparticle preparation technique essentially depends on the physicochemical properties of the polymeric nanoparticle matrix material intended to be used and on the active compound to be encapsulated in the nanoparticles [25, 26]. Regarding the polymeric nanoparticle matrix material, criteria such as biocompatibility and degradability determine its selection [27–29]. Moreover, for an effective nanoparticulate drug delivery system, sufficient drug loading and controlled drug release over a predetermined period of time must be ensured. The characteristics of drug release, that is, release mechanism and release rate, from drug-delivery systems vary according to the type of employed encapsulation technique and the physicochemical properties (interaction) of drug and polymer. The release from polymeric nanoparticles *in vitro* is normally fast (several minutes to hours) due to the short distance drugs have to cover to diffuse out of the particles. The release rate of drugs from nanoparticles is also strongly influenced by the biological environment. Nanoparticles may interact with biological components like proteins and cells that alter the release rate of drugs from nanoparticles. As a consequence, the *in vitro* drug release characteristics may not predict the release situation *in vivo*. Moreover, a precise assessment of the *in vitro* drug release from nanoparticles is technically difficult to achieve, which is mainly attributed to the inability of rapid separation of the nanoparticles from the dissolved or released drug in the surrounding medium [17, 30, 31].

Different methods have been used to characterize the behavior of pulmonary administered drug-loaded carriers in biological systems. These range from *in vitro* cell culture methods to *in vivo* pharmacokinetic analysis. *Ex vivo* isolated, perfused, and ventilated lung models have been utilized in numerous pharmacological and toxicological studies to elucidate the fate of inhaled drugs or toxic substances. In *ex vivo* lung models, lung-specific pharmacokinetic effects, like drug absorption and distribution profiles, can be investigated without the contribution of systemic absorption, distribution, and elimination of the drug. Moreover, it is possible to elucidate the effect of the interaction of nanoparticles with the natural pulmonary environment on the release of encapsulated drugs. Accordingly, more reliable drug release and distribution data are obtained that are closer to the *in vivo* situation [6, 32–34].

It is interesting to note that the first investigations regarding the use of polymeric nanoparticles as drug carriers for the controlled and targeted delivery of drugs to the desired site of action have been reported in the mid 1970's [35]. It was shown that the "natural" drug distribution after systemic application was altered by the encapsulation of drug into polymeric nanoparticles. Since then, great efforts have been made in this field, and several treatment modalities for cancer on the basis of polymeric nanostructured drug delivery vehicles have been developed and made clinically available, for example, Abraxane, Transdrug, and Genexol-PM [25, 36, 37]. In contrast to systemic administration, the regional application of drug-loaded nanoparticles to the respiratory tract has been so far incompletely investigated. This is attributed on one hand to the limited efficiency of conventional devices to generate nanoparticle-containing aerosols and on the other hand to the lack of methods to assess the drug release form and the distribution behavior of pulmonary administered nanoparticulate drug-delivery systems [38, 39]. Meanwhile, technological advances have led to improved designs for aerosol-generation devices that solve the main drawbacks, and the key attributes associated with successful nanoparticle aerosolization have been identified [40–42]. However, the prediction of drug release and distribution from pulmonary administered nanoparticulate-delivery systems remains a major challenge.

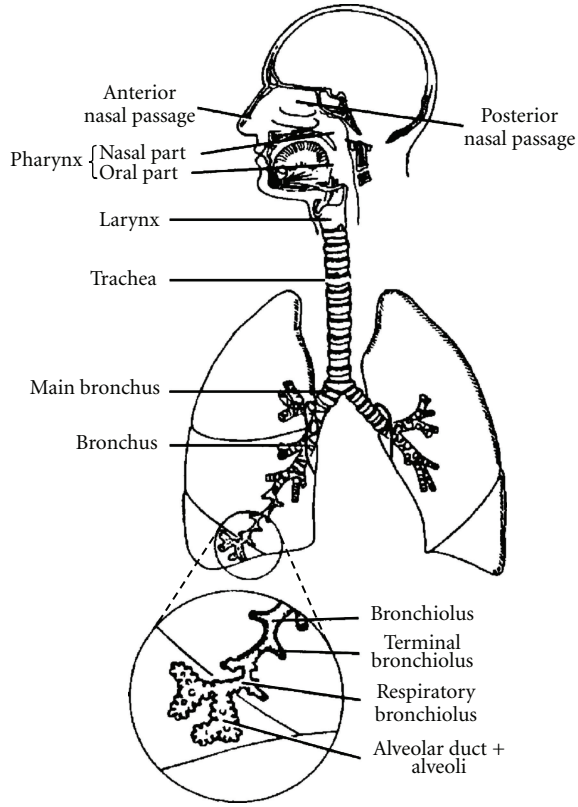

Figure 1: Schematic of the human respiratory system (Adapted from [43]).

2. Structure and Function of the Lung

The development of drug delivery systems for pulmonary application requires a detailed knowledge of the lung in its healthy, as well as various diseased states. The lung is composed of more than 40 different cell types, of which approximately one-third are epithelial cells [44, 45]. The conducting zone includes the nasal cavity, pharynx, larynx, trachea, bronchi, and bronchioles, while the respiratory zone, where the gas exchange takes place, includes respiratory bronchioles and alveoli (Figure 1). The conducting airways

exhibit 16 bifurcations, comprising the trachea, the bronchi, and the bronchioles. The terminal bronchioles represent the passage to the respiratory region, which exhibits another 6 bifurcations. The respiratory region includes the respiratory bronchioles, from which the alveolar ducts with alveolar sacs branch off [46]. The airways also fulfill some other essential functions, such as warming, humidifying, and cleaning of the inhaled air. Warming and humidifying of the inspired air predominantly take place in the nasal cavity and the pharynx. In the deeper airways this process continues, so that the air finally reaching the alveoli has body heat and is completely saturated with water. Also the cleaning of the inspired air partly takes place in the nose; dust, bacteria, and particles are caught by impaction. Further inhaled substances deposit on the mucus layer which coats the walls of the conducting airways. The mucus is secreted by goblet and submucosal gland cells and forms a gel like layer consisting of mucin as the major component [47]. Ciliated cells are another important type of cells which predominate in the bronchial epithelia of the conducting region. Their major function is the propulsion of mucus upwards and out of the lung (bronchotracheal escalator), thus the lung will be cleared of foreign substances [48, 49]. Beneath, in the respiratory bronchioles the epithelium consists of ciliated cells and Clara cells.

In the alveolar space there is no mucus layer, but a complex surfactant lining that covers the alveolar epithelium and reduces the surface tension to prevent collapse of the alveoli during breathing [50]. It contains approximately 90% lipids and 10% proteins [51]. The lipids in the surface lining material consist mainly of phospholipids (~80–90%) and a minor portion of neutral lipids (~10–20%). Among the phospholipids, phosphatidylcholines (~70–80%) and phosphatidylglycerols (~10%) represent the predominant classes, with minor amounts of phosphatidylinositols, phosphatidylserines, and phosphatidylethanolamines [52]. About half of the protein mass of the alveolar lining layer is composed of the surfactant-associated proteins SP-A and SP-D, which are high molecular weight hydrophilic proteins, and SP-B and SP-C, which are low molecular weight hydrophobic proteins [53]. The surfactant proteins SP-A and SP-D have been identified as playing a fundamental role in innate immunity. A complex interaction between phospholipids and SP-B and SP-C enables the decrease of surface tension in the alveolar region to values of ~0 mN/m during compression/expansion cycles [54, 55]. Pulmonary surfactant is secreted by type II pneumocytes, which cover only 5% of the total alveolar surface. Beside the production of pulmonary surfactant, alveolar type II cells play a role in alveolar fluid balance, coagulation and fibrinoylsis, host defense and proliferation, and differentiation into type I cells [56]. Type I pneumocytes are very thin (≤200 nm) with a large extension (~200 μm), covering over ~95% of the alveolar epithelial surface [57]. They form the primary diffusion barrier between air and blood which is highly permeable for water, gases, and hydrophobic molecules, while it is poorly permeable for large hydrophilic substances (peptides and proteins) or ionic species. Macromolecules pass this barrier by active transport mechanisms [7, 58, 59]. In addition to epithelial cells, the alveoli contain macrophages that engulf particles, potentially digest them, and slowly migrate with their payload out of the respiratory tract, either following along the mucociliary escalator or (to a lesser degree) the lymphatic system. Thus, the pulmonary endocytosis by macrophages represents the main mechanism of removing solid particles in the alveolar region [60, 61].

Nanoparticles have been praised for their advantageous drug delivery properties to the lung, such as avoidance of mucociliary and macrophage clearance and long residence times until degradation or translocation by epithelial cells takes place [6, 62–70].

3. Pulmonary Drug Delivery

3.1. The Lung as a Route of Application for Systemic and Local Therapy. Although the lung represents effective barrier systems and clearance mechanisms much attention has been raised in the last decades to this organ for drug delivery applications. One reason is its large absorption area. The lung build up a total surface of ~100 m^2 that is enveloped by an equally large capillary network, from which many agents can be readily absorbed to the bloodstream avoiding a first-pass-effect of the liver. Another reason is the known instability and low permeability of proteins and peptides when these biopharmaceuticals are administered through the widely preferred oral route. Consequently, most proteins and peptides on the market are administered intravenously. But the parenteral route of application does generally not meet with patients' convenience and compliance, in particular because the indication for the use of these agents is usually treatment of a chronic disease requiring frequent injections. Thus, the pulmonary route of application offers a noninvasive alternative for systemic therapy [71–77]. However, systemic macromolecule delivery via the lung has suffered setbacks as for example demonstrated for pulmonary administered insulin (Exubera) that was withdrawn from the market in 2008 for commercial and health-risk reasons [78, 79].

A large number of small molecular weight drugs are employed for the targeted treatment of respiratory diseases following inhalation. This basic concept of targeted drug therapy has been followed for a long time in the treatment of airway diseases. In particular, the application of β_2-agonists and corticosteroids by means of inhalation has improved the therapy of bronchial asthma and chronic obstructive pulmonary disease targeting the smooth musculature of the bronchi and immunologically competent intrapulmonary cells [80, 81]. In addition, the endothelial cells or the smooth muscle cells surrounding the pulmonary vessels present a target of inhalative drug therapy. As an example, prostaglandin derivatives have been recently introduced for aerosol therapy of pulmonary arterial hypertension [82, 83].

3.2. Devices for Aerosol Generation. Over the past decades several devices have been conceived and developed for the administration of drugs to the respiratory tract, namely pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs), and nebulizers [84–87]. pMDIs are hand-held devices that use pressurized propellants to atomize

the drug solution, suspension, or emulsion. These devices generally require a coordinative inhalation by the patient [88]. DPIs do not only differ in the principle of aerosol particle generation and delivery, but also with regard to design differences such as discrete or reservoir drug containment and the number of doses [89]. While the drugs are released from pMDI by the utilization of propellants, DPIs operate by using the inspiratory flow of the patient for disintegration of the powder and dose entrainment. Thus, reproducibility of the inhaled dose from these devices is extremely dependant on the patient [90]. Several types of nebulizers are available for aerosol generation for pulmonary drug delivery, namely jet nebulizers, ultrasonic nebulizers, and nebulizers that use a vibrating-mesh technology for aerosol generation [91]. Jet nebulizers are driven by compressed air. The liquid is dispersed into small droplets (<5-6 μm) by passing through a narrow nozzle orifice and multiple impactions on a baffle structure. In general, the droplet size distribution of a nebulizer and the output rate are also influenced by the physical properties of the drug solution and the air flow rate from the compressor [92]. Ultrasonic nebulizers use a piezoelectric transducer in order to create droplets from an open liquid reservoir. As the energy is transferred through the liquid container it becomes evident that the properties of the drug formulation have strong effects on the aerosol particle size and the output rate [93]. Vibrating-mesh nebulizers use perforated membranes actuated by an annular piezoelement to vibrate in resonant bending mode. The holes in the membrane have a large cross-section size on the liquid supply side and a narrow cross-section size on the side from where the droplets emerge. Depending on the therapeutic application, the hole sizes (2 μm and upwards) can be adjusted, as well as the number of holes [40, 41, 94].

