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Computational and bioengineered lungs as alternatives to whole animal, isolated organ, and cell-based lung models

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Patel B, Gauvin R, Absar S, Gupta V, Gupta N, Nahar K, Khademhosseini A, Ahsan F. Computational and bioengineered lungs as alternatives to whole animal, isolated organ, and cell-based lung models. *Am J Physiol Lung Cell Mol Physiol* 303: L733–L747, 2012. First published August 10, 2012; doi:10.1152/ajplung.00076.2012.—Development of lung models for testing a drug substance or delivery system has been an intensive area of research. However, a model that mimics physiological and anatomical features of human lungs is yet to be established. Although in vitro lung models, developed and fine-tuned over the past few decades, were instrumental for the development of many commercially available drugs, they are suboptimal in reproducing the physiological microenvironment and complex anatomy of human lungs. Similarly, intersubject variability and high costs have been major limitations of using animals in the development and discovery of drugs used in the treatment of respiratory disorders. To address the complexity and limitations associated with in vivo and in vitro models, attempts have been made to develop in silico and tissue-engineered lung models that allow incorporation of various mechanical and biological factors that are otherwise difficult to reproduce in conventional cell or organ-based systems. The in silico models utilize the information obtained from in vitro and in vivo models and apply computational algorithms to incorporate multiple physiological parameters that can affect drug deposition, distribution, and disposition upon administration via the lungs. Bioengineered lungs, on the other hand, exhibit significant promise due to recent advances in stem or progenitor cell technologies. However, bioengineered approaches have met with limited success in terms of development of various components of the human respiratory system. In this review, we summarize the approaches used and advancements made toward the development of in silico and tissue-engineered lung models and discuss potential challenges associated with the development and efficacy of these models.

bioengineered lung; computational modeling; in silico; lung models; tissue engineering

LUNG DISEASES ARE THE THIRD leading cause of deaths in the United States that translate into one in six deaths. More than 400,000 Americans die of lung disease every year and around 35 million are now living with chronic lung problems (1). Therefore, there is an important need to understand as to how a new drug entity or a novel formulation may influence the anatomy, physiology, and pathogenesis of lungs and vice versa.

The human lung has an approximate volume of six liters, contains about 300 million alveoli, and provides a total surface area of 100 square meters as blood-air interface, which is packed into an elastic dynamic structure responsible for gas exchange (159). The human airway wall is a connective tissue that includes smooth muscle cells (SMC), mucus glands, blood vessels, and fibroblasts surrounded by a collagen-rich extracellular matrix (ECM). Epithelial cells, responsible for the efficient oxygen and carbon dioxide transfer, are at the interface between air and the connective tissue and are attached to an underlying basement membrane. The respiratory system is the site of a variety of pulmonary diseases such as asthma, bronchitis, pneumonia, cystic fibrosis, emphysema, and cancer (137). The treatment of these pathologies often necessitates the use of drugs or antibiotics to reestablish tissue homeostasis. Extensive research is going on both in academia and in indus-

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tries to come up with new drugs as well as new delivery systems that might improve the efficacy, safety, or compliance of existing therapeutic strategies.

Preclinical studies involve extensive use of animals to assess pharmacological and toxicological aspects of an investigational drug candidate. In fact, animal research has been routinely carried out to obtain important information concerning many physiological and pathological processes that are relevant to humans and thus has been fundamental in the development of many drugs. However, animal models have never been perfect and their application is always a controversial aspect of medical research (111). Because diseased animal models are usually created by administration of chemical agents, such physiological milieu may not entirely be translated into human disease conditions. Also, the methods by which animals are used during the experiment can rarely be applied to humans. Hence diseased animal models are able to achieve only ~60% of correlation with actual pathological conditions in humans (65). The lack of correlation between animal models and humans was evidenced by the tragic event experienced in 2006 when the TeGenero anti-CD28 antibody caused multiple organ failure within hours in human volunteers, despite having been tested in monkeys at 500 times higher concentrations (147). Furthermore, the correlation is adversely affected by the interspecies anatomical and physiological variability (64). Besides, maintaining a running animal facility with trained personnel adds a significant economic burden for researchers (56). As a result, scientists tend to select the smallest number of animals per group to achieve statistically significant outcomes. Also, acute strategies are usually performed to create even chronic disease models that might not closely resemble the exact pathological conditions. All these attempts aimed to reduce the cost contribute significantly in the variability or lack of correlation with humans.

An experimental model that can closely mimic the clinical conditions, can allow required manipulations to fit the extent of severity of a disease in an individual, and can be used as an alternative to animal models has potential to gain significant interests for both practical and ethical reasons. Research into this direction could minimize the use of animal models to investigate drug candidates and/or delivery systems to treat lung disorders. One of these approaches, i.e., in silico models, is an extension of in vitro models and utilizes computer simulation to model a physiological or pharmacological process (31). In silico models combine the benefits of both in vitro and in vivo models and can include parameters that are difficult to achieve in experimentally. On the other hand, bioengineered lung models are extensively being investigated to replace live organ models (113). Along with its use to test a particular drug candidate in research laboratories, engineered lungs have a high potential to be used as alternatives to organ replacement therapy. This review summarizes the work carried out toward the development of in silico and bioengineered human lung models along with the future direction toward development of sophisticated artificial lung models.

IN SILICO MODELING OF AEROSOL DEPOSITION IN LUNGS

In silico modeling of airway mechanics, airflow dynamics, and particle deposition in human lungs could be established as noteworthy tool in the areas of inhalation toxicity, propagation of infectious lung diseases, and drug delivery for local or systemic pharmacological actions. Precise quantification of aerosol fraction deposited into the lungs is important to advance the respiratory route as a way to deliver drugs and to relate certain lung diseases with the extent of exposure to hazardous airborne particulate matter and pathogens (42, 57). Furthermore, it is essential to obtain accurate quantitative data on aerosol deposition in different regions of lung and link them with airway responses for enhanced understanding of the intrinsic characteristics of various lung diseases (100). However, accurate computational estimation of aerosol deposition is very complicated because of the intricate airway geometry, inconsistent nature of the airflow during respiration, complex airway mechanics, presence of surfactant layer on the luminal airway wall, and significant patient-to-patient variability. In fact, the inconsistencies in the airflow pattern in the lungs are observed because of differences in the numbers and angles of divisions of airways and changes in airflow from laminar in upper airways to turbulent in terminal airways. Disease conditions also make the overall airway mechanics very complex. In addition, earlier computational fluid dynamics (CFD) models were required to use simple airway geometries incorporating only few generations of tracheobronchial tree because of computational limitations (32, 33, 47, 76, 86, 94, 98, 171–173). Lately, experiments conducted by Nowak et al. (124) demonstrated that a general model of airway tree based on standardized geometry is not suitable to predict aerosol deposition and concluded that more practical models generated from imaging techniques are required, since geometry of the airways has a strong impact on the aerosol deposition. Hence a realistic model of human tracheobronchial tree to study airflow patterns and particle deposition must carry all these complexities to achieve successful and reliable prediction of the fate of inhaled particles in the lungs. In silico models are aimed to incorporate such complex parameters to predict the deposition pattern of particulate carriers in the airways. However, it is always of significant concern whether inclusion of all the parameters in a predictive model is practically possible or not and, even when it is possible, how precise the predictability would be. There is also skepticism about the dynamic nature of such models required to respond to the pathological changes associated with each disease. Additionally, pragmatic application of such models to predict deposition profile in the lower airways is also a critical concern. Thus the following section reviews these aspects of in silico lung models seeking answers to such issues. Furthermore, since development of in silico models utilizes raw information gathered from in vivo or in vitro experimental systems, brief discussion of such systems is also presented here.

