Materials Today Advances 14 (2022) 100216

Contents lists available at ScienceDirect

Materials Today Advances

journal homepage: www.journals.elsevier.com/materials-today-advances/

Airborne toxicological assessment: The potential of lung-on-a-chip as an alternative to animal testing

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ARTICLE INFO

Article history:
Received 22 October 2021
Received in revised form
29 January 2022
Accepted 1 February 2022
Available online xxx

Keywords:
Lung-on-a-chip
Alveoli
Small airway
Suspended particles
Particle toxicology
Toxicity assessment

ABSTRACT

Recent studies have shown that there exists a direct relationship between environmental pollutants (PM2.5, smog), the respiratory system, and the morbidity and mortality of cardiovascular diseases. However, the mechanism and principle of how these harmful substances are deposited in lung tissues and impair lung function remain unclear. It is important to gain improved understanding of the interaction between environmental pollutants and human lungs. Owing to the complexity of air pollution and toxicological risks, it is difficult to predict and evaluate the response of human lungs toward the damage caused by air pollution. Although animal models can be used as a basis for toxicological classification, the toxic effect on the human body could be very different from that on animals owing to the distinctive features of different species. This article provides a comprehensive review of in vitro lung-on-a-chip technologies and their application in the toxicological assessment of environmental pollutants. A lung-on-a-chip uses a bionic structure mimicking the physiological characteristic of lungs, features of a real airway, and condition of the physiological airflow. Accordingly, it can be used to reveal the intrinsic interaction between lung tissues and particulate matter and provide new insights into the effect of the toxicology of environmental particles on lungs. In addition, the development of novel and optimized lung-on-a-chip devices and their application devices in the health assessment of air pollution are expected to overcome the limitations of the current in vitro toxicological tests. They are also anticipated to provide effective and accurate methods for drug screening and toxicity testing. Finally, the application potential of in vitro lung-on-a-chip models is emphasized in this review.

1. Introduction

Common air pollutants from the environment such as SiO_2 nanoparticles, diesel exhaust particles (DEPs), and E-cigarette can cause pulmonary toxicity and chronic lung diseases such as chronic obstructive pulmonary disease (COPD), chronic bronchitis, and asthma [1–3]. Exposure to air pollutants has a profound adverse impact on human lung health [4]. Therefore, it is very important to understand (1) how pollutants are transferred from the respiratory tract to the alveolar tissues, impairing lung function and (2) how particle deposition in lung tissues affects human health.

Many environmental toxicants and their toxicological effects have been identified and tested in animals [5,6]. However, the results obtained from animal testing in the past studies were frequently biased for use on human bodies owing to the differences in the structures and functions of the respiratory tracts of different species [7]. Furthermore, it is difficult to conclude the potential harm on the human body based on animal inhalation test results, because numerous animals are required for an inhalation toxicity test. Therefore, several regulations have been incorporated in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) plan based on the objectives defined by the 3R principle: reduction (promote the use of fewer laboratory animals), refinement (refine the experimental procedures to minimize animal suffering), and replacement (use test systems that do not involve

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https://doi.org/10.1016/j.mtadv.2022.100216

animals or only involve species with a low phylogenetic rate) [8]. The general concept is to support the use of alternative non-animal testing to the maximum extent and avoid unnecessary animal testing. Therefore, many in vitro models have been developed to be used in air pollution toxicity tests to replace animal tests [9]. The corresponding test standards have been proposed to provide experimenters with guidelines to follow in the in vitro model testing process [10,11].

In recent years, the focus of research on environmental air pollution toxicity has shifted to the use of microfluidics and its integration with advanced three-dimensional (3D) tissue engineering structures, "organ on chip" methods was created in vitro to capture more of the complexity of the cellular microenvironment of humane body [12]. Among them, lung-on-a-chip (LoC) system can not only have the biomimicry of the dynamic air-liquid interface, but can even create the important human lung function of pulmonary cilia and directional mucociliary clearance, which provides new opportunities for environmental toxicology research [13]. In this review, we introduced the effects of common environmental pollutants on human lungs and provided a complete explanation of the potential of lung-on-a-chip system in assessing environmental pollutants.