3.3. Polymeric Nanoparticles as Inhalative Drug-Delivery Vehicles. Nanomaterials exploit novel physical, chemical, and biological properties [14–16]. The general aim of controlled release formulations is the modification of pharmacokinetics and thus, improved pharmacodynamic characteristics at the target site. A successful drug delivery system needs to demonstrate optimal drug loading and release properties, and low toxicity [17, 20, 23, 24, 95–98]. Nanoparticle formulations for this purpose with a mean size between 50 and 300 nm normally consist of polymeric materials. Polymers with particular physical or chemical characteristics, such as biocompatibility, degradability, or responsiveness to environmental changes have been predominantly used [99]. In addition to biocompatibility and degradability of the applied polymer, sufficient association of the therapeutic agent with the carrier particles and controlled and targeted drug release properties, nanoparticles need to meet further standards, such as protection of the drug against degradation, ability to be transferred into an aerosol, and stability against forces generated during aerosolization. Nanoparticles composed of biodegradable polymers fulfill the stringent requirements placed on these delivery systems [22–24].

Due to their well-established biocompatibility and biodegradability, aliphatic polyesters like polylactide (PLA) and poly(lactide-*co*-glycolide) (PLGA) are the most extensively used materials for biomedical applications [27]. However, linear polyesters have many limitations as nanoparticle matrix materials. Firstly, PLGA nanoparticles degrade over a period of weeks to months, but typically deliver drugs for a much shorter period of time. Slow or nondegrading polymers may lead to an unwanted accumulation in the lung when repeated administrations are needed, and may cause inflammatory processes [20, 95]. One way to overcome this problem is to synthesize polymers with faster degradation rates. Fast-degrading polymers are obtained by grafting of short PLGA chains onto polyvinyl alcohol backbones [100, 101]. The adjustable properties of these branched polyesters make them highly suitable for pulmonary formulations, especially with regard to biodegradation rates and *in vitro* cytotoxicity [102, 103]. Moreover, these types of biodegradable polyester revealed no signs of inflammatory response *in vivo* [104]. Their amphiphilic properties allows the generation of nanoparticles without the use of additional surfactant stabilizers [105, 106]. Another type of biodegradable polymer suitable for pulmonary application is based on ether-anhydride terpolymers consisting of poly(ethylene glycol), sebacic acid, and 1,3-bis(carboxyphenoxy)propane. These polymers are known to form aerosolizable particles and to exhibit fast degradation rates (half-life <12 h) [107–109].

Secondly, for an effective nanoparticulate-delivery system, sufficient drug-loading and tailored release properties must be ensured. Nanoparticles prepared from hydrophobic polymers, like PLGA, often incur the drawback of poor incorporation of low molecular weight hydrophilic drugs due to the low affinity of the drug compounds to the polymers [110, 111]. The introduction of charged functional groups within the polymer structure, like for example described by Wittmar et al. and Wang et al., promotes electrostatic interactions with oppositely charged drugs, thereby improving the design of nanoparticulate carriers [112, 113].

The release rate and release mechanism from drug-delivery systems vary according to the carrier vehicle, as well as to the properties of the employed drug and polymer combination. The *in vitro* release pattern from polymeric nanoparticles used in the field of medicine and pharmacy is of importance for characterization purposes and for quality control reasons. The release of drug compounds from nanoparticulate drug delivery systems is a result of the direct interaction of nanoparticles with their environment and is thought to be dependent upon desorption of the surface-bound, adsorbed drug, diffusion through the nanoparticle matrix, and rate of polymer degradation. Thus, diffusion and biodegradation govern the process of drug release from polymeric nanoparticles [17, 30, 31].

Several manufacturing techniques are known for the production of drug-loaded polymeric nanoparticles, allowing extensive modulation of their characteristics and control of their behavior at the target site. Conventionally, two groups of preparation methods can be distinguished. The first involves polymerization of monomers whereas the second is based on precipitation of preformed, well-defined natural or synthetic polymers, as for example used in salting out,

emulsion evaporation, emulsification diffusion, and solvent displacement. The choice of the nanoparticle preparation technique essentially depends on the physicochemical properties of the polymeric nanoparticle matrix material intended to be used and on the active compound to be encapsulated in the nanoparticles. One way to encapsulate the drug into the nanoparticles is accomplished by the preparation of nanoparticles in the presence of the therapeutic agent, what leads to a "homogeneous" distribution of drug within the polymer matrix. Another way to associate drug and polymer is achieved by subsequent sorption of the drug to unloaded nanoparticles either to the surface or the bulk of nanoparticles. The type of binding may also result in different release mechanisms and release rates [16, 17, 25, 26, 28, 29, 31].

Overall, the final choice of the appropriate polymer, manufacturing technique, and nanoparticle characteristics will primarily depend on the biocompatibility and degradability of the polymer, secondarily on the physicochemical characteristics of the drug, and thirdly on the therapeutic goal to be reached [31].

Owing to the advantageous drug delivery properties of polymeric nanoparticles, researchers were encouraged to find suitable application forms for pulmonary delivery. Their small size limits pulmonary deposition as nanoparticles alone are expected to be exhaled after inhalation [114]. In general, aerosol particle size is characterized by the mass median aerodynamic diameter (MMAD). The MMAD is used to describe the particle size distribution of any aerosol statistically based on the weight and size of the particles. Thus, a group of very dense particles will exhibit a larger MMAD than that of a group of less dense particles, despite an identical geometric size. It is well understood that pulmonary deposition is achieved by three principal mechanisms: inertial impaction, sedimentation, and diffusion. Impaction predominates during the passage through the oropharynx and large conducting airways if the particles possess a MMAD of $>5\,\mu$m, or have a high velocity. Gravitational force leads to sedimentation of smaller particles (MMAD of $<3\,\mu$m) in the smaller airways. Additionally, sedimentation increases by breath holding. In the range below a MMAD of $1\,\mu$m, particles are deposited by diffusion, which is based on Brownian motion. Thus, extent and efficiency of drug deposition is influenced by particle-specific and physiological factors, such as particle size and geometry, lung morphology, and breathing pattern [43, 115]. Common methods to deposit drug-loaded nanoparticles in the deeper lung are the nebulization of nanosuspensions and the aerosolization of nanoparticle-containing microparticles (composite microparticles) [21, 64, 66].

A number of nanoparticle formulations were found to be accessible for nebulization with common nebulizers [42, 105, 106, 116, 117]. One major advantage of this method is that regardless of the aerodynamic properties of the nanoparticles themselves, alveolar deposition can be easily achieved by generating adequate droplet sizes. Over the past decades, the generation of therapeutic aerosols has primarily been reserved to pneumatic- and ultrasound-driven nebulizers. Recent technological advances have led to improved nebulizer designs employing vibrating-mesh technology for aerosol generation [40, 41]. Vibrating mesh nebulizers have been shown to overcome the main drawbacks of pneumatic- and ultrasound-driven nebulizers, that is, concentration of medicaments, temperature changes, and high residual volumes inside the nebulizer reservoir. The aggregation of nanoparticles during aerosolization is dependent on both the nanoparticle surface characteristics and the technique for aerosol generation. The aggregation tendency was reduced for nanoparticles exhibiting a more hydrophilic surface [42]. Coating of nanoparticle surfaces with hydrophilic polymers was also shown to improve the nebulization stability of biodegradable nanoparticles [105, 106]. Furthermore, the use of vibrating mesh nebulizers is suitable for the delivery of "delicate" structures, like biodegradable nanoparticles due to avoidance of high shear stress during aerosolization [118].

As an alternative to nebulization of a nanosuspension, polymeric nanoparticles can be delivered to the lung by means of dry powder aerosolization. For this reason, nanoparticles need to be encapsulated into composite microparticles using standard techniques like spray drying or agglomeration [119, 120]. The composite microparticles must display defined aerodynamic properties (MMAD) to obtain peripheral lung deposition of inhaled particles [114]. The delivery of nanoparticles as part of microparticles has been intensively investigated for several reasons. A common obstacle that limits the use of biodegradable polymeric nanoparticles is their chemical and physical instability in aqueous suspension [121, 122]. Nanoparticles tend to aggregate when stored over an extended period of time. Furthermore, hydrolytic degradation of the polymeric nanoparticle matrix material and drug leakage from nanoparticles into the aqueous medium take place. Thus, for stabilization of biodegradable polymeric nanoparticles a subsequent drying step needs to be carried out to remove water from these systems. The most commonly used methods to convert a colloidal suspension into solid powders of sufficient stability are freeze- and spray drying [123, 124]. Spray drying offers the advantage over freeze-drying that nanoparticles are transformed to respirable microparticle-containing powders in a one-step process. Freeze-drying would cause additional disintegration to form microparticles suitable for pulmonary application. The addition of stabilizers like sugars or polymers has shown to prevent unwanted nanoparticle aggregation during drying and storage [125]. Spontaneous redispersion of nanoparticles is a key desideratum in the development of successful composite drug delivery systems to the lung. Composite microparticles should release their therapeutic payload (drug-loaded nanoparticles) when they get into contact with aqueous media, and the unaffected nanoparticles can carry out their therapeutic benefit at the target site. Typical examples for preparation of composite microparticles by spray drying can be found in the literature. Drug-loaded PLGA nanoparticles and trehalose as microparticle matrix material were used to prepare composite microparticles suitable for inhalation [126]. The use of "porous nanoparticle-aggregate particles" (PNAPs) as dry powder delivery vehicles to the lung was investigated by Sung et al.. Drug-loaded PLGA nanoparticles were prepared using

a solvent evaporation method and subsequently converted to PNAPs using a spray drying technique [127]. Effervescent powder formulations containing nanoparticles were recently introduced for pulmonary drug delivery. These formulations were composed of poly(butyl cyanoacrylate) nanoparticles and as effervescent components sodium carbonate and citric acid stabilized with ammonia were employed. The active release mechanism (effervescent reaction) of the composite microparticles was observed when the carrier particles were exposed to humidity and unaffected nanoparticles were released [128].

Another interesting method to obtain nanoparticle containing microparticles is enabled by controlled agglomeration of oppositely charged nanoparticle populations. Positively- and negatively charged biodegradable nanoparticles are brought into contact under vigorous stirring, and spontaneous composite microparticle formation takes place. Nanoparticle aggregation is driven by electrostatic attraction/forces in this case [129, 130].