In Vivo Experimental Methods

Several experimental and mathematical studies have been conducted to determine the fraction of aerosol deposited in different regions of human lungs. It has been established that aerosol deposition in lungs not only is a function of particle size but also is influenced by airflow patterns and collective geometry of airways. Thus it is difficult to rationalize the use of animal models to predict aerosol deposition patterns in human lungs largely because of significant differences in the anatomy of lungs of animal species compared with human lungs. Hence earlier attempts involved in vivo investigations

using radionuclide imaging techniques for quantification of regional deposition in human lungs. Tossici-Bolt et al. (152) developed an analytical algorithm to translate planar scintillation images [two-dimensional (2D)] of aerosol distribution in the lungs into equivalent three-dimensional (3D) images. Similarly, Usmani et al. (156) used technetium-99m-labeled monodispersed albuterol aerosols to determine regional drug deposition in 12 asthma patients using planar gamma-scintigraphy. However, these studies provided only elementary information about aerosol deposition in large anatomical segments such as the extrathoracic, thoracic, and alveolar regions. Subsequent studies were attempted to gather the information about aerosol deposition at much smaller scales, for instance, in each generation of bronchial tree. Fleming et al. (49) and Hashish et al. (66) utilized multimodality imaging techniques such as computed tomography and magnetic resonance imaging to measure 3D distribution of radiolabeled aerosol and transformed the information to obtain aerosol distribution by airway generations with the help of airway dimensions described in the Weibel model (158). Another study, focused on improving spatial discrimination of aerosol deposition patterns, used single photon emission computed tomography (SPECT) and mathematical models to create a concentrically sliced 3D lung model to quantify the radiolabeled aerosol deposition in each of the airways in lungs (107). Some researchers have also reported indirect method of measuring total lung deposition by quantifying the concentration of aerosol retained in mouth or nose (39, 40, 82), whereas others have attempted to measure aerosol deposition in different regions of lung on the basis of their volumetric depth using serial bolus delivery of an inert aerosol (81, 83).

Basic methodologies to determine particle deposition in the lungs are either pharmacokinetic, providing estimation of total lung deposition based on plasma and/or urine drug concentrations, or radionuclide imaging providing information on particle deposition in different regions of the lungs. The main advantage of this imaging technique is the visual conformation of an expected aerosol deposition pattern for which it has been designed. Previously, 2D gamma-scintigraphy was widely used as an imaging technique to characterize inhaled drug deposition. Equipped with either one or two gamma cameras, this instrument can supply information about allocation of radiolabeled agent in the specific organ (119). The quantification of amount of particles deposited in different lung regions is achieved by spatially dividing the bronchial tree into regions of interest and calculating the ratio of the amount of particle deposition in peripheral region (P) to that of central region (C), also known as planar index. It actually indicates the proportion of aerosol deposition in small bronchioles and alveolar region compared with that in tracheobronchial region (27, 118). However, since this technique projects a 3D organ such as lungs in planar images and estimates particles deposition on the basis of (2D) images, it lacks 3D resolution and overestimates particle deposition in overlapping airways. Hence attenuation for the radioactivity in the chest wall is required to be corrected for accurate estimation (114).

Recent advances in the field of nuclear imaging have made possible much accurate measurement of deposited radiolabeled particles in human lungs (12, 41, 44, 50, 51, 93, 107, 118). SPECT and PET (positron emission tomography) are now used to create tomographic 3D lung structure by utilizing the information gathered by revolving detector cameras around the subject. However, the clarity of the images depends on the lung and patient movement and on the number of observations during the scanning procedures.

SPECT. SPECT uses a gamma camera rotating completely around the subject to provide 3D image of the lungs (26). In SPECT, the single camera rotating around the chest of the patient laid in supine position takes a longer time for scanning and may lead to erroneous quantification of deposited particles due to higher absorption, mucociliary clearance, or cough (20, 93). To resolve this issue and increase accuracy, double- or even triple-headed cameras have been employed. Newman et al. (118) used this technique to construct a 3D model of particle deposition in lungs using a mathematical algorithm, whereas Eberl et al. (44) used it to separately quantify the aerosol deposition between small and large airways. 3D lung structures, generated by this technique, are usually divided into different regions of interest, called voxels (Fig. 1) (105). On the other hand, several other researchers have also divided lung regions into concentric shells, considering main bronchial bifurcation as central axis, as shown Fig. 2 (8, 106). These shells are further transformed into spatial arrangements with the help of radioactivity counts to derive a parameter called penetration index, which is a ratio of P to C or C to P, as described above.

PET. The fundamental principle of PET is the annihilation of a positron and production of two identical photons upon interacting with radionuclide, which travel in opposite directions from the site of origin. The signal is identified by pairing a photon with an identical photon detected at 180° in the opposite direction. This significantly increases signal-to-noise ratio and puts PET a step ahead compared with traditional gamma-scintigraphy.

In previous studies, radiolabeled albumin spheres were administered along with desired aerosol formulations to determine particle deposition, instead of attaching radionuclides on the inhaled particles of interest (126). However, modern development of sophisticated PET techniques have made it possible to directly label drug particles with positron emitters. Hence radiolabeled aerosol particles can be formulated through the same procedure as normal formulation and the same delivery system can be employed for testing in humans. Radionuclide decay time needs to be taken into consideration as these radionuclide have short half-lives (for instance, half-life of ^{11}C is 20 min and that of ^{18}F is 110 min) and the preparation of the radiolabeled drug into inhalable dosage form and its loading into the delivery device may consume appreciable amount of time. Furthermore, the procedure of radiolabeling may influence the size of the inhaled particle and significantly affect the observations while investigating the lung deposition patterns of aerosol particles. Therefore, changes occurring in particle size after incorporation of the radionuclide need to be supervised and, if required, suitable corrections ought to be made. Otherwise, underestimation of particle deposition may be observed (18).