2. Traditional method: animal models, advantages and disadvantages

Most toxicological testing is conducted on animal models following the regulatory testing requirements. In acute toxicology testing, the entire body or the nasal inhaler of rodents is exposed to a high concentration of the target substance for 24 h or less. The research data can also be obtained in a short time. In subacute toxicology testing, a repeated dose 28-day toxicity study is frequently conducted, and the level of toxicity is determined based on the non-observed adverse effect level. The response dose is calculated based on the experimental data considering the differences between animals and humans as well as between different individuals. Finally, the response dose is converted into the lowest reference dose of human exposure to the toxicant. Carcinogenic analysis of chronic toxicology requires a repeated doses study of more than 24 weeks [14]. It is possible to conduct histopathological analysis on tumor tissues to evaluate toxicant-induced tumorigenesis in organisms and the impact of toxicant accumulation in organs. The results obtained from chronic toxicity tests are used to evaluate the potential carcinogenicity of the tested substance, which can provide important data for analyzing the carcinogenic risk of human exposure to that substance [14] (see Fig. 1).

Owing to the differences between different species, assessing the hazards and risks of chemicals on the human body by testing on rodents has been debated (Fig. 2A) [7]. The local toxicity of inhaled substances in the respiratory tract is known to be affected by two parameters: (1) the deposition method of the test substance and (2) the specific immune response of the lung environment when removing the harmful substance (Fig. 2B) [15]. However, the deposition of inhaled substances is dependent on the dynamics of the airflow. In particular, the difference in the deposition behavior of particulate matter in animal models and humans is affected by the different features of their lungs (Fig. 2C) [16]. Some examples are the anatomical structure and organ geometry of the upper and lower respiratory tracts, airway size, breathing pattern, and ventilation volume. In addition, different species exhibit numerous distinct physiological characteristics, including the cell type and its growth location in the respiratory tract, composition and distribution of mucus in the airway, clearance function of macrophages, and physiological mechanism and metabolic process of the airway [17]. In many past studies, the toxicant detection and toxicological evaluation results obtained from animal tests were directly used to calculate the minimum human exposure dose. This approach frequently yields erroneous results. How to provide an effective and accurate in vitro model of human tissues and re-explain the interaction between toxicological substances and a real human lung environment remain important problems to be solved urgently in future toxicology testing [18].

3. Current in vitro environmental toxicity testing models

3.1. 3D cell culture insert models

Many environmental toxicity studies have already started to investigate in vitro test models (Fig. 3A) [22]. At present, a commercial in vitro organ model of the human mucociliary epithelium (EpiAirway™) is available in the market for toxicological and drug testing. In one past study, 59 chemical substances classified by the European Chemicals Agency were used in in vitro testing and evaluation. The tests were conducted using four test chemical concentrations and the MTT viability assay to obtain dose/response curves. The toxicity of each chemical on the in vitro model was further determined and compared with rat test data. As shown by the final results, the sensitivity and specificity ranges were 87.5%–100% and 56%–89%, respectively. This study suggested that toxicity research should focus on overcoming the drawbacks of animal models to enable in vitro models to provide improved prediction of acute inhalation toxicity. The study also emphasized the effectiveness and major potential of the use of in vitro models in toxicological tests [23].

The U.S. Environmental Protection Agency (EPA) evaluated a novel in vitro model assay (MucilAir™), which can be used as an alternative to animal testing. This method provides improved inhalation risk assessment of chlorothalonil, a pesticide. To obtain the toxicity equivalency concentration for human health risk assessment, the above in vitro model was tested against chlorothalonil particle deposition, with the surface concentration calculated from computation fluid dynamics simulation. The MucilAir™ assay method was proposed in 2014, which provides improved inhalation risk assessment of chlorothalonil. After understanding the relevance of using in vitro models for the toxicity testing of pesticides or other contact irritants, the U.S. EPA started to support their use instead of traditional animal tests for toxicology testing. The corresponding test standards were further provided by the EPA as guidelines for conducting in vitro model tests [26].