4. Methods to Evaluate the Controlled Release Properties of Inhaled Nanoparticles

A basic concern in the field of nanomedicine is the development of successful nanoparticulate controlled release formulations with the aim to improve the characteristics of the therapeutic agent at the target site. Biological environments are known to strongly influence the release properties of nano-sized drug delivery vehicles. An insistent problem in the development of nanoparticulate drug delivery systems is the lack of systems to follow the drug release after contact with an external medium. As a consequence, conventional *in vitro* drug release studies may have very little in common with the delivery and release situation *in vivo* and the development of more sophisticated controlled drug release carriers to the lung is precluded [31].

Different preclinical models are used to account for the drug release mechanisms, as well as the rate and extent of drug absorption after pulmonary administration [6, 32, 34]. The complexity of the employed models increases from *in vitro* cell culture methods and *in silico* models, which are primarily used as screening tools, to *in vivo* pharmacokinetic analysis that provide fundamental information about the fate of the released drug by monitoring drug levels in plasma, lung fluid, and tissue. Several cell culture models of the respiratory tract are described using both continuous and primary cells to explore drug transport mechanisms under precise experimental conditions [45, 57, 131–133]. Continuous cell cultures using alveolar or bronchial epithelial cells like A549 and Calu-3 are often employed as simple *in vitro* models for pulmonary drug delivery studies. In contrast to continuous cell cultures primary cultures consisting of alveolar epithelial cells present cell morphologies and biochemical characteristics closer to the *in vivo* situation. However, time-consuming isolation and cultivating, as well as limited cell lifetimes are the main drawbacks of primary cell culture models. The estimation of drug absorption from the respiratory tract based on physicochemical properties and permeability of drugs using computational and experimental models led to the extension of the biopharmaceutical classification system (BCS) [134]. The pulmonary BCS (pBCS) takes into consideration the specific biology of the respiratory tract, particle deposition, and the subsequent process of drug absorption and depicts an alternative to the currently and widely used studies in animals. Drug structure-permeability relationships may contribute to reliable prediction of pulmonary pharmacokinetics used for the development of novel inhalable drugs [135]. *Ex vivo* isolated, perfused, and ventilated lung models allow the investigation of lung-specific pharmacokinetic effects on the fate of inhaled therapeutics [32–34]. These preparations maintain structural integrity of the lung tissue and allow careful control of the experimental regimen of the isolated lung. Drugs can be administered directly to the respiratory tract in a quantitative and reproducible manner, and simple sampling and analysis of perfusate provide the absorptive profile. The fundamental information about the fate of the inhaled therapeutics gained from *in vivo* pharmacokinetic analysis are accompanied by reduced screening capacity, increased expense, ethical considerations, and the potential for nonlinear dose-response relationships between the *in vitro* and *in vivo* situation as described for inhaled toxic substances [136, 137]. Overall, the application and comparison of different models to elucidate the drug behavior at the target site is needed to establish reliable *in vitro-in vivo* correlations [32, 34].

4.1. Basic Techniques of Isolated, Perfused, and Ventilated Lung Preparations. Isolated, perfused and ventilated lung (IPL) preparations have long been used by investigators interested in the respiratory, as well as nonrespiratory functions of this complex organ [138–141]. Recently, this technique has also been adopted to assess pulmonary pharmacokinetics of inhaled therapeutics [6, 32–34]. Areas in which IPL models have not been extensively used include the evaluation of controlled release properties of pulmonary drug delivery formulations like polymeric nanoparticles. With suitable modifications, application of IPL preparations for these investigations has become technically feasible. A schematic of an IPL using a rabbit lung is depicted in Figure 2. The basic techniques of IPL for pharmacokinetic measurements include the lung isolation, perfusion, and ventilation, the delivery of the formulation to the air-space by an appropriate method, and an adequate sample analytic. Differences between simple *in vitro* tests and intact lung models are to be expected on the basis of direct interaction of nanoparticles with their environment. Accordingly, more reliable drug release and distribution data are obtained that adequately reflect the dynamic effects occurring *in vivo* and thus enhance our knowledge on the fate of nanoparticulate drug delivery formulations at the target site [31].

The choice of an appropriate organ donor animal in IPL studies is influenced by several factors [139]. Size and airway geometry of the lung govern the selection of a particular species. Relevant anatomical and physiological characteristics, as well as respiratory parameters of appropriate organ donor animals for IPL preparations can be found in the literature [33, 34]. The most popular species

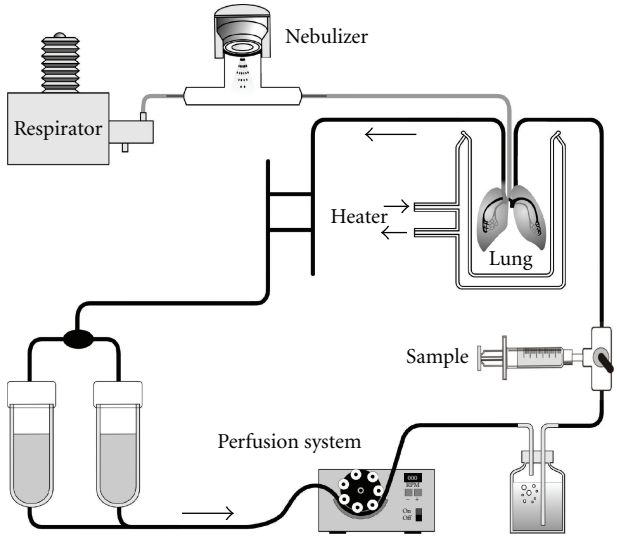

Figure 2: Schematic depiction of the basic arrangement of the isolated, perfused, and ventilated rabbit lung model useful for absorption and distribution studies of pulmonary-administered controlled release formulations (Modified from [106]).

have been the rat, the guinea pig, and the rabbit as donors for IPL experiments intended to assess the pulmonary pharmacokinetics of inhaled therapeutics (Table 1). Small animals have several disadvantages like tiny blood vessels that pose difficulties in surgical procedures and a lung geometry that impedes high lung deposition of therapeutic aerosols. An additional factor to be considered might be the volume and the number of perfusate samples needed for analytical tests during the course of the experiment. These difficulties are clearly overcome by using larger experimental animals having, however, the distinct disadvantage of increased animal and experimental costs.

The IPL approach has several advantages but also numerous limitations [33, 138–141]. IPL preparations offer several advantages to experimentation with animals. Perfusion experiments allow a definitive evaluation of lung-specific effects on the fate of inhaled drug substances. Experimental parameters remain controllable in IPL preparations, while in the intact animal these are likely to change with time especially in response to the administration of a drug formulation. The delivery methods for pulmonary drug administration to experimental animals are highly limited and often associated with low dosing efficiency (<10%) [164–167]. In contrast, the aerosol delivery to IPL models can be easily controlled by adjustment of the ventilation regimen [142, 165]. The release of drugs from its formulation can be monitored by sequential analysis of the (synthetic) perfusate medium. Unlike in the intact animal, it is possible to take frequent samples from the perfusate. This allows a complete qualitative and quantitative analysis of drug release from the test formulation. The determination of accurate and complete mass-balance of drugs is possible throughout the perfusion experiment.

Unlike *in vitro* cell culture, pharmacokinetic studies in *ex vivo* models display the advantage of structural and functional integrity of the organ, for example, cell-to-cell contacts, native extracellular matrix, and pulmonary surfactant lining layer. Lung-specific factors that remain functional in perfusion studies govern drug absorption and distribution profiles and influence the final results in the intact perfused organ, and, therefore, enable a realistic extrapolation of the results to the *in vivo* situation [34].

The principal limitation imposed by IPL preparations is the comparatively short duration of study time. Long-term studies (>6 h) cannot be performed since physiological and biochemical integrity of the lung preparation deteriorates with time [139, 141, 168, 169]. Often, it is not possible to determine the effect of therapeutic agents on the lung tissue in such a short period of time. Furthermore, only *in vitro* cell culture allows detailed analysis of cellular transport processes [45, 57, 131–133].

Finally, a practical consideration is the level of expertise in all aspects of the surgical procedures, as well as in all other technical aspects required in setting up and conducting of successful perfusion experiments.

4.2. Surgical Procedure for IPL Preparation: Isolation, Perfusion, and Ventilation. The preparation of the IPL using a rabbit as organ donor animal is described briefly hereafter and interested readers are referred to excellent reviews on this topic [139–141]. In the following, the method of Seeger et al. is briefly described [141]. For lung isolation animals need to be deeply anesthetized and anticoagulated. Then a median incision is made to expose the trachea by blunt dissection, and a cannula is inserted into the trachea. Subsequently, the animals are ventilated with room air, using a respirator. After mid-sternal thoracotomy, the ribs are spread, the right ventricle is incised, and a fluid-filled perfusion catheter is immediately placed into the pulmonary artery. Immediately after insertion of the catheter, perfusion with cold buffer fluid is started, and the heart is then cut open at the apex. Next, the trachea, lungs, and heart are excised *en bloc* from the thoracic cage. A second perfusion catheter with a bent cannula is introduced via the left ventricle into the left atrium and is fixed in this position. After rinsing the lungs with buffer fluid for washout of blood, the perfusion circuit is closed for recirculation. Meanwhile, the flow is slowly increased, and left atrial pressure is set to 1.5 mmHg. In parallel with the onset of artificial perfusion, ventilation is changed (5% CO_2, 16% O_2, and 79% N_2) to maintain the pH of the recirculating buffer at 7.4. Tidal volume is 10 ml/kg body weight with a frequency of 30 strokes/min. The IPL is placed in a temperature-equilibrated housing chamber (37°C), freely suspended from a force transducer for continuous monitoring of organ weight [170]. Pressures in the pulmonary artery, the left atrium, and the trachea are registered by means of small-diameter tubing threaded into the perfusion catheters and the trachea and connected to pressure transducers. Only lungs that have a homogeneous white appearance with no signs of hemostasis, edema, or atelectasis, a constant mean pulmonary artery and peak ventilation pressure in the normal range (4–10 and 5–8 mmHg, resp.), and are isogravimetric during an initial

Table 1: Typical examples of pulmonary drug delivery studies employing isolated, perfused, and ventilated lung models.