Both SPECT and PET techniques offer certain advantages and have some drawbacks, which make the process of selecting one over the other rather difficult. Other factors like the field of view of the cameras, the radioactivity dose required, as well as the scattering and attenuation correction procedure need to be considered when choosing between these

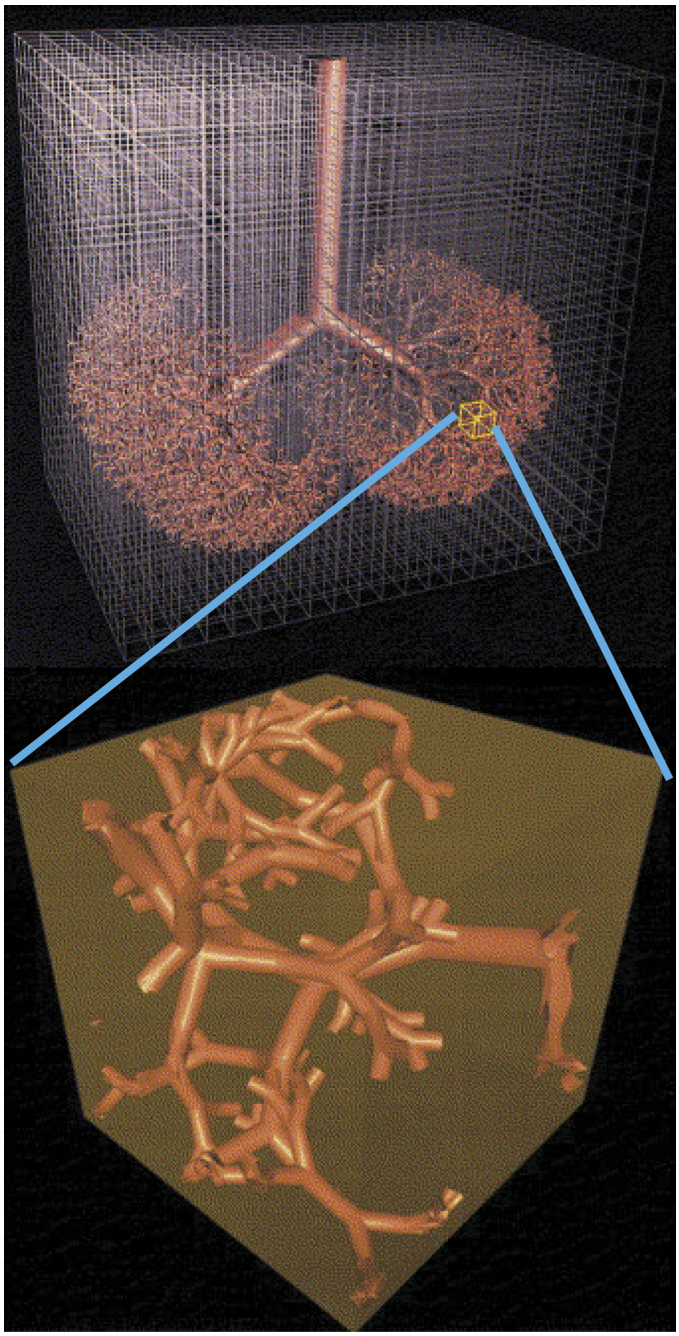

Fig. 1. A matrix of voxels corresponding to the single photon emission computed tomography (SPECT) format was superimposed on the complex 3D network of airways (*top*). The *bottom* image (zoomed in) shows the airway composition in an individual voxel (105).

two techniques. SPECT has significantly higher field of view (>45 cm in the *Z*-direction) and can completely scan the designated region in a single image. On the other hand, PET has limited field of view (~15 cm in the *Z*-direction) and multiple images are required to obtain a complete 3D structure. Additionally, higher doses of radioactive substances are administered during PET imaging compared with SPECT scan on human lungs. However, this radioactive dose is neither too high nor unsafe for humans and significantly increases signal-to-noise ratio (3).

In Vitro Experimental Methods

Several other attempts of measuring aerosol deposition in human lungs were performed by using airway hollow cast models. Plastic cast models of lungs for many species have been developed by several researchers using a three-step process (17, 21, 23, 30, 46, 60–62, 138–141). During lung cast preparation, excised lung is air dried until the parenchyma becomes completely dehydrated and rigid. Subsequently, a solvent-dissolvable silicon resin is poured into the dried lung through the trachea and allowed to harden. The dried tissues are then removed by dissolving them in acidic solution, leaving the resin cast behind. This cast is then dipped into a curable resin solution, which is then cross-linked. Finally, the internal resin is dissolved away, creating a hollow cast of the lungs. During quantitative particle deposition experiments, these hollow casts are usually suspended in an artificial thoracic chamber where physiological respiratory pressure conditions are maintained and aerosol is administered through the trachea. Determination of aerosol deposition in these models usually involves a destructive method whereby the cast is carefully segmented and the amount of aerosol deposited in each segment is measured. Chan et al. (21) studied the regional particle deposition in a hollow cast model of human lung airways lengthening up to six generations and compared the observations with the one obtained in nonsmoker healthy volunteers. On the basis of their experience, they introduced a new anatomical parameter, called the bronchial deposition size, which allowed them to classify different individuals or populations on the basis of their tracheobronchial deposition efficiencies. Gurman et al. (61) examined the deposition of monodispersed aerosol in human upper airway cast model under constant and cyclic inspiratory flow conditions and observed higher bronchial deposition during cyclic flow. It suggested that a realistic model for particle deposition in the lungs needs respiratory

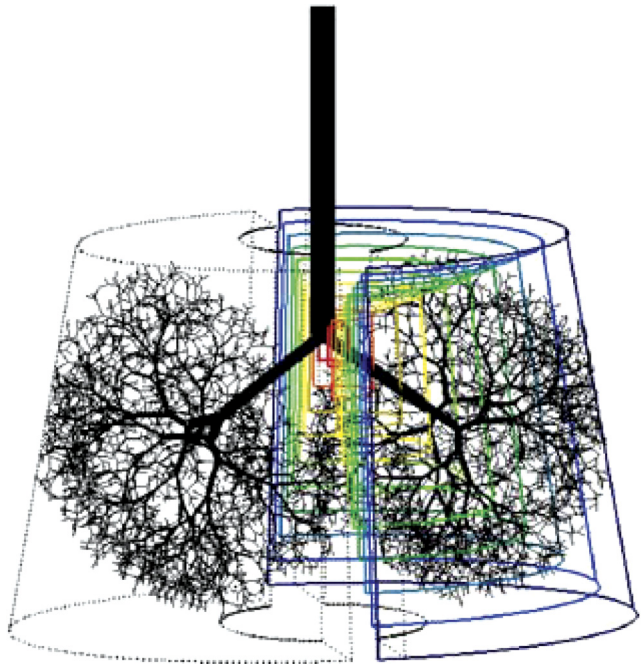

Fig. 2. Three-dimensional computer-generated lung airways were subdivided by superimposing nested truncated shells (106).

airflow patterns mimicking the conditions that occur in vivo. Hence mechanically operating larynx with changeable opening was attached to the tracheobronchial tree for subsequent particle deposition studies (62). Similar observations were also reported by Schlesinger et al. (138), where significantly higher deposition of particles was measured in replicate hollow casts of a human tracheobronchial tree under cyclic inspiratory flow compared with constant flow. Particle deposition patterns and their relation to inspiratory airflow were also investigated in a hollow cast model of donkey trachea which exhibited similar deposition patterns to those obtained from an in vivo model (139). In another study, the effects of particle size and inspiratory patterns were investigated on particle deposition in cast model of human upper airway starting from the oral cavity and extending up to three bronchial generations. The observed particle deposition was found to be a function of Stokes number and suggested that impaction is the major mechanism for particle deposition (23). Stokes number, also known as inertial parameter, is an index of impactability of an aerosol particle. The value for the Stokes number determines whether the airborne aerosol particle will follow the fluid stream or travel straight and impact on the wall when fluid stream is deviated.