3.2. Organ-on-a-chip models

Organ-on-a-chip (OoC) is a multichannel 3D microfluidic system used for culturing cells. By combining cell biology, engineering, and biomaterial technology, tissue–environment interfaces and mechanical stimulations can be provided to such a chip to simulate the micro-environment of organs [27]. OoC is a promising method for simulating human pathology and physiology in an in vitro environment, which overcomes the limitations of the current cell and animal models (Fig. 3B) [28,25].

Although the animal models used in the past studies have provided considerable experimental results and insights on lung physiology and pathophysiology, they have significant limitations in reflecting the structure, disease symptoms, and responses of the human respiratory system. Therefore, it is important to develop in vitro models for analyzing the pathogenesis of lung diseases, drug efficacy, and inhalation toxicology. Lung-on-a-chip (LoC) provides a strong support to the use of in vitro human lung models for disease modeling, drug discovery, and drug testing [29].

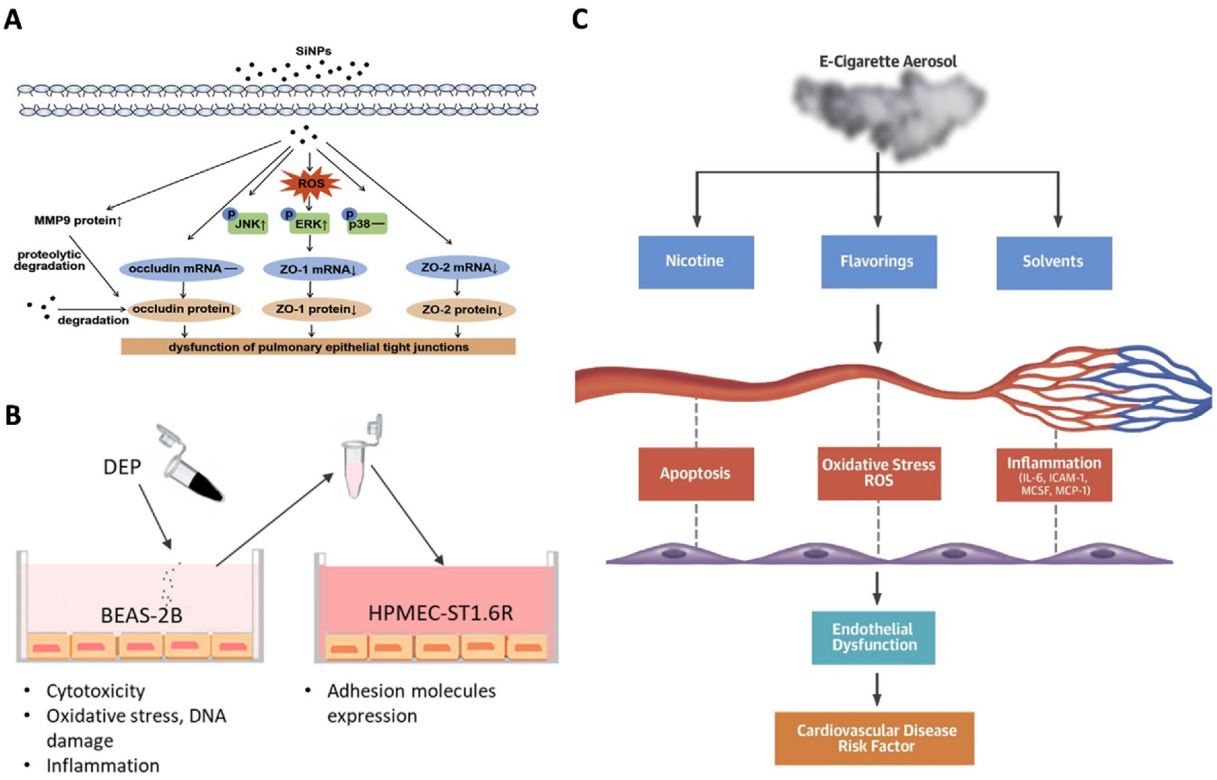


Fig. 1. Mechanism of nanoparticle-induced pulmonary dysfunction. A: Hypothetical mechanism for SiNP-induced tight junction dysfunction [19]. B: Graphical scheme of the co-culture model systems and the analysis of DEP-induced responses on epithelial and endothelial cells, and their interaction in the resulting inflammatory effects [20]. C: Overview of the mechanisms by which e-cigarette use might cause acute endothelial dysfunction [21].

4. Lung-on-a-chip (LoC)

4.1. Dynamic air-liquid interface culture

A multifunctional microfluidic device was designed in a reported study to simulate the human alveolar–microvascular interface [30]. This microfluidic system consisted of two polydimethylsiloxane (PDMS) films separated by a thin polyester film containing 400-nm-holes. Human alveolar epithelial cells and human lung microvascular endothelial cells were cultured on the opposite sides of the extracellular matrix (ECM) coating film in the microfluidic device, thereby creating two separate compartments. The cells were arranged in each compartment. After the arranged cells formed a double-layer tissue, the epithelial compartment was exposed to air to form a unique type of air–liquid interface (ALI) that mimicked the interface in lungs [24]. This interface allows exchange of gas with air in an external environment, which typically occurs at the air–liquid barrier in the alveolar structure. By manipulating the fluid flow in the microfluidic device, nutrients can be delivered independently to the epithelium and the endothelium [31,32].