Organ donor animal	Perfusion system	Ventilation system	Drug/formulation	Formulation application	Analytics	References
Rat	Recirculating flow, 15 mL/min; Krebs-Ringer/Krebs-Henseleit buffer + 4% BSA	Alternating "negative" pressure; tidal volume: ~1 mL, ventilation frequency: 7–28 strokes/min	Fluorescent dyes, labeled dextrans, polypeptides (polyaspartamide, insulin); solution	"Forced solution instillation"	Fluorescence spectroscopy, ELISA	[142–152]
	Recirculating or single-pass flow, 7–11 mL/min; Krebs-Ringer buffer + 4.5% BSA	Alternating "negative" pressure; tidal volume: ~1 mL, ventilation frequency: 70 strokes/min	Budesonide; solution	Instillation	HPLC, liquid scintillation counting	[153]
	Recirculating flow, 5 mL/min; Krebs-Henseleit buffer + 3% BSA	"Positive" pressure inflation; tidal volume: 2, 4 mL, ventilation frequency: 60, 30 strokes/min	Levofloxacin; solution	Nebulization	HPLC	[154]
	Single-pass flow, ~17 mL/min; Krebs-Ringer buffer + 2% BSA	Alternating "negative" pressure; tidal volume: ~1 mL, ventilation frequency: 75 strokes/min	Budesonide, formoterol, terbutaline; powder	Powder aerosolization (DustGun® technology)	LC-MS/MS	[155]
	Recirculating flow, 10–12 mL/min; Krebs-Ringer buffer + 4.5% BSA	Alternating "negative" pressure; tidal volume: ~1 mL, ventilation frequency: 80 strokes/min	Diverse low molecular weight therapeutic agents, labelled dextrans, oligopeptides; solution	Nebulization, instillation (Aeroprobe® technology)	Fluorescence spectroscopy, LC-MS/MS	[156, 157]
Guinea pig	Single-pass flow, 10 mL/min; Krebs-Ringer buffer + 4.5% BSA	Alternating "negative" pressure; tidal volume: ~2 mL, ventilation frequency: 80 strokes/min	Xanthines; solution	Instillation	liquid scintillation counting	[158]
Rabbit	Recirculating flow, 100 mL/min; Krebs-Henseleit buffer (+ 4% hydroxyethyl-amylopectine)	"Positive" pressure inflation; tidal volume: 30 mL, ventilation frequency: 30 strokes/min	Fluorescent dyes, salbutamol, iloprost; solution, nanosuspension	Nebulization, instillation	Fluorescence spectroscopy; HPLC; RIA	[106, 159–161]
	Recirculating flow; Krebs-Ringer buffer + 4.5% BSA	Alternating "negative" pressure	Isoproterenol, isoproterenol prodrugs; solution	Nebulization, instillation	HPLC	[162, 163]

BSA: bovine serum albumin; ELISA: enzyme-linked immunosorbent assay; HPLC: high-pressure liquid chromatography; LC-MS/MS: liquid chromatography-tandem mass spectrometry; RIA: radioimmunoassay.

steady state period of at least 30 min are considered for experiments.

A number of visual and physiological parameters can be determined to ascertain the viability of the IPL preparation [139, 171]. Under optimized preparation and perfusion conditions, greater than 85% of all excised lungs fulfill these criteria, and lungs may be perfused for ~6 h without changes in physiological aspects [141, 168, 169].

For analysis of pulmonary pharmacokinetics of inhaled therapeutics, such as pulmonary absorption and distribution characteristics, formulations need to be delivered to the IPL by the intratracheal route. Intratracheal delivery can be carried out by dry powder insufflation or inhalation or by means of fluid instillation or nebulization [32–34]. For nebulization purpose, a nebulizer unit is connected to the inspiratory tubing between the ventilator and the lung to pass the produced aerosol through by the inspiration gas [106, 159]. In order to determine the absorption of drug from the lung into the perfusate, samples are taken from the venous part of the system (Figure 2). Additionally, the analysis of the drug distribution characteristics to the different compartments of the lung is performed by lavage for the amount of drug remaining in the lung-lining fluid and by extraction or microscopic techniques for the amount of drug remaining in the lung tissue at the end of the experiment.

4.3. Application of Drug Formulations to the IPL. The choice of an appropriate IPL preparation set-up for the analysis of the fate of inhaled therapeutics at the target site is influenced by several factors. The particular problem determines the selection of experimental parameters like ventilation method, perfusion characteristic, and perfusate type [139, 140]. The ventilation of the IPL can be realized by two modes, namely, "positive" pressure and "negative" (subatmospheric) pressure ventilation. During "positive" pressure ventilation a connection of a respirator directly to the lung, which pushes bolus volumes of air into the lung, is required. Subatmospheric pressure ventilation is accomplished by using a reverse connected respirator to cycle subatmospheric pressures inside the chamber in which the lung is suspended. While "negative" pressure ventilation is generally preferred for drug absorption and distribution studies (Table 1), as it prevents water loss, tissue drying, and improves organ viability (i.e., reduced risk of architecture destruction by overinflation with subsequent edema formation and progressive atelectasis), "positive" pressure ventilation enables a highly efficient and homogeneous deposition of therapeutic aerosols to the isolated lung as described above [33, 106, 140, 159]. Beside the morphology of the respiratory tract, ventilation pattern is generally recognized to have a high impact for the successful aerosol delivery to isolated lungs. To avoid low dosing efficiency and nonreproducible aerosol deposition pattern in the lung, a synchronization of aerosol application and inspiration needs to be adjusted (tidal volume ↑, ventilation frequency ↓) [142, 165].

The applied perfusion technique is dependent on the experimental design [32, 33, 140]. The perfusion may be performed in a single-pass or recirculating manner. Single-pass perfusion systems have the advantage of being less sophisticated; however, depending on the flow rate and the duration of the experiment, they come along with higher consumption of perfusate. Moreover, a sensitive sample analytics is required [155]. In pharmacokinetic experiments of inhaled therapeutics, the use of artificial perfusion medium, for example Krebs-Henseleit buffer fluid, with addition of hydroxyethylamylopectin or dextran as oncotic agent is only appropriate for hydrophilic drug substances. Hydrophobic drug analysis is relieved in the presence of albumin owing to binding of hydrophobic compounds. For example, Liu et al. investigated the effect of different perfusion buffers on the pharmacokinetics of several drugs with distinct physicochemical properties that were administered to the circulation of an isolated rat lung model [172]. The total recovery of the lipophilic drug propranolol was found to be significantly decreased when dextran was used as oncotic agent instead of albumin. Moreover, the measurement of pulmonary disposition of the potent glucocorticoid budesonide after administration to the air-space or the pulmonary circulation of the isolated rat lung was only feasible in the presence of 4.5% albumin in the perfusion medium [153]. These studies emphasize the use of albumin as oncotic agent in perfusion buffers when the pulmonary disposition of hydrophobic drugs is under investigation. In addition, depending on the experimental design, heparinized animal plasma may be added to the buffer fluid (10–15%). Use of heparinized animal blood as perfusion fluid most closely resembles the *in vivo* state. However, analysis of drugs and interpretation of results are rendered much more difficult by the presence of such a "complex" perfusion medium, thereby negating some of the advantages of the isolated lung technique [140, 141].

The use of IPL preparations to study the lung disposition of several inhaled therapeutics was pioneered by Byron et al. and Ryrfeldt et al. in the mid 1980's (Table 1) [32, 33]. The pulmonary absorption and distribution of low molecular and high molecular weight drugs was addressed in several studies [143–152]. Ryrfeldt et al. investigated the pulmonary disposition of the glucocorticoid budesonide in an isolated rat lung after instillation [153]. The drug absorption from the air-space into the perfusate was characterized by two distinct phases: after a rapid initial absorption phase about half of the instilled dose was slowly transferred into the perfusate. This study points out the high lung affinity of budesonide, no biotransformation of this compound was found in the lung. The high affinity to the lung together with an absence of lung metabolism was shown to be an important factor to explain the clinical benefits seen with budesonide [80, 81]. Kröll et al. used the isolated guinea pig lung model for the measurement of the pulmonary fate of two antiasthmatic drugs (xanthines) [158]. After intratracheal instillation of theophylline, the peak concentration in the lung perfusate appeared within a short period of time, and after 10 and 60 minutes, ~68 and ~87% of the given dose had been absorbed, respectively. The rapid disappearance of locally administered theophylline may explain the lack of success of inhalation therapy with this therapeutic agent.

The pulmonary disposition of the antibiotic levofloxacin was evaluated after systemic application and inhalation in a model of the isolated rat lung. Different experimental conditions including higher or lower respiratory frequency with lower or higher tidal volume were tested. Comparison of systemic and pulmonary administration revealed statistically significant differences between partition coefficients showing much higher values for the latter route. Thus, inhalation compared to systemic administration improves levofloxacin access to the lung tissue [154].

Only limited information is available regarding the administration, deposition, and absorption of dry powder aerosols to IPL preparations. For this purpose, Ewing et al. and Byron et al. established an isolated rat lung model and reported the absorption profiles of a variety of test compounds [143, 155]. Using the recently developed DustGun aerosol technology, Ewing et al. exposed the IPL model to respirable dry powder aerosols of three drugs at high concentrations [155]. Other interesting techniques for reproducible aerosol application to IPL preparations include the miniaturized nebulization catheter (AeroProbe) and the "forced solution instillation" technique [142, 165].

Lahnstein et al. investigated the pulmonary absorption and distribution of fluorescent dyes in an isolated rabbit lung model [159]. Three structurally diverse probes were administrated intrapulmonary by nebulization of dye solutions. The authors found that the absorption of the model compounds from the air-space into the perfusate was mainly affected by the physicochemical properties (octanol/water partition coefficient) of the employed dyes. While for the hydrophobic dye only a marginal appearance in the perfusate was observed due to accumulation in the lung tissue, a rapid increase in perfusate concentration (with stable plateau concentration) was obtained for both hydrophilic dyes.

Rapid absorption and capacity-limited metabolism of isoproterenol and prodrugs thereof were observed following intrabronchial and aerosol administration of drug to the isolated rabbit lung [162, 163].

The IPL preparation is a valuable model for the analysis of pharmacokinetic profiles of pulmonary administered drugs. Upgrading of the *ex vivo* models to pharmacodynamic investigations has recently become technically feasible. As an example, the pharmacokinetics and vasodilatory effect of nebulized iloprost were investigated in a model of experimental pulmonary hypertension employing the isolated rabbit lung [160]. The nebulization of different amounts of iloprost caused a dose-dependent pulmonary vasodilatation. In addition, a similar dose-dependent appearance of iloprost in the recirculating perfusate was noted.

The effect of polymeric nanoparticles on the microvascular permeability and translocation across the alveolar barrier was tested in isolated rabbit lungs after nanoparticle instillation [173, 174]. The increase in pulmonary microvascular permeability was related to the number of administered nanoparticles. Moreover, positively charged nanoparticles were more effective in the microvascular permeability response than negatively charged nanoparticles. The authors concluded that the surface properties and the total surface area need to be considered to interpret the changes of the microvascular permeability upon nanoparticle challenge. The applied polymeric nanoparticles were mainly located in the alveolar space and in macrophages after instillation, and no translocation of nanoparticles from the alveoli into the perfusion medium was observed. However, the relevance of these findings for the *in vivo* translocation of inhaled ultrafine particles remains to be established, owing to the fact that polymeric nanoparticles are currently under investigation as potential drug delivery systems to the lung [95].

Beck-Broichsitter et al. compared the pulmonary disposition characteristics of the hydrophilic model drug 5(6)-carboxyfluorescein after aerosolization as solution or entrapped into polymeric nanoparticles in an isolated rabbit lung model [106]. Nanoparticles were of spherical shape with a mean particle size of ~200 nm. Nebulization of the nanosuspension using a vibrating mesh nebulizer led to negligible changes of nanoparticle properties. The drug release *in vitro* was fast. Nevertheless, after deposition of equal amounts of 5(6)-carboxyfluorescein in the isolated rabbit lung model, less 5(6)-carboxyfluorescein was detected in the perfusate for loaded nanoparticles (~10 ng/ml) when compared to 5(6)-carboxyfluorescein aerosolized from solution (~18 ng/ml).