Furthermore, Grgic et al. (60) demonstrated that, in a situation of idealized geometry of the mouth and throat and for a constant Stokes number, deposition pattern differs along with Reynolds number owing to variation in the flow field. The Reynolds number is a dimensionless parameter characterizing different type of fluid flow around an object. It is a ratio of inertial forces to viscous forces acting on an aerosol. A Reynolds number below 1 represents a laminar flow, whereas value above 1,000 leads to a turbulent flow.

Although hollow cast lung models represent the most accurate geometry of tracheobronchial tree, several discrepancies have been reported in the estimation of aerosol deposition in various regions of the lungs. The absence of a surfactant layer on the luminal surface of the airway wall drastically decreases the ability of airway to adsorb hydrophilic particles, which eventually lead to erroneous measurement of deposition. Additionally, there is a higher probability that the particles exiting from the outlet may deposit on the outer surface of the cast because of the application of even pressure in the enclosing chamber. Moreover, the process of using plastic casts for the determination of particle deposition is destructive, and a new replica is required for each experiment, resulting into higher interexperimental error.

In Silico Lung Modeling

Thus far, most of the attempts in modeling aerosol deposition in lungs were carried out either using highly simplified geometry models of human lungs (4, 5) or using anatomy-based models of lung with fewer generations and limited number of airways (60, 97, 98, 109, 110). Morphometric models of human airways that have been developed to compute aerosol deposition included typical-path models (168, 169) based on idealized geometry and multipath models comprised of asymmetric airway geometry (5). Although these studies have common interest of extrapolating the results obtained in artificial lung models to the actual human lungs, accurate geometries of human airways were not recapitulated by these models.

Earlier models of human airways have excluded mouth and larynx anatomy while performing particle deposition studies. However, a literature survey demonstrated that the throat plays an important role in determining lung deposition of inhaled particles and that the inconsistency observed in lung deposition is often due to the inconsistency occurring in the throat deposition (14). To evaluate the effects of upper airway on the lungs, a realistic human upper airway model was simulated by a computational method and compared with results obtained in an airway model starting from the trachea (95). This study demonstrated that the airway model containing oral cavity and larynx produced turbulent airflow in the trachea, whereas turbulence was negligible in the model starting from the trachea. This observation suggested that earlier studies, in which upper airways had not been included, are unreliable estimates and may not be utilized to quantify lung deposition following inhalation. Another study, designed to evaluate influence of extrathoracic airways on the regional and local microaerosol deposition characteristics, also showed that oral cavity has a significant impact on the lung deposition. Hence oral airway geometry needs to be included in experimental models of human airways (166).

On the basis of computational fluid dynamics, Nowak et al. (124) were among few of the researchers to predict particle deposition in whole lung. The authors concluded that the differences in aerosol deposition into the lungs due to intersubject variability were largely observed in proximal airways and negligible in the deep lung. However, the limitations of this study included the use of Weibel's idealized geometry and the exclusion of upper airways (124). Hence to create a highly realistic model of lung deposition, a CFD model of proximal airways and an analytical model of deep lung need to be combined, which can predict an aerosol deposition in whole lung taking subject-specific anatomical variability in account when required and ignoring it for deep lung regions. Annapragada and Mischchiy (3) supported this approach because they believed that it can largely decrease the required computational power and an accurate and total human lung aerosol deposition model can be constructed by putting together a CFD for proximal airways and an analytical or semiempirical computational model for deep lung. In a study, Ma et al. (101) constructed a human airway model extending from the mouth up to generation 10 of the tracheobronchial tree based on the anatomical and geometrical information obtained by medical imaging on healthy human subjects. The observations demonstrated that the computed extrathoracic deposition, the ratio of fraction of aerosol deposited in left to right lung, and the deposition efficiency at the level of each generation correlated with available in vivo and in vitro data and that micrometer-size aerosol particles were largely deposited in large-medium airways.

It is apparent that the above discussion was mainly focused on the development of predictive models of total and regional aerosol deposition in upper airways; understanding and estimating particle transport and deposition in alveolar region are very important to efficiently design an aerosol-based delivery system. However, because of its smaller size and lack of accessibility, earlier attempts to model the structure and dynamics of an acinar airway have shown limited success. Fun-

damental understanding of aerodynamic behavior of aerosolized particles suggests significant impact of alveolar geometries and airflow on particle deposition in this region. An earlier report (63) described the 3/4 spheroid as being the most common shape of an alveoli among a number of other proposed shapes. Furthermore, the 3D structure of acinar airways has been also modeled in different ways such as polyhedron models (149), full or partial annular rings representing alveoli surrounding cylindrical models (38, 154), and more interesting honeycomb-like polygonal models (88). These 3D acinar airway models, still being idealized, are more realistic and can incorporate irregularities in alveolar geometry and respiration. Hence they represent a significant step forward in modeling particle transport compared with the 2D models with complex branching structure. Additionally, in an acinar region, continuous changes in shape and size of the airways occur during breathing, which influence airflow pattern and subsequently the particle transport. Thus several researchers have included the moving wall feature of acinar airways while modeling aerosol deposition (6, 37, 68, 99). Furthermore, few studies suggest that airflow recirculation and irreversibility play important role in convective mixing of aerosol particles between tidal air and residual air in acinar airways (68, 155). Taking all together, it seems crucial to incorporate moving wall and ventilation features while modeling acinar airways for close-to-real estimation of aerosol deposition.

Although a realistic and complete experimental or computational model to predict regional and subject-specific particle deposition in lungs is yet not available, these attempts show remarkable progress toward developing computational models of aerosol deposition by including all possible factors. In silico models can offer significant advantages over conventional models, since these will be based on accurately captured geometries of airways and applicable to all the species (3). Additionally, these models can also incorporate hypothetical scenarios, such as disease-induced geometrical alterations. Indeed, advances in medical imaging techniques and computational capabilities are needed to significantly improve the analysis of the regional deposition of inhaled particles and to establish 3D in silico modeling itself as a powerful tool to elucidate particle deposition patterns in the human lungs. Furthermore, these computational models need to be validated in terms of their applicability in different individuals and in different disease conditions before incorporating these models for routine use. With the astonishing current rate of advancements in computational and imaging technologies, incorporation and modulation of complexities of human lungs in an in silico models seem imminent in the near future.