As shown in the past studies, dynamic culture can provide cells with sufficient nutrients and satisfy the nutrient load at a high cell density in a metabolic environment. These features suggest that a flowing system presents excellent culture performance, which allows epithelial cells to grow rapidly. In addition, the shear stress generated by the flowing fluid allows the cells to adjust their morphology, proliferate, and differentiate [33]. It has been shown in studies that in a shear stress environment, endothelial cells demonstrate low pro-inflammatory response and high expression of carbon monoxide compounds. A low-shear environment can also be used to simulate the microenvironment of vascular atherosclerosis [34,35]. A flow system presents excellent blood delivery performance for the entire body; therefore, thrombosis induced by inflammation can be analyzed quantitatively [36].

It is generally agreed in the studies on in vitro models of lungs that culturing cells at the ALI is more representative of the real physiological environment in lungs and more similar to the complex in vivo microenvironment [37,38]. The cells in lung tissues cultured in vitro under ALI and immersion conditions were found to have very different features. In particular, A549 epithelial cells cultured under the ALI condition presented a greater expression of surface-active proteins and a higher surface tension than those under the immersion condition. However, surface-active proteins are required in the physiological alveolar environment to resist the surface tension and support the establishment of the lung immune environment [39]. In addition, an ALI is a necessary differentiation condition required in the in vitro culturing of small human airways such that the cells can differentiate into functional airway cells (Fig. 4A) [40].

4.2. Tissue barrier function

Human lungs contain numerous epithelial tissues, which are in constant contact with the external environment (Fig. 4B) [41]. The barrier function of lung tissues serves as the first line of defense against harmful environmental factors and infectious substances in our daily life [44]. This barrier function is provided by the junctional complex formed between the adherens junction and tight junction proteins in closely arranged cells [45]. Past studies have shown that inhaled toxins such as cigarette smoke and air pollution particles can damage the connections between tissue cells, thereby leading to excessive cell proliferation and abnormal epithelial differentiation. The destruction of the barrier function may cause irreversible