Although IPL studies are conducted with an intact organ close to the physiological state, it is to a large extent unresolved if pharmacokinetic data obtained from IPL preparations are consistent with that measured *in vivo* [32, 33]. Recently, a linear relationship between the drug absorption kinetics of diverse low molecular weight drugs (<700 g/mol) in a vertically positioned IPL system and from the lung *in vivo* was demonstrated [156, 175]. Moreover, drugs for which air-to-perfusate absorption kinetics were evaluated *ex vivo* and *in vivo* were also tested in epithelial cell culture models (Caco-2 and 16HBE14o) regarding their transport characteristics. Permeability in intestinal and airway cell culture models were found to be in excellent agreement with the physicochemical properties of the investigated drugs, as well as the rate of absorption measured in the IPL [156, 157, 176]. The absence of a bronchial circulation in horizontally positioned IPL preparations and therefore a lack of tracheobronchial absorption pathways have been attributed as the likely cause of a substantial difference in the absorption kinetics of low molecular weight drugs between the IPL preparation and *in vivo*. The absorption of low molecular weight drugs *in vivo* takes place from alveolar, as well as tracheobronchial regions at effective and comparable rates. As a result, the IPL preparation underestimates the absorption of low molecular weight drugs compared to the *in vivo* situation. However, macromolecules show poor to insignificant absorption across the thicker tracheobronchial membranes and thus, the absorption profiles of macromolecules derived from IPL preparations have been reported as statistically indistinguishable from those obtained *in vivo* [32, 149]. Clearly, studies regarding the difference between the vertically and horizontally positioned IPL systems on drug absorption need to be carried out.

In recent studies, the development and performance of a novel nanoparticle-based formulation for pulmonary

delivery of salbutamol has been characterized systematically through all steps beginning from the particle preparation process, over the *in vitro* testing of drug release, drug transport in cell culture, pulmonary absorption, and distribution characteristics in an isolated rabbit lung model, to *in vivo* bronchoprotection studies in anaesthetized guinea pigs. Sustained salbutamol release from the drug-loaded nanoparticles was observed for 2.5 h *in vitro*. Drug transport experiments conducted with primary cultured human alveolar epithelial cells revealed a delayed transport of salbutamol across the cell monolayer for nanoparticle formulations. In parallel, a sustained salbutamol release profile was observed after aerosol delivery of nanoparticles to the IPL as reflected by a distinct absorption profile and lower salbutamol recovery in the perfusate (~40%) when compared to salbutamol solution (~63%). Moreover, a prolonged pharmacological effect was observed for 120 min *in vivo* when salbutamol-loaded nanoparticles were administered to guinea pigs [161, 177]. Overall, these results demonstrate good agreement between *in vitro*, *ex vivo,* and *in vivo* tests, serve as examples for the potential of the IPL to be used to predict drug absorption from the intact animal and, therefore, present a solid basis for future advancement in nanomedicine strategies for pulmonary drug delivery.

5. Conclusion and Perspective

Implementation of nanotechnology offers new concepts for development of optimized therapeutic and diagnostic tools in medicine. Biodegradable polymeric nanoparticles hold great promise to improve controlled and targeted drug delivery to the desired site of action. Various nanoparticle-containing formulations for drug delivery to the lung are currently under investigation. Existing analytical protocols allow the accurate analysis of the physicochemical properties of these drug delivery systems, but a lack of methods that elucidate the controlled release properties of polymeric nanoparticles constrict the development of improved drug-delivery vehicles. Isolated, perfused, and ventilated lung models are promising tools to evaluate the controlled release characteristics of nanoparticles intended for pulmonary application. *Ex vivo* models allow the determination of the fate of nanoparticulate drug delivery formulations at the target site. As a consequence, more reliable drug release and distribution data are obtained that adequately reflect the dynamic effects occurring *in vivo*. The first promising results that were made by the analysis of the release properties of drug-loaded polymeric nanoparticles by the *ex vivo* technique emphasize this strategy and will hopefully promote progress in nanomedicine.

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References

[1] G. Taylor and I. Kellaway, "Pulmonary drug delivery," in *Drug Delivery and Targeting*, A. Hillery, A. W. Lloyd, and J. Swabrick, Eds., pp. 269–300, Routledge Chapman & Hall, London, UK, 2001.

[2] H. Bisgaard, C. O'Callaghan, and G. C. Smaldone, Eds., *Drug Delivery to the Lung*, Informa Healthcare, New York, NY, USA, 2002.

[3] H. M. Courrier, N. Butz, and TH. F. Vandamme, "Pulmonary drug delivery systems: recent developments and prospects," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 19, no. 4-5, pp. 425–498, 2002.

[4] D. A. Groneberg, C. Witt, U. Wagner, K. F. Chung, and A. Fischer, "Fundamentals of pulmonary drug delivery," *Respiratory Medicine*, vol. 97, no. 4, pp. 382–387, 2003.

[5] T. Gessler, W. Seeger, and T. Schmehl, "Inhaled prostanoids in the therapy of pulmonary hypertension," *Journal of Aerosol Medicine*, vol. 21, no. 1, pp. 1–12, 2008.

[6] C. Mobley and G. Hochhaus, "Methods used to assess pulmonary deposition and absorption of drugs," *Drug Discovery Today*, vol. 6, no. 7, pp. 367–375, 2001.

[7] N. R. Labiris and M. B. Dolovich, "Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications," *British Journal of Clinical Pharmacology*, vol. 56, no. 6, pp. 588–599, 2003.

[8] N. R. Labiris and M. B. Dolovich, "Pulmonary drug delivery. Part II: the role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications," *British Journal of Clinical Pharmacology*, vol. 56, no. 6, pp. 600–612, 2003.

[9] X. M. Zeng, G. P. Martin, and C. Marriott, "The controlled delivery of drugs to the lung," *International Journal of Pharmaceutics*, vol. 124, no. 2, pp. 149–164, 1995.

[10] J. G. Hardy and T. S. Chadwick, "Sustained release drug delivery to the lungs. An option for the future," *Clinical Pharmacokinetics*, vol. 39, no. 1, pp. 1–4, 2000.

[11] Y. Xie, P. Zeng, and T. S. Wiedmann, "Disease guided optimization of the respiratory delivery of microparticulate formulations," *Expert Opinion on Drug Delivery*, vol. 5, no. 3, pp. 269–289, 2008.

[12] R. Langer, "New methods of drug delivery," *Science*, vol. 249, no. 4976, pp. 1527–1533, 1990.

[13] R. Langer, "Drug delivery and targeting," *Nature*, vol. 392, no. 6679, supplement, pp. 5–10, 1998.

[14] D. A. La Van, D. M. Lynn, and R. Langer, "Moving smaller in drug discovery and delivery," *Nature Reviews Drug Discovery*, vol. 1, no. 1, pp. 77–84, 2002.

[15] O. C. Farokhzad and R. Langer, "Impact of nanotechnology on drug delivery," *ACS Nano*, vol. 3, no. 1, pp. 16–20, 2009.

[16] B. Mishra, B. B. Patel, and S. Tiwari, "Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery," *Nanomedicine*, vol. 6, no. 1, pp. e9–e24, 2010.

[17] K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni, and W. E. Rudzinski, "Biodegradable polymeric nanoparticles as drug delivery devices," *Journal of Controlled Release*, vol. 70, no. 1-2, pp. 1–20, 2001.

[18] J. Panyam and V. Labhasetwar, "Biodegradable nanoparticles for drug and gene delivery to cells and tissue," *Advanced Drug Delivery Reviews*, vol. 55, no. 3, pp. 329–347, 2003.

[19] O. M. Koo, I. Rubinstein, and H. Onyuksel, "Role of nanotechnology in targeted drug delivery and imaging: a concise review," *Nanomedicine*, vol. 1, no. 3, pp. 193–212, 2005.

[20] P. G. A. Rogueda and D. Traini, "The nanoscale in pulmonary delivery. Part 1: deposition, fate, toxicology and effects,"

Expert Opinion on Drug Delivery, vol. 4, no. 6, pp. 595–606, 2007.

[21] P. G. A. Rogueda and D. Traini, "The nanoscale in pulmonary delivery. Part 2: formulation platforms," *Expert Opinion on Drug Delivery*, vol. 4, no. 6, pp. 607–620, 2007.

[22] S. Azarmi, W. H. Roa, and R. Löbenberg, "Targeted delivery of nanoparticles for the treatment of lung diseases," *Advanced Drug Delivery Reviews*, vol. 60, no. 8, pp. 863–875, 2008.

[23] E. Rytting, J. Nguyen, X. Wang, and T. Kissel, "Biodegradable polymeric nanocarriers for pulmonary drug delivery," *Expert Opinion on Drug Delivery*, vol. 5, no. 6, pp. 629–639, 2008.

[24] T. Lebhardt, S. Roesler, M. Beck-Broichsitter, and T. Kissel, "Polymeric nanocarriers for drug delivery to the lung," *Journal of Drug Delivery Science and Technology*, vol. 20, no. 3, pp. 171–180, 2010.

[25] C. Vauthier and K. Bouchemal, "Methods for the preparation and manufacture of polymeric nanoparticles," *Pharmaceutical Research*, vol. 26, no. 5, pp. 1025–1058, 2009.

[26] C. E. Mora-Huertas, H. Fessi, and A. Elaissari, "Polymer-based nanocapsules for drug delivery," *International Journal of Pharmaceutics*, vol. 385, no. 1-2, pp. 113–142, 2010.

[27] J. M. Anderson and M. S. Shive, "Biodegradation and biocompatibility of PLA and PLGA microspheres," *Advanced Drug Delivery Reviews*, vol. 28, no. 1, pp. 5–24, 1997.

[28] I. Bala, S. Hariharan, and M. N. V. R. Kumar, "PLGA nanoparticles in drug delivery: the state of the art," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 21, no. 5, pp. 387–422, 2004.

[29] C. E. Astete and C. M. Sabliov, "Synthesis and characterization of PLGA nanoparticles," *Journal of Biomaterials Science, Polymer Edition*, vol. 17, no. 3, pp. 247–289, 2006.

[30] C. Washington, "Drug release from microdisperse systems: a critical review," *International Journal of Pharmaceutics*, vol. 58, no. 1, pp. 1–12, 1990.

[31] J. Kreuter, *Colloidal Drug Delivery Systems*, Marcel Dekker, New York, NY, USA, 1994.

[32] M. Sakagami, "In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery," *Advanced Drug Delivery Reviews*, vol. 58, no. 9-10, pp. 1030–1060, 2006.

[33] A. Tronde, C. Bosquillon, and B. Forbes, "The isolated perfused lung for drug absorption studies," in *Biotechnology Pharmaceutical Aspects VII*, C. Erhardt and K. J. Kim, Eds., vol. 7 of *Drug Absorption Studies*, pp. 135–163, Springer, Berlin, Germany, 2008.

[34] C. A. Fernandes and R. Vanbever, "Preclinical models for pulmonary drug delivery," *Expert opinion on drug delivery*, vol. 6, no. 11, pp. 1231–1245, 2009.

[35] J. Kreuter, "Nanoparticles-a historical perspective," *International Journal of Pharmaceutics*, vol. 331, no. 1, pp. 1–10, 2007.

[36] D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, and R. Langer, "Nanocarriers as an emerging platform for cancer therapy," *Nature Nanotechnology*, vol. 2, no. 12, pp. 751–760, 2007.

[37] M. J. Hawkins, P. Soon-Shiong, and N. Desai, "Protein nanoparticles as drug carriers in clinical medicine," *Advanced Drug Delivery Reviews*, vol. 60, no. 8, pp. 876–885, 2008.

[38] M. Bur, A. Henning, S. Hein, M. Schneider, and C. M. Lehr, "Inhalative nanomedicine-opportunities and challenges," *Inhalation Toxicology*, vol. 21, supplement 1, pp. 137–143, 2009.