Application of In Silico Modeling in Lung Diseases

Several lung diseases are associated with a series of physiological and morphological changes in the lungs. In silico lung modeling may provide an opportunity to include such changes and assist in predicting outcomes. Relevant computer codes can be introduced in the already developed computational models to account for such changes occurring in lungs during disease conditions. In this review, an example of in silico modeling of asthmatic airway has been described. Asthma is a complex lung disease affecting both the airway morphology and the ventilation patterns in a patient. It eventually leads to modified airflow patterns in the airway lumen, which in turn influence the flight of inhaled particles and their deposition in the different regions of the lungs. Thus, to study the altered behavior of the particles in an asthmatic lung, Martonen et al. (105) proposed a mathematical model with the intention of exploring the various factors influencing particle deposition in disease-induced lung model.

In this in silico model of particle deposition in asthma, Martonen et al. (105) incorporated various changes occurring in human lungs during progression of the disease into a previously developed in silico model of healthy human respiratory system (85, 93). In this model, computer codes were developed in such a way that they had immediate applicability to the delivery of inhaled aerosol and could be customized to each patient. Narrowing of the airway lumen is the major characteristic of asthma and is attributed to the combined effect of bronchoconstriction of the airway smooth muscle, inflammation of the airway walls, and the thickening of the mucus layer. Along with this, critical issues usually associated with asthma such as the heterogeneity and severity were simulated in this model. The heterogeneity was incorporated into this model by physically inducing symptoms of the disease at large, central, and small airways of the tracheobronchial tree. Furthermore, severity in this model was included in the form of reduction in airway diameters by 20 and 40% due to bronchoconstriction, inflammation, and mucus. Newly developed computer code describing asthma morphology algorithm was integrated into the source code of an aerosol deposition model designed for the targeted delivery of inhaled drugs. This asthma model was used to measure the effects of disease on the deposition of aerosol particles. Additionally, different ventilation patterns were also simulated with the help of the computer and incorporated in this model to account for the intersubject variability in the breathing patterns. As a result, this model was able to simulate the alterations occurring during deposition of the inhaled drug particles in the diseased airways. In conclusion, authors suggested that this mathematical model should be utilized for the safe and efficacious delivery of the pharmaceutical drugs to the desired sites in asthma patients.

CELL-BASED 3D MODELS FOR LUNG TISSUE ENGINEERING

Introduction to Tissue-Engineered Models

Since lung tissue does not regenerate, the only way to replace permanently damaged and dysfunctional airways is by transplantation. The actual shortage of suitable organs available for transplantation results into a constant increase in number of patients on waiting lists, and current technical limitations of medical devices provide only temporary benefit to the patients. Therefore, there is a need for lung tissue analogs for both in vitro assays for drug development and in vivo application for lung regeneration (131). Over the last 20 years, the combination of medicine, biology, engineering, and material science has led to the development of multiple engineered tissue models such as skin (7, 59, 167), blood vessels (89–91, 122, 162), cartilage (54), ligaments (58), heart valves (174), and bladder (125) that are currently used for clinical applications and fundamental studies. However, complex organs such as lung and liver are still at their early stage of development since current tissue engineering strategies have

shown limited success in the fabrication of functional 3D tissues.

The most common tissue engineering strategy consists of harvesting cells from a patient, expanding them in culture, and seeding them into a biodegradable scaffolding material. This material provides an adequate 3D environment for the cells to proliferate and can later be implanted into the patient or used for in vitro studies. When implanted, these cells would produce their own ECM and form a new tissue in vivo, which will gradually replace the scaffold. Scaffolds must be biocompatible and capable of reproducing the characteristics of the local microenvironment found in native ECM. Their design can be based on porosity, elasticity, transport, and diffusion capabilities, as well as other physical, biological, and chemical requirements to build a functional tissue. In the case of lung tissue, the scaffolding material needs to provide a framework adequate for cell growth and tissue development without compromising the elastic recoil of the engineered tissue (120).

Increasing the Complexity of Tissue-Engineered Lung Models

The ultimate goal of tissue engineering is the repair and the regeneration of damaged tissue. However, applications outside the clinical field such as the replacement of in vitro models for fundamental research, safety testing, and drug screening are in need of more physiologically relevant human tissues (9). The high cost of pharmaceuticals and the difficulty of rapidly identifying new environmental toxins result from the lack of efficient experimental models that can replace costly and time-consuming animal studies (72). Animal models have provided a great extent of information related to the fundamental behavior of lung tissue. However, they remain complex and difficult to control in terms of parameters susceptible to modulate the results in comparative studies. Tissue-engineered models have been primarily developed to overcome interindividual variations among animals used in experimental protocols. They present significant advantages since multiple variables can be discriminated from one another and their usage reduce the number of subjects needed to perform a specific study, which can be considerable especially in the drug development business. This industry is striving to develop methods allowing precise evaluation of newly developed product regarding safety standards and side effects (144). Therefore the use of tissue engineering strategies to mimic lung function in vitro will help to further investigate the interactions between multiple factors or drugs contributing to long-term lung repair and remodeling in response to physical, biological, or chemical factors.

Multiple biocompatible and biodegradable scaffolding materials have been developed to engineer 3D lung tissue. Natural ECM such as collagen (19, 69, 128, 129, 145), Matrigel (115, 116), and other naturally occurring polymers (2) have been used for both in vitro and in vivo studies. Similarly, synthetic polymers such as polyglycolic acid (PGA) (142), poly(lactic-co-glycolic acid) (PLGA) (115), and pluronic F-127 (PF-127) (34) have shown great potential for lung reconstruction. The main advantage of synthetic scaffolds over natural ECM is the capability to process them to tailor their mechanical, chemical, and biological properties. Porous PF-127 scaffolds seeded with lung progenitor cells produced alveolar structures morphologically similar to lung tissue following in vivo implantation (34). Others have achieved similar results using an injectable polymer or a collagen-glycosaminoglycan scaffold, showing the capability of lung cells to reproduce lung structures when seeded in an open porous scaffold (2, 22).

From a clinical perspective, growing lungs by combining a scaffold seeded with autologous cells from a patient could decrease the chance of rejection and potentially improve the success and patency of the transplant. The use of dermal fibroblasts, a readily available autologous cell source, has proven to be efficient for repairing lung tissue in vivo (77, 137). Using a scaffold-free approach similar to previously published studies (92) in combination with temperature responsive culture dishes, Kanzaki and coworkers (77) have shown the capability of cell sheets to effectively seal air leaks following thoracic surgery. Moreover, cells sheets were fully integrated within the native lung tissue 4 wk postsurgery and the entire surface of the implanted cell sheet was found to be covered by mature mesothelial cells with microvilli 3 mo after transplantation. Decellularization of the lung tissue using detergent has been also proven to be efficient to produce a 3D structure that allows cell seeding (120). Figure 3 summarizes key processing steps to obtain decellularized lung matrix suitable for cell seeding, growth, and eventually implantation (130). The generation of a tissue-engineered airway patch from autologous muscle cells and fibroblasts seeded on a decellularized porcine matrix resulted in the successful treatment of a

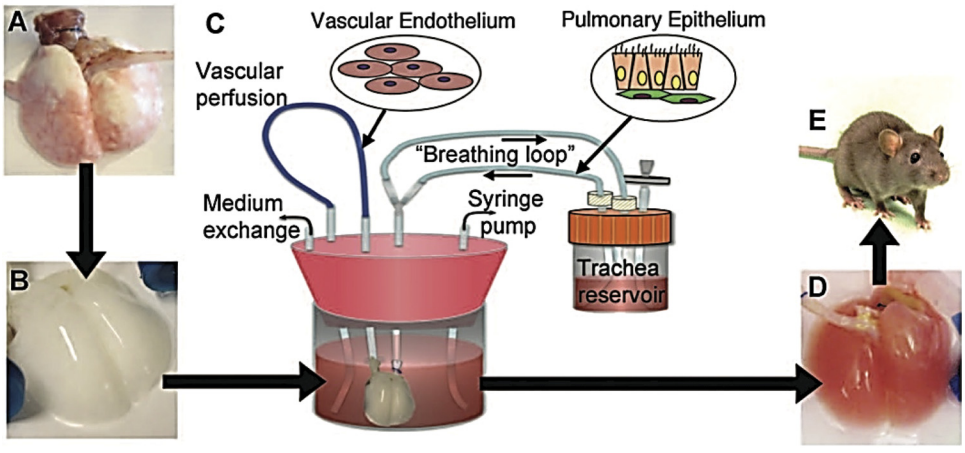

Fig. 3. Schematic representation of the key processing steps involved in lung tissue engineering. A decellularization solution is infused into an isolated rat lung through cannulated pulmonary artery and trachea (*A*) to obtain acellular lung matrix devoid of any cells (*B*), which is subsequently mounted on a bioreactor for seeding of respective endothelial cells (*C*). The engineered lung is collected from the bioreactor after 4–8 days of culture (*D*) and is ready to be implanted into synergistic rat recipient (*E*). Reprinted with permission from Ref. 130.

human patient and completely restored the functional airways as required (103, 160). Tissue engineering approaches were also investigated to perform bronchoscopic lung volume reduction, a procedure aiming to improve the respiratory function in patients suffering from advanced emphysema (75). In this study, the promotion of scar tissue formation using a fibrin hydrogel sealant and the resulting resizing of the lung enhanced the respiratory function without the major trauma usually associated with surgery. However, recent findings have shown that regeneration of the damaged airways is regulated by multiple cell types and processes, dependent on the synergistic interactions between the different cell types present in lung tissue (170). These results reinforce the concept that the use of adequate cell types displaying appropriate phenotype could improve engineered lung function and promote organotypic tissue regeneration.

Engineering tissues comprising the entire complexity of human lungs is technically difficult and very challenging. Although there is significant effort to reproduce this configuration in vitro, most models lack the dynamic component such as the breathing movement, a characteristic of lung tissue. Tissue-engineered models including this dynamic environment have clearly shown the direct influence of mechanical stimuli on tissue behavior (29, 148, 150, 153). Since various cell types sense biological, chemical, and physical cues differently, the behavior of airway wall needs to recapitulate the physiological tissue structure and biomimetic environment of lung tissue (24, 25, 129). The importance of the ECM composition has also been shown to play an important role in the behavior of lung cells and tissue (55, 165). Airway wall remodeling observed in pathologies such as asthma are characterized by subepithelial thickening and remodeling of the ECM. Thus cell-cell and cell-matrix interaction are essential for airways formation and it is necessary to reproduce these conditions in vitro to obtain relevant engineered lung tissue for pharmacokinetics. The adequate formation of features such as the basal lamina, which comprises type IV collagen, laminin, and specific glycosaminoglycans and where endothelial cells reside is therefore essential for pharmacological applications and drug screening.

Since it is very difficult to interpret the relevance of tissue response to various stimuli in the absence of a dynamic and biomimetic 3D environment, it would be of great interest to reproduce these features in vitro to enhance the biofidelity of the experimental models. For example, the complex 3D architectural structure of lung parenchyma requires connection of alveolar units to the airways and to the pulmonary circulation. Most existing models do not recreate this active interface where solute transport and nutrient exchange takes place. The flexible and dynamic structure of the lung, in which mechanical forces play an essential role, would require the design of often-complex bioreactors (53). Physical stimuli involved in the action of breathing were shown to be involved in the initiation and maintenance of lung regeneration of mature lungs (133). Therefore, the understanding of this dynamic balance and its application to tissue-engineered models will depend on the ability to integrate the biology, the chemistry, the physics, and the surrounding physiological environment to efficiently produce lung substitutes in vitro (28, 70, 134). Recent development in the field showed that lung cells seeded in a dynamically strained 3D scaffold result in the formation of morphologically relevant alveolar structures (120, 151). The use of a bioreactor to condition the engineered lungs in vitro also allowed the cells to proliferate, differentiate, and regenerate the tissue (123, 151). A biomimetic microdevice reproducing the structural, functional, and mechanical behavior of human alveolar-capillary interface by precisely mimicking the lining of the alveolar air space has been recently published (71, 72). This microsystem reproduces the dynamic distortion of the alveolar-capillary interface and shows that, when cultured in the microdevice, epithelial cells increase their production of surfactant, as does the native lung to avoid drying of the tissue (72). This model was also shown to be sensitive to bacterial infections such as *Escherichia coli*, demonstrating the capability of the bioinspired system to effectively recapitulate the normal cellular response to microbial infections in human lung alveoli. Although it remains challenging to include immune cells in tissue-engineered models, this study has demonstrated that breathing motion accentuates proinflammatory activities and contributes to the development of acute lung inflammation (72).

Tissue-Engineered Lung Models in Drug Development and Discovery

The main difference between engineered lung tissues designed for therapeutic vs. pharmacological applications resides in the targeted functionality. Tissue-engineered lung for therapeutic applications can follow the traditional approach of cells seeded in a 3D scaffold favoring growth and differentiation, to generate the assembly of a functional tissue (112). As mentioned previously, this method has proven to be suitable to reestablish tissue function in various in vivo studies (34, 77, 103, 160). In contrast, engineered lung for pharmacological applications has to include a variety of specialized cells present in native tissue such as epithelial cells, SMCs, endothelial cells, and pneumocytes, assembled in a precise and hierarchical architecture. This level of complexity is responsible for the slow progress in this field but is essential to provide adequate and reliable tissue for the study of pharmacological agents and for drug screening.