[39] I. Roy and N. Vij, "Nanodelivery in airway diseases: challenges and therapeutic applications," *Nanomedicine*, vol. 6, no. 2, pp. 237–244, 2010.

[40] R. Dhand, "Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol," *Respiratory Care*, vol. 47, no. 12, pp. 1406–1416, 2002.

[41] J. C. Waldrep and R. Dhand, "Advanced nebulizer designs employing vibrating mesh/aperture plate technologies for aerosol generation," *Current Drug Delivery*, vol. 5, no. 2, pp. 114–119, 2008.

[42] L. A. Dailey, T. Schmehl, T. Gessler et al., "Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features," *Journal of Controlled Release*, vol. 86, no. 1, pp. 131–144, 2003.

[43] W. C. Hinds, *Aerosol Technology: Properties, Behavior, and Measurements of Airborne Particles*, John Wiley & Sons, New York, NY, USA, 1999.

[44] J. D. Crapo, B. E. Barry, P. Gehr, M. Bachofen, and E. R. Weibel, "Cell number and cell characteristics of the normal human lung," *American Review of Respiratory Disease*, vol. 126, no. 2, pp. 332–337, 1982.

[45] B. Forbes and C. Ehrhardt, "Human respiratory epithelial cell culture for drug delivery applications," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 60, no. 2, pp. 193–205, 2005.

[46] E. R. Weibel, Ed., *Morphometry of the Human Lung*, Springer, Berlin, Germany, 1963.

[47] C. M. Evans and JA. S. Koo, "Airway mucus: the good, the bad, the sticky," *Pharmacology and Therapeutics*, vol. 121, no. 3, pp. 332–348, 2009.

[48] C. Marriott, "Mucus and mucociliary clearance in the respiratory tract," *Advanced Drug Delivery Reviews*, vol. 5, no. 1-2, pp. 19–35, 1990.

[49] A. B. Lansley, "Mucociliary clearance and drug delivery via the respiratory tract," *Advanced Drug Delivery Reviews*, vol. 11, no. 3, pp. 299–327, 1993.

[50] L. A. J. M. Creuwels, L. M. G. Van Golde, and H. P. Haagsman, "The pulmonary surfactant system: biochemical and clinical aspects," *Lung*, vol. 175, no. 1, pp. 1–39, 1997.

[51] J. Goerke, "Pulmonary surfactant: functions and molecular composition," *Biochimica et Biophysica Acta*, vol. 1408, no. 2-3, pp. 79–89, 1998.

[52] R. Veldhuizen, K. Nag, S. Orgeig, and F. Possmayer, "The role of lipids in pulmonary surfactant," *Biochimica et Biophysica Acta*, vol. 1408, no. 2-3, pp. 90–108, 1998.

[53] J. Pérez-Gil and K. M. W. Keough, "Interfacial properties of surfactant proteins," *Biochimica et Biophysica Acta*, vol. 1408, no. 2-3, pp. 203–217, 1998.

[54] R. Wüstneck, J. Perez-Gil, N. Wüstneck, A. Cruz, V. B. Fainerman, and U. Pison, "Interfacial properties of pulmonary surfactant layers," *Advances in Colloid and Interface Science*, vol. 117, no. 1-3, pp. 33–58, 2005.

[55] J. Pérez-Gil, "Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions," *Biochimica et Biophysica Acta*, vol. 1778, no. 7-8, pp. 1676–1695, 2008.

[56] H. Fehrenbach, "Alveolar epithelial type II cell: defender of the alveolus revisited," *Respiratory Research*, vol. 2, no. 1, pp. 33–46, 2001.

[57] M. Ehrhardt, M. Laue, and K. J. Kim, "In vitro models of the alveolar epithelial barrier," in *Biotechnology Pharmaceutical*

Aspects VII, C. Erhardt and K. J. Kim, Eds., Drug Absorption Studies, pp. 258–282, Springer, Berlin, Germany, 2008.

[58] J. S. Patton, "Mechanisms of macromolecule absorption by the lungs," *Advanced Drug Delivery Reviews*, vol. 19, no. 1, pp. 3–36, 1996.

[59] M. Gumbleton, "Caveolae as potential macromolecule trafficking compartments within alveolar epithelium," *Advanced Drug Delivery Reviews*, vol. 49, no. 3, pp. 281–300, 2001.

[60] J. D. Brain, "Mechanisms, measurement, and significance of lung macrophage function," *Environmental Health Perspectives*, vol. 97, pp. 5–10, 1992.

[61] A. Palecanda and L. Kobzik, "Receptors for unopsonized particles: the role of alveolar macrophage scavenger receptors," *Current Molecular Medicine*, vol. 1, no. 5, pp. 589–595, 2001.

[62] J. Yu and Y. W. Chien, "Pulmonary drug delivery: physiologic and mechanistic aspects," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 14, no. 4, pp. 395–453, 1997.

[63] U. Pison, T. Welte, M. Giersig, and D. A. Groneberg, "Nanomedicine for respiratory diseases," *European Journal of Pharmacology*, vol. 533, no. 1–3, pp. 341–350, 2006.

[64] J. C. Sung, B. L. Pulliam, and D. A. Edwards, "Nanoparticles for drug delivery to the lungs," *Trends in Biotechnology*, vol. 25, no. 12, pp. 563–570, 2007.

[65] M. Smola, T. Vandamme, and A. Sokolowski, "Nanocarriers as pulmonary drug delivery systems to treat and to diagnose respiratory and non respiratory diseases," *International Journal of Nanomedicine*, vol. 3, no. 1, pp. 1–19, 2008.

[66] W. Yang, J. I. Peters, and R. O. Williams, "Inhaled nanoparticles. A current review," *International Journal of Pharmaceutics*, vol. 356, no. 1-2, pp. 239–247, 2008.

[67] M. M. Bailey and C. J. Berkland, "Nanoparticle formulations in pulmonary drug delivery," *Medicinal Research Reviews*, vol. 29, no. 1, pp. 196–212, 2009.

[68] H. M. Mansour, Y. S. Rhee, and X. Wu, "Nanomedicine in pulmonary delivery," *International journal of nanomedicine*, vol. 4, pp. 299–319, 2009.

[69] G. Oberdörster, E. Oberdörster, and J. Oberdörster, "Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles," *Environmental Health Perspectives*, vol. 113, no. 7, pp. 823–839, 2005.

[70] M. S. Cartiera, K. M. Johnson, V. Rajendran, M. J. Caplan, and W. M. Saltzman, "The uptake and intracellular fate of PLGA nanoparticles in epithelial cells," *Biomaterials*, vol. 30, no. 14, pp. 2790–2798, 2009.

[71] J. S. Patton and R. M. Platz, "Pulmonary delivery of peptides and proteins for systemic action," *Advanced Drug Delivery Reviews*, vol. 8, no. 2-3, pp. 179–196, 1992.

[72] P. R. Byron and J. S. Patton, "Drug delivery via the respiratory tract," *Journal of Aerosol Medicine*, vol. 7, no. 1, pp. 49–75, 1994.

[73] R. W. Niven, "Delivery of biotherapeutics by inhalation aerosol," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 12, no. 2-3, pp. 151–231, 1995.

[74] R. U. Agu, M. I. Ugwoke, M. Armand, R. Kinget, and N. Verbeke, "The lung as a route for systemic delivery of therapeutic proteins and peptides," *Respiratory Research*, vol. 2, no. 4, pp. 198–209, 2001.

[75] G. Scheuch, M. J. Kohlhaeufl, P. Brand, and R. Siekmeier, "Clinical perspectives on pulmonary systemic and macromolecular delivery," *Advanced Drug Delivery Reviews*, vol. 58, no. 9-10, pp. 996–1008, 2006.

[76] J. S. Patton and P. R. Byron, "Inhaling medicines: delivering drugs to the body through the lungs," *Nature Reviews Drug Discovery*, vol. 6, no. 1, pp. 67–74, 2007.

[77] R. Siekmeier and G. Scheuch, "Systemic treatment by inhalation of macromolecules—principles, problems, and examples," *Journal of Physiology and Pharmacology*, vol. 59, no. 6, pp. 53–79, 2008.

[78] C. Bailey and A. Barnett, "Why is Exubera being withdrawn?" *British Medical Journal*, vol. 335, no. 7630, p. 1156, 2007.

[79] J. Kling, "Inhaled insulin's last gasp?" *Nature Biotechnology*, vol. 26, no. 5, pp. 479–480, 2008.

[80] T. Volsko and M. D. Reed, "Drugs used in the treatment of asthma: a review of clinical pharmacology and aerosol drug delivery," *Respiratory care clinics of North America*, vol. 6, no. 1, pp. 41–55, 2000.

[81] D. D. Sin and S. F. Paul Man, "Inhaled corticosteroids in the long-term management of patients with chronic obstructive pulmonary disease," *Drugs and Aging*, vol. 20, no. 12, pp. 867–880, 2003.

[82] T. Gessler, T. Schmehl, H. Olschewski, F. Grimminger, and W. Seeger, "Aerosolized vasodilators in pulmonary hypertension," *Journal of Aerosol Medicine*, vol. 15, no. 2, pp. 117–122, 2002.

[83] H. Olschewski, G. Simonneau, N. Galiè et al., "Inhaled iloprost for severe pulmonary hypertension," *New England Journal of Medicine*, vol. 347, no. 5, pp. 322–329, 2002.

[84] A. R. Clark, "Medical aerosol inhalers: past, present, and future," *Aerosol Science and Technology*, vol. 22, no. 4, pp. 374–391, 1995.

[85] I. Gonda, "The ascent of pulmonary drug delivery," *Journal of Pharmaceutical Sciences*, vol. 89, no. 7, pp. 940–945, 2000.

[86] P. J. Anderson, "History of aerosol therapy: liquid nebulization to MDIs to DPIs," *Respiratory Care*, vol. 50, no. 9, pp. 1139–1150, 2005.

[87] D. E. Geller, "Comparing clinical features of the nebulizer, metered-dose inhaler, and dry powder inhaler," *Respiratory Care*, vol. 50, no. 10, pp. 1313–1321, 2005.

[88] S. P. Newman, "Principles of metered-dose inhaler design," *Respiratory Care*, vol. 50, no. 9, pp. 1177–1190, 2005.

[89] P. J. Atkins, "Dry powder inhalers: an overview," *Respiratory Care*, vol. 50, no. 10, pp. 1304–1312, 2005.

[90] J. B. Fink, "Metered-dose inhalers, dry powder inhalers, and transitions," *Respiratory Care*, vol. 45, no. 6, pp. 623–635, 2000.

[91] P. P. H. Le Brun, A. H. De Boer, H. W. Frijlink, and H. G. M. Heijerman, "A review of the technical aspects of drug nebulization," *Pharmacy World and Science*, vol. 22, no. 3, pp. 75–81, 2000.

[92] O. N. M. McCallion, K. M. G. Taylor, P. A. Bridges, M. Thomas, and A. J. Taylor, "Jet nebulisers for pulmonary drug delivery," *International Journal of Pharmaceutics*, vol. 130, no. 1, pp. 1–11, 1996.

[93] K. M. G. Taylor and O. N. M. McCallion, "Ultrasonic nebulisers for pulmonary drug delivery," *International Journal of Pharmaceutics*, vol. 153, no. 1, pp. 93–104, 1997.