Experimental access to the human airways for the development of pharmacological products is very challenging. Postmortem analyses on biopsies have provided their share of information, but supply of these tissues is limited and the control of experiments involving these samples is also limited. Traditional monolayer cell culture have also provided extensive fundamental knowledge regarding cell response to different biological and biochemical stimuli, but they still lack the cell-cell and cell-ECM interactions, as well as the dynamic environment present in vivo. In vitro studies of respiratory disorders have been facilitated by the development of tissue-engineered lung models using different cell populations isolated from asthmatic and healthy individuals (128). However, there is a marked interest to develop culture systems in which a physiological environment can be reproduced to facilitate the testing of different physical, chemical, or biological stimuli or pharmacological agents on engineered lung to study cellular response to those stimulants. On the basis of the fact that mechanical forces can directly influence cell function via mechanotransduction pathways (16, 164), tissue-engineered models have been designed to characterize the effect of dynamic strain on lung tissue (24, 25). Results showed that mechanical strain induces the upregulation of collagen III and

IV, as well as MMP-2 and -9 expressions by lung fibroblasts, indicating a remodeling response of the tissue in vitro. Furthermore, studies have reported that the 3D computational models can be very useful in predicting cell-ligand or cell-cell interactions as well as in modeling mechanobiological responses such as epithelial layer disruption under the influence of hydraulic stresses generated by the movement of air-liquid interface or microbubble flow (36, 87).

Microfabricated models have also been established to study bronchial response to various agents and to understand the mechanisms responsible for disorders associated with mucosal inflammation and airways hyperresponsiveness such as asthma (71). Nanoparticles are known to stimulate pulmonary epithelial cells to produce inflammatory cytokines (117). Recently a 3D model of the human airways using a coculture of human bronchial and fibroblastic cells has been developed to investigate the effects and evaluate the risk assessment of carbon nanotubes (CNTs) on the respiratory system (143). Results have shown increased production of nitric oxide (inflammatory marker) and decreased cell viability as a function of CNT concentration in an aqueous solution. This model presented a physiologically relevant arrangement of epithelial and fibroblasts cells separated by a thin semiporous membrane in a 3D collagenous ECM, similar to the basement membrane found in vivo. Similarly, biomimetic pulmonary-alveolar-capillary barriers reproducing the support of gas exchange in the lung are currently gaining speed as the new approaches to study lung disease and physiology (69, 72, 120).

Advantages and Limitations of Currently Available Tissue-Engineered Lung Models

The human airways act as a barrier and consist of organized tissue layers comprising multiple cell types and depending on cell-cell and cell-ECM interactions. In the absence of ECM, cells grown in monolayer or in 2D coculture cannot adopt an organization comparable to native lung tissue. In comparison, 3D tissue-engineered models can provide access for these interactions to occur through soluble factor expression and exchanges. Therefore, they more closely mimic lung tissue and they represent relevant platforms for the study of fundamental mechanisms such as diffusion, mechanotransduction, and remodeling, among others (24, 25). One of the most common methods used for the determination of in vitro development of pulmonary tissue is the formation of alveolar-like structures, observed by traditional histological analyses. Although widely accepted, this method is exclusively based on cell morphology and remains simplistic. It could benefit from more comprehensive identification and evaluation methods of the functional characteristics of lung tissue. Because the lung comprises a heterogeneous cell population having different levels of specialization, it is of the utmost importance to develop tools that reproduce functional 3D tissue displaying adequate organization, since it is responsible for the interaction between the multiple cell types that trigger physiological response to external perturbations (74, 146).

The synthesis of new biomaterials and the development of new fabrication strategies have led to increased complexity in the structure and biological functions of engineered tissue constructs. However, some significant challenges such as vascularization and biomimetic properties still need to be further addressed and tailored depending on the required application. The optimization of scaffold architecture and bioactivity also needs to be adapted depending on the application, such as fundamental research, regenerative medicine, or drug screening. Microscale technologies have proven to be powerful for both tissue engineering and fundamental biology applications since they allow the fabrication of materials incorporating biological and physical cues that influence cell fate and behavior. A better understanding of the cellular interactions with the microenvironment and the mechanisms determining stem cells differentiation combined with novel fabrication strategies will allow the integration of complexity into engineered tissues.

Emerging Trends and Opportunities in Lung Tissue Engineering Development

To develop tissue substitutes that restore the normal function of living tissue, it is important to establish appropriate design criteria. These design features need to be based on an extensive understanding of the cellular and molecular mechanisms regulating the tissue of interest. They also need to account for the role that ECM proteins and mechanical stresses play in tissue repair (131). Recent advances in microtechnologies have resulted into new tissue engineering approaches based on the replication of cell-scale complexities into 3D structures. These parameters are critical since the spatial organization and behavior of the cells depend on these factors and regulate tissue development (73). Therefore the use of in vitro lung models showing physiologically relevant cell and tissue organization, such as a pseudo-stratified epithelium and a basement membrane, and functionality such as contractility, oxygen exchange, and mucus production, is of great interest for the field of pharmacology. Lung tissue combines the requirements for adequate structure, vasculature and function to perform its duty. It is expected that new research fields such as micro- and nanotechnology, stem cell biology, systems biology, tissue engineering, and regenerative medicine will converge to generate products that are urgently needed for the treatment of patients and the development of useful pharmaceutical tools.

Scale-up of in vitro models. The main challenge is the requirement for scalable engineered constructs reproducing the microenvironment found in vivo. Microscale technologies are currently studied as potential tools for addressing these issues. The cell-seeded scaffold approach, which led to significant advances over the past decade, is currently shifting from empirical approaches to precisely engineered systems based on mechanistic models, structures, and chemistries (80). Microfabrication techniques such as soft lithography, micromolding, and photolithography have recently emerged as powerful approaches to generate precisely engineered scaffolds. These techniques have provided a broad set of tools capable of probing and controlling cell behavior by producing cell-scale features into materials (11, 52, 78, 79, 163). Two distinct approaches have emerged from the use of microfabrication techniques in tissue engineering. Those can be classified as either "top-down" or "bottom-up" depending whether the process is used to control the microscale features of a bulk material (top-down) or to fabricate microscale functional tissue units that can be used as building blocks that can assemble together to generate larger constructs (bottom-up). Top-down approaches aim to control the microscale features of large

constructs. Some significant advances have been made by use of top-down techniques, and it has been demonstrated that microvasculature can be engineered in biomaterials and hydrogels by using micromolding (10, 13, 84). Bottom-up approaches, on the other hand, aim at generating large-scale tissues by assembling small building blocks. These repeatable blocks, comprising controllable and microengineered features, mimic the characteristics of native tissues, which are often made of multiple functional units. These building blocks can later be assembled in an organized fashion, resulting in functional engineered tissues. For example, it was shown that cell-laden microgels could be molded in multiple complementary microunits and assembled into self-organizing larger patterns, allowing to engineer tissue complexity (43, 48, 67). By using microtechnologies, it is also possible to create the patterning of multiple cell types, chemicals, and stiffness gradients with high-resolution spatial control (80, 132). These approaches are greatly contributing to the actual efforts in the field of tissue engineering to reproduce cell-cell and cell-ECM interactions with fidelity in engineered tissues. Therefore translation of these technologies to complex tissues such as the lung will help engineer biomimetic tissue.