[94] M. Knoch and M. Keller, "The customised electronic nebuliser: a new category of liquid aerosol drug delivery systems," *Expert Opinion on Drug Delivery*, vol. 2, no. 2, pp. 377–390, 2005.

[95] S. Gill, R. Löbenberg, T. Ku, S. Azarmi, W. Roa, and E. J. Prenner, "Nanoparticles: characteristics, mechanisms of action, and toxicity in pulmonary drug delivery—a review," *Journal of Biomedical Nanotechnology*, vol. 3, no. 2, pp. 107–119, 2007.

[96] J. W. Card, D. C. Zeldin, J. C. Bonner, and E. R. Nestmann, "Pulmonary applications and toxicity of engineered nanoparticles," *American Journal of Physiology*, vol. 295, no. 3, pp. L400–L411, 2008.

[97] W. H. De Jong and P. J. A. Borm, "Drug delivery and nanoparticles: applications and hazards," *International Journal of Nanomedicine*, vol. 3, no. 2, pp. 133–149, 2008.

[98] K. R. Vega-Villa, J. K. Takemoto, J. A. Yáñez, C. M. Remsberg, M. L. Forrest, and N. M. Davies, "Clinical toxicities of nanocarrier systems," *Advanced Drug Delivery Reviews*, vol. 60, no. 8, pp. 929–938, 2008.

[99] M. Caldorera-Moore and N. A. Peppas, "Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems," *Advanced Drug Delivery Reviews*, vol. 61, no. 15, pp. 1391–1401, 2009.

[100] L. A. Dailey and T. Kissel, "New poly(lactic-co-glycolic acid) derivatives: modular polymers with tailored properties," *Drug Discovery Today: Technologies*, vol. 2, no. 1, pp. 7–13, 2005.

[101] L. A. Dailey, M. Wittmar, and T. Kissel, "The role of branched polyesters and their modifications in the development of modern drug delivery vehicles," *Journal of Controlled Release*, vol. 101, no. 1–3, pp. 137–149, 2005.

[102] F. Unger, M. Wittmar, F. Morell, and T. Kissel, "Branched polyesters based on poly[vinyl-3-(dialkylamino)alkylcarbamate-co-vinyl acetate-co-vinyl alcohol]-graft-poly(d,l-lactide-co-glycolide): effects of polymer structure on in vitro degradation behaviour," *Biomaterials*, vol. 29, no. 13, pp. 2007–2014, 2008.

[103] F. Unger, M. Wittmar, and T. Kissel, "Branched polyesters based on poly[vinyl-3-(dialkylamino)alkylcarbamate-co-vinyl acetate-co-vinyl alcohol]-graft-poly(d,l-lactide-co-glycolide): effects of polymer structure on cytotoxicity," *Biomaterials*, vol. 28, no. 9, pp. 1610–1619, 2007.

[104] L. A. Dailey, N. Jekel, L. Fink et al., "Investigation of the proinflammatory potential of biodegradable nanoparticle drug delivery systems in the lung," *Toxicology and Applied Pharmacology*, vol. 215, no. 1, pp. 100–108, 2006.

[105] L. A. Dailey, E. Kleemann, M. Wittmar et al., "Surfactant-free, biodegradable nanoparticles for aerosol therapy based on the branched polyesters, DEAPA-PVAL-g-PLGA," *Pharmaceutical Research*, vol. 20, no. 12, pp. 2011–2020, 2003.

[106] M. Beck-Broichsitter, J. Gauss, C. B. Packhaeuser et al., "Pulmonary drug delivery with aerosolizable nanoparticles in an ex vivo lung model," *International Journal of Pharmaceutics*, vol. 367, no. 1-2, pp. 169–178, 2009.

[107] J. Fu, J. Fiegel, E. Krauland, and J. Hanes, "New polymeric carriers for controlled drug delivery following inhalation or injection," *Biomaterials*, vol. 23, no. 22, pp. 4425–4433, 2002.

[108] J. Fu, J. Fiegel, and J. Hanes, "Synthesis and characterization of PEG-based ether-anhydride terpolymers: novel polymers for controlled drug delivery," *Macromolecules*, vol. 37, no. 19, pp. 7174–7180, 2004.

[109] J. Fiegel, J. Fu, and J. Hanes, "Poly(ether-anhydride) dry powder aerosols for sustained drug delivery in the lungs," *Journal of Controlled Release*, vol. 96, no. 3, pp. 411–423, 2004.

[110] J. M. Barichello, M. Morishita, K. Takayama, and T. Nagai, "Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method," *Drug Development and Industrial Pharmacy*, vol. 25, no. 4, pp. 471–476, 1999.

[111] T. Govender, S. Stolnik, M. C. Garnett, L. Illum, and S. S. Davis, "PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug," *Journal of Controlled Release*, vol. 57, no. 2, pp. 171–185, 1999.

[112] M. Wittmar, F. Unger, and T. Kissel, "Biodegradable brush-like branched polyesters containing a charge-modified poly (vinyl alcohol) backbone as a platform for drug delivery systems: synthesis and characterization," *Macromolecules*, vol. 39, no. 4, pp. 1417–1424, 2006.

[113] X. Wang, X. Xie, C. Cai, E. Rytting, T. Steele, and T. Kissel, "Biodegradable branched polyesters poly(vinyl sulfonate-covinyl alcohol)-graft poly(D,L-lactic-coglycolic acid) as a negatively charged polyelectrolyte platform for drug delivery: synthesis and characterization," *Macromolecules*, vol. 41, no. 8, pp. 2791–2799, 2008.

[114] J. Heyder, J. Gebhart, G. Rudolf, C. F. Schiller, and W. Stahlhofen, "Deposition of particles in the human respiratory tract in the size range 0.005–15 μm," *Journal of Aerosol Science*, vol. 17, no. 5, pp. 811–825, 1986.

[115] C. Jaafar-Maalej, V. Andrieu, A. Elaissari, and H. Fessi, "Assessment methods of inhaled aerosols: technical aspects and applications," *Expert Opinion on Drug Delivery*, vol. 6, no. 9, pp. 941–959, 2009.

[116] J. Liu, T. Gong, H. Fu et al., "Solid lipid nanoparticles for pulmonary delivery of insulin," *International Journal of Pharmaceutics*, vol. 356, no. 1-2, pp. 333–344, 2008.

[117] J. Hureaux, F. Lagarce, F. Gagnadoux et al., "Lipid nanocapsules: ready-to-use nanovectors for the aerosol delivery of paclitaxel," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 73, no. 2, pp. 239–246, 2009.

[118] T. Ghazanfari, A. M. A. Elhissi, Z. Ding, and K. M. G. Taylor, "The influence of fluid physicochemical properties on vibrating-mesh nebulization," *International Journal of Pharmaceutics*, vol. 339, no. 1-2, pp. 103–111, 2007.

[119] A. H. L. Chow, H. H. Y. Tong, P. Chattopadhyay, and B. Y. Shekunov, "Particle engineering for pulmonary drug delivery," *Pharmaceutical Research*, vol. 24, no. 3, pp. 411–437, 2007.

[120] P. C. Seville, H. Y. Li, and T. P. Learoyd, "Spray-dried powders for pulmonary drug delivery," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 24, no. 4, pp. 307–360, 2007.

[121] D. Lemoine, C. Francois, F. Kedzierewicz, V. Preat, M. Hoffman, and P. Maincent, "Stability study of nanoparticles of poly(ε-caprolactone), poly(D,L-lactide) and poly(D,L-lactide-co-glycolide)," *Biomaterials*, vol. 17, no. 22, pp. 2191–2197, 1996.

[122] M. S. Muthu and SI. S. Feng, "Pharmaceutical stability aspects of nanomedicines," *Nanomedicine*, vol. 4, no. 8, pp. 857–860, 2009.

[123] W. Abdelwahed, G. Degobert, S. Stainmesse, and H. Fessi, "Freeze-drying of nanoparticles: formulation, process and storage considerations," *Advanced Drug Delivery Reviews*, vol. 58, no. 15, pp. 1688–1713, 2006.

[124] P. Tewa-Tagne, S. Briançon, and H. Fessi, "Preparation of redispersible dry nanocapsules by means of spray-drying: development and characterisation," *European Journal of Pharmaceutical Sciences*, vol. 30, no. 2, pp. 124–135, 2007.

[125] G. Pilcer and K. Amighi, "Formulation strategy and use of excipients in pulmonary drug delivery," *International Journal of Pharmaceutics*, vol. 392, no. 1-2, pp. 1–19, 2010.

[126] K. Tomoda, T. Ohkoshi, K. Hirota et al., "Preparation and properties of inhalable nanocomposite particles for treatment of lung cancer," *Colloids and Surfaces B*, vol. 71, no. 2, pp. 177–182, 2009.

[127] J. C. Sung, D. J. Padilla, L. Garcia-Contreras et al., "Formulation and pharmacokinetics of self-assembled rifampicin

nanoparticle systems for pulmonary delivery," *Pharmaceutical Research*, vol. 26, no. 8, pp. 1847–1855, 2009.

[128] L. Ely, W. Roa, W. H. Finlay, and R. Löbenberg, "Effervescent dry powder for respiratory drug delivery," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 65, no. 3, pp. 346–353, 2007.

[129] L. Shi, C. J. Plumley, and C. Berkland, "Biodegradable nanoparticle flocculates for dry powder aerosol formulation," *Langmuir*, vol. 23, no. 22, pp. 10897–10901, 2007.

[130] L. J. Peek, L. Roberts, and C. Berkland, "Poly(D,L-lactide-co-glycolide) nanoparticle agglomerates as carriers in dry powder aerosol formulation of proteins," *Langmuir*, vol. 24, no. 17, pp. 9775–9783, 2008.

[131] N. R. Mathias, F. Yamashita, and V. H. L. Lee, "Respiratory epithelial cell culture models for evaluation of ion and drug transport," *Advanced Drug Delivery Reviews*, vol. 22, no. 1-2, pp. 215–249, 1996.

[132] A. Steimer, E. Haltner, and C. M. Lehr, "Cell culture models of the respiratory tract relevant to pulmonary drug delivery," *Journal of Aerosol Medicine*, vol. 18, no. 2, pp. 137–182, 2005.

[133] J. L. Sporty, L. Horálková, and C. Ehrhardt, "In vitro cell culture models for the assessment of pulmonary drug disposition," *Expert Opinion on Drug Metabolism and Toxicology*, vol. 4, no. 4, pp. 333–345, 2008.

[134] G. L. Amidon, H. Lennernas, V. P. Shah, and J. R. Crison, "A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability," *Pharmaceutical Research*, vol. 12, no. 3, pp. 413–420, 1995.

[135] H. Eixarch, E. Haltner-Ukomadu, C. Beisswenger, and U. Bock, "Drug delivery to the lung: permeability and physicochemical characteristics of drugs as the basis for a pulmonary biopharmaceutical classification system (pBCS)," *Journal of Epithelial Biology and Pharmacology*, vol. 3, pp. 1–14, 2010.

[136] S. A. Cryan, N. Sivadas, and L. Garcia-Contreras, "In vivo animal models for drug delivery across the lung mucosal barrier," *Advanced Drug Delivery Reviews*, vol. 59, no. 11, pp. 1133–1151, 2007.