Stem cells and lung tissue engineering. Considerable excitement has been generated by the possibility that human tissues and perhaps organs such as the lung could be regenerated following stem cell activation by means of regulated developmental processes. Stem cells have the capacity to maintain themselves indefinitely, while simultaneously dividing to generate daughter progenitor cells, which will continue to divide and eventually give rise to differentiated cell lineages. The use of stem cells requires an extensive knowledge about lung development and organogenesis, which has been extensively reviewed elsewhere (161). Most common lung diseases result in emphysema or fibrosis, consisting in continued pathological alteration of natural tissue morphology and leading to complete loss of lung function. Adult stem cells currently represent a potential cell source that could be used clinically for lung repair (102, 104, 121, 137). The possibility that the lung epithelium may contain endogenous populations of progenitor cells, located in the basal layer and in the upper airways, has been suggested to be responsible for maintenance and repair of the alveolar epithelium following injury (15, 45). This would support the idea that the loss or failure of these progenitor cells during senescence and disease could be accountable for the correlation between the diminution in diffusion capacity with aging and pathology (104). Three dimensional biomimetic scaffolds used in combination with lung progenitors and stem cells, specific growth factors and physical stimuli were shown to induce organotypic differentiation and lung tissue morphogenesis (115, 136). Similarly embryonic stem cells, which are known to maintain high proliferation and self-renewal capabilities, are currently being investigated for their potential use with these types of scaffolds (35, 96). Since cells can adapt and remodel in response to environmental cues, cocultures are increasingly being used to drive differentiation toward desired lineages. It has been shown that providing an appropriate microenvironment in the form of embryonic lung mesenchyme promotes the formation of pulmonary epithelium in vitro (135, 157). Comparative studies have also shown that decellularized lung and coatings of specific ECM proteins on tissue engineering scaffolds could lead to the differentiation of embryonic stem cells into pulmonary cells in vitro (35, 96).

Lab-on-a-chip approach for in vitro lung studies. The development of viable alternatives to evaluate the efficiency of drugs and identify the cellular and molecular mechanisms responsible for cell response and gene expression represents the future of pharmacological studies. Development of cell-based biochips that closely mimic physiological and pathological tissue response could tremendously improve current toxicology studies and the development of pharmaceuticals, which currently rely on animal testing and clinical trials. Better understanding of factors promoting lung repair and regeneration is needed to improve the efficiency of novel in vitro models designed for pharmaceutical screening. Breakthroughs such as the lung-on-a-chip (72), which integrates the chemical, biological, and mechanical structures and functions of native

Table 1. *Overview of the features, applications, and requirements of in silico and tissue-engineered lung models compared with conventional models*

Topic	In Vitro Models	In Vivo Models	In Silico Models	Tissue-Engineered Models
Advantage	Pharmacopeially accepted, a number of commercial drugs have been developed using these models	Human and animal tests possible; extensive history in drug development	Applicable to any geometry/species/patient; parametric studies possible	Possible to test a drug in a system that closely resembles the human lung structure
Disadvantage	Casts are destructive, not possible on humans; CT based; potential application to human model preparation is very involved; parametric studies are not possible	Radioactive tracers and concerns about exposure to ionizing radiation; parametric studies are technically feasible but not practical owing to radiation concerns	Accuracy demands imaging-based geometry; computationally highly intensive	Materials to build all the component of the lung structure are still to be developed
Best use of model	Early-stage feasibility testing of drug preparations; comparative, head-to-head studies of multiple preparations	Recent developments in PET tracers synthesis allow labeling of actual drug molecules in real formulation	What-if scenarios are easy to address; parametric variations are simple; excellent as a design tool	Once developed, distribution of drug and its efficacy could be analyzed in a condition that resembles the human lung
Access	Widely available with routine laboratory facility requirements	Via sophisticated medical centers	CT imaging required; high-performance computation required	Bioengineering facility is required

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lung, demonstrate the potential strength of these models. Microengineered approaches offer new opportunities to model specialized physical and biological microenvironment found in multiple organs. These devices could also lead to novel high throughput analysis and screening of cell and tissue response to drugs, chemicals, micro- and nano-particles, toxins, pathogens, and other physiologically relevant stimuli applicable to pharmaceutical applications. They represent a low-cost screening platform that could replace in vivo assays and substantially improve the predictive capability of pharmacological studies. To provide with a comparative perspective, the basic features and applicability of in vitro, in vivo, in silico, and tissue-engineered lung models have been summarized in Table 1.

CONCLUDING REMARKS

Development of a lung model in which pharmacological and toxicological studies of a therapeutic drug and delivery system can be effectively performed has gained significant advancement by applying in silico and tissue engineering strategies. Emergence of such systems has been inspired from the shortcomings of conventional in vitro and in vivo models. In vitro models are pharmacopeially accepted and a number of commercially available drugs were developed by using these models. However, the casts that are used to prepare these models are nonreusable and impractical for human use. Analysis of the efficiency, toxicity, and pharmacokinetic behavior of drug candidates is widely conducted in animal models, which, however, raise concerns regarding the outcomes because of intersubject variability and noticeable lack of correlation with humans. Development of in silico models, on the other hand, has shown significantly promising applicability to humans by including the critical parameters that are difficult to incorporate in both in vitro and in vivo systems. However, accurate acquisition of imaging-based geometry and computational capabilities are still required to increase the precision of these techniques.

Prominent advancement has also been achieved in developing a tissue-engineered lung model. However, since lung is an organ and is therefore more elaborate than a tissue, there is a considerable gap between the currently available technologies and the clinical feasibility of implanting a bioengineered lung. Previous studies have demonstrated potential of repair for small sections of damaged lung tissue in vitro and in vivo, but no method has provided evidence of being able to reproduce a whole lung structure with full functional capabilities. The ongoing research focusing on designing new biocompatible materials, studying the influence of dynamic mechanical forces and mechanotransduction on lung development, and minimizing the immune response following implantation all represents great projects for thorough investigations, but it would be unlikely that the synthesis of a completely new material presenting lunglike properties would enable the fabrication of a 3D bioengineered lung. Similarly, the use of bioreactor culture of a tissue-engineered lung in vitro prior to its implantation would represent a complex and elaborate step that many would consider as impractical and inefficient for clinical applications. Recent trends in 3D organ reconstruction have shown that short-term achievements toward the production of a functional bioengineered lung suitable for transplantation are likely to result from the decellularization and the recellularization of a whole lung (127). This approach will eventually become compatible with stem cell technologies, thus reducing the risk of adverse immune response and increasing the success rate of bioengineered lung reconstruction. There is also a need for improved biomaterials and the development of biomimetic scaffolds to reproduce the complex microenvironment of human lung for in vitro studies. A better understanding of lung physiology as well as advances in micro- and nanotechnology, stem cell biology, tissue engineering, and regenerative medicine will lead to the successful development of models and therapies by enhancing the performance of currently available techniques used both in the laboratory for drug development purposes and in the clinic.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: B.P., A.K., and F.A. conception and design of research; B.P. prepared figures; B.P., R.G., S.A., V.G., N.G., and K.N. drafted manuscript; B.P. and F.A. edited and revised manuscript; F.A. approved final version of manuscript.

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