[137] P. Gerde, "Animal models and their limitations: on the problem of high-to-low dose extrapolations following inhalation exposures," *Experimental and Toxicologic Pathology*, vol. 57, no. 1, supplement, pp. 143–146, 2005.

[138] R. W. Niemeier, "Isolated perfused rabbit lung: a critical appraisal," *Environmental Health Perspectives*, vol. 16, pp. 67–71, 1976.

[139] H. M. Mehendale, L. S. Angevine, and Y. Ohmiya, "The isolated perfused lung. A critical evaluation," *Toxicology*, vol. 21, no. 1, pp. 1–36, 1981.

[140] R. W. Niemeier, "The isolated perfused lung," *Environmental Health Perspectives*, vol. 56, pp. 35–41, 1984.

[141] W. Seeger, D. Walmrath, F. Grimminger et al., "Adult respiratory distress syndrome: model systems using isolated perfused rabbit lungs," *Methods in Enzymology*, vol. 233, pp. 549–584, 1994.

[142] P. R. Byron and R. W. Niven, "A novel dosing method for drug administration to the airways of the isolated perfused rat lung," *Journal of Pharmaceutical Sciences*, vol. 77, no. 8, pp. 693–695, 1988.

[143] P. R. Byron, N. S. R. Roberts, and A. R. Clark, "An isolated perfused rat lung preparation for the study of aerosolized drug deposition and absorption," *Journal of Pharmaceutical Sciences*, vol. 75, no. 2, pp. 168–171, 1986.

[144] R. W. Niven and P. R. Byron, "Solute absorption from the airways of the isolated rat lung. I. The use of absorption data to quantify drug dissolution or release in the respiratory tract," *Pharmaceutical Research*, vol. 5, no. 9, pp. 574–579, 1988.

[145] R. W. Niven and P. R. Byron, "Solute absorption from the airways of the isolated rat lung II. Eeffect of surfaccants on absorption of fluorescein," *Pharmaceutical Research*, vol. 7, no. 1, pp. 8–13, 1990.

[146] R. W. Niven, F. Rypacek, and P. R. Byron, "Solute absorption from the airways of the isolated rat lung. III. Absorption of several peptidase-resistant, synthetic polypeptides: poly-(2-hydroxyethyl)-aspartamides," *Pharmaceutical Research*, vol. 7, no. 10, pp. 990–994, 1990.

[147] P. R. Byron, Z. Sun, H. Katayama, and F. Rypacek, "Solute absorption from the airways of the isolated rat lung. IV. mechanisms of absorption of fluorophore-labeled poly-α,β-[N(2-hydroxyethyl)-DL-aspartamide]," *Pharmaceutical Research*, vol. 11, no. 2, pp. 221–225, 1994.

[148] J. Z. Sun, P. R. Byron, and F. Rypacek, "Solute absorption from the airways of the isolated rat lung. V. Charge effects on the absorption of copolymers of N(2-hydroxyethyl)-DL-aspartamide with DL-aspartic acid or dimethylaminopropyl-DL-aspartamide," *Pharmaceutical Research*, vol. 16, no. 7, pp. 1104–1108, 1999.

[149] M. Sakagami, P. R. Byron, J. Venitz, and F. Rypacek, "Solute disposition in the rat lung in vivo and in vitro: determining regional absorption kinetics in the presence of mucociliary escalator," *Journal of Pharmaceutical Sciences*, vol. 91, no. 2, pp. 594–604, 2002.

[150] M. Sakagami, P. R. Byron, and F. Rypacek, "Biochemical evidence for transcytotic absorption of polyaspartamide from the rat lung: effects of temperature and metabolic inhibitors," *Journal of Pharmaceutical Sciences*, vol. 91, no. 9, pp. 1958–1968, 2002.

[151] Y. Pang, M. Sakagami, and P. R. Byron, "The pharmacokinetics of pulmonary insulin in the in vitro isolated perfused rat lung: implications of metabolism and regional deposition," *European Journal of Pharmaceutical Sciences*, vol. 25, no. 4-5, pp. 369–378, 2005.

[152] Y. Pang, M. Sakagami, and P. R. Byron, "Insulin self-association: effects on lung disposition kinetics in the airways of the isolated perfused rat lung (IPRL)," *Pharmaceutical Research*, vol. 24, no. 9, pp. 1636–1644, 2007.

[153] A. Ryrfeldt, G. Persson, and E. Nilsson, "Pulmonary disposition of the potent glucocorticoid budesonide, evaluated in an isolated perfused rat lung model," *Biochemical Pharmacology*, vol. 38, no. 1, pp. 17–22, 1989.

[154] M. J. Jesús Valle, F. González López, and A. Sánchez Navarro, "Pulmonary versus systemic delivery of levofloxacin. The isolated lung of the rat as experimental approach for assessing pulmonary inhalation," *Pulmonary Pharmacology and Therapeutics*, vol. 21, no. 2, pp. 298–303, 2008.

[155] P. Ewing, S. J. Eirefelt, P. Andersson, A. Blomgren, A. Ryrfeldt, and P. Gerde, "Short inhalation exposures of the isolated and perfused rat lung to respirable dry particle aerosols; the detailed pharmacokinetics of budesonide, formoterol, and terbutaline," *Journal of Aerosol Medicine*, vol. 21, no. 2, pp. 169–180, 2008.

[156] A. Tronde, BO. Nordén, A. B. Jeppsson et al., "Drug absorption from the isolated perfused rat lung—correlations with drug physicochemical properties and epithelial permeability," *Journal of Drug Targeting*, vol. 11, no. 1, pp. 61–74, 2003.

[157] A. Tronde, E. Krondahl, H. Von Euler-Chelpin et al., "High airway-to-blood transport of an opioid tetrapeptide in the

isolated rat lung after aerosol delivery," *Peptides*, vol. 23, no. 3, pp. 469–478, 2002.

[158] F. Kroll, J. A. Karlsson, E. Nilsson, A. Ryrfeldt, and C. G. A. Persson, "Rapid clearance of xanthines from airway and pulmonary tissues," *American Review of Respiratory Disease*, vol. 141, no. 5, pp. 1167–1171, 1990.

[159] K. Lahnstein, T. Schmehl, U. Rüsch, M. Rieger, W. Seeger, and T. Gessler, "Pulmonary absorption of aerosolized fluorescent markers in the isolated rabbit lung," *International Journal of Pharmaceutics*, vol. 351, no. 1-2, pp. 158–164, 2008.

[160] R. T. Schermuly, A. Schulz, H. A. Ghofrani et al., "Comparison of pharmacokinetics and vasodilatory effect of nebulized and infused iloprost in experimental pulmonary hypertension: rapid tolerance development," *Journal of Aerosol Medicine*, vol. 19, no. 3, pp. 353–363, 2006.

[161] M. Beck-Broichsitter, J. Gauss, T. Gessler, W. Seeger, T. Kissel, and T. Schmehl, "Pulmonary targeting with biodegradable salbutamol-loaded nanoparticles," *Journal of Aerosol Medicine*, vol. 23, no. 1, pp. 47–57, 2010.

[162] R. K. Brazzell, R. B. Smith, and H. B. Kostenbauder, "Isolated perfused rabbit lung as a model for intravascular and intrabronchial administration of bronchodilator drugs I: isoproterenol," *Journal of Pharmaceutical Sciences*, vol. 71, no. 11, pp. 1268–1274, 1982.

[163] R. K. Brazzell and H. B. Kostenbauder, "Isolated perfused rabbit lung as a model for intravascular and intrabronchial administration of bronchodilator drugs II: isoproterenol prodrugs," *Journal of Pharmaceutical Sciences*, vol. 71, no. 11, pp. 1274–1281, 1982.

[164] O. G. Raabe, H. S. Yeh, G. J. Newton, R. F. Phalen, and D. J. Velasquez, "Deposition of inhaled monodispers aerosols in small rodents," in *Inhaled Particles IV*, W. H. Walton, Ed., pp. 3–21, Pergamon Press, Oxford, UK, 1977.

[165] A. Tronde, G. Baran, S. Eirefelt, H. Lennernäs, and U. H. Bengtsson, "Miniaturized nebulization catheters: a new approach for delivery of defined aerosol doses to the rat lung," *Journal of Aerosol Medicine*, vol. 15, no. 3, pp. 283–296, 2002.

[166] C. Hofstetter, M. Flonder, E. Thein et al., "Aerosol delivery during mechanical ventilation to the rat," *Experimental Lung Research*, vol. 30, no. 7, pp. 635–651, 2004.

[167] R. J. MacLoughlin, B. D. Higgins, J. G. Laffey, and T. O'Brien, "Optimized aerosol delivery to a mechanically ventilated rodent," *Journal of Aerosol Medicine*, vol. 22, no. 4, pp. 323–332, 2009.

[168] R. W. Niemeier and E. Bingham, "An isolated perfused lung preparation for metabolic studies," *Life Sciences*, vol. 11, no. 16, pp. 807–820, 1972.

[169] F. Kroll, J.-A. Karlsson, and E. Nilsson, "Lung mechanics of the guinea-pig isolated perfused lung," *Acta Physiologica Scandinavica*, vol. 128, no. 1, pp. 1–8, 1986.

[170] S. Uhlig and O. Heiny, "Measuring the weight of the isolated perfused rat lung during negative pressure ventilation," *Journal of Pharmacological and Toxicological Methods*, vol. 33, no. 3, pp. 147–152, 1995.

[171] S. Uhlig and L. Wollin, "An improved setup for the isolated perfused rat lung," *Journal of Pharmacological and Toxicological Methods*, vol. 31, no. 2, pp. 85–94, 1994.

[172] X. Liu, J. Y. Wang, A. M. Khlentzos et al., "Influence of perfusate composition on drug disposition in the in-situ perfused rat lung," *International Journal of Pharmaceutics*, vol. 382, no. 1-2, pp. 192–197, 2009.

[173] J. Hamoir, A. Nemmar, D. Halloy et al., "Effect of polystyrene particles on lung microvascular permeability in isolated perfused rabbit lungs: role of size and surface properties," *Toxicology and Applied Pharmacology*, vol. 190, no. 3, pp. 278–285, 2003.

[174] A. Nemmar, J. Hamoir, B. Nemery, and P. Gustin, "Evaluation of particle translocation across the alveolo-capillary barrier in isolated perfused rabbit lung model," *Toxicology*, vol. 208, no. 1, pp. 105–113, 2005.

[175] A. Tronde, BO. Nordén, H. Marchner, A. K. Wendel, H. Lennernäs, and U. H. Bengtsson, "Pulmonary absorption rate and bioavailability of drugs in vivo in rats: structure-absorption relationships and physicochemical profiling of inhaled drugs," *Journal of Pharmaceutical Sciences*, vol. 92, no. 6, pp. 1216–1233, 2003.

[176] F. Manford, A. Tronde, A. B. Jeppsson, N. Patel, F. Johansson, and B. Forbes, "Drug permeability in 16HBE14o- airway cell layers correlates with absorption from the isolated perfused rat lung," *European Journal of Pharmaceutical Sciences*, vol. 26, no. 5, pp. 414–420, 2005.

[177] E. Rytting, M. Bur, R. Cartier et al., "In vitro and in vivo performance of biocompatible negatively-charged salbutamol-loaded nanoparticles," *Journal of Controlled Release*, vol. 141, no. 1, pp. 101–107, 2010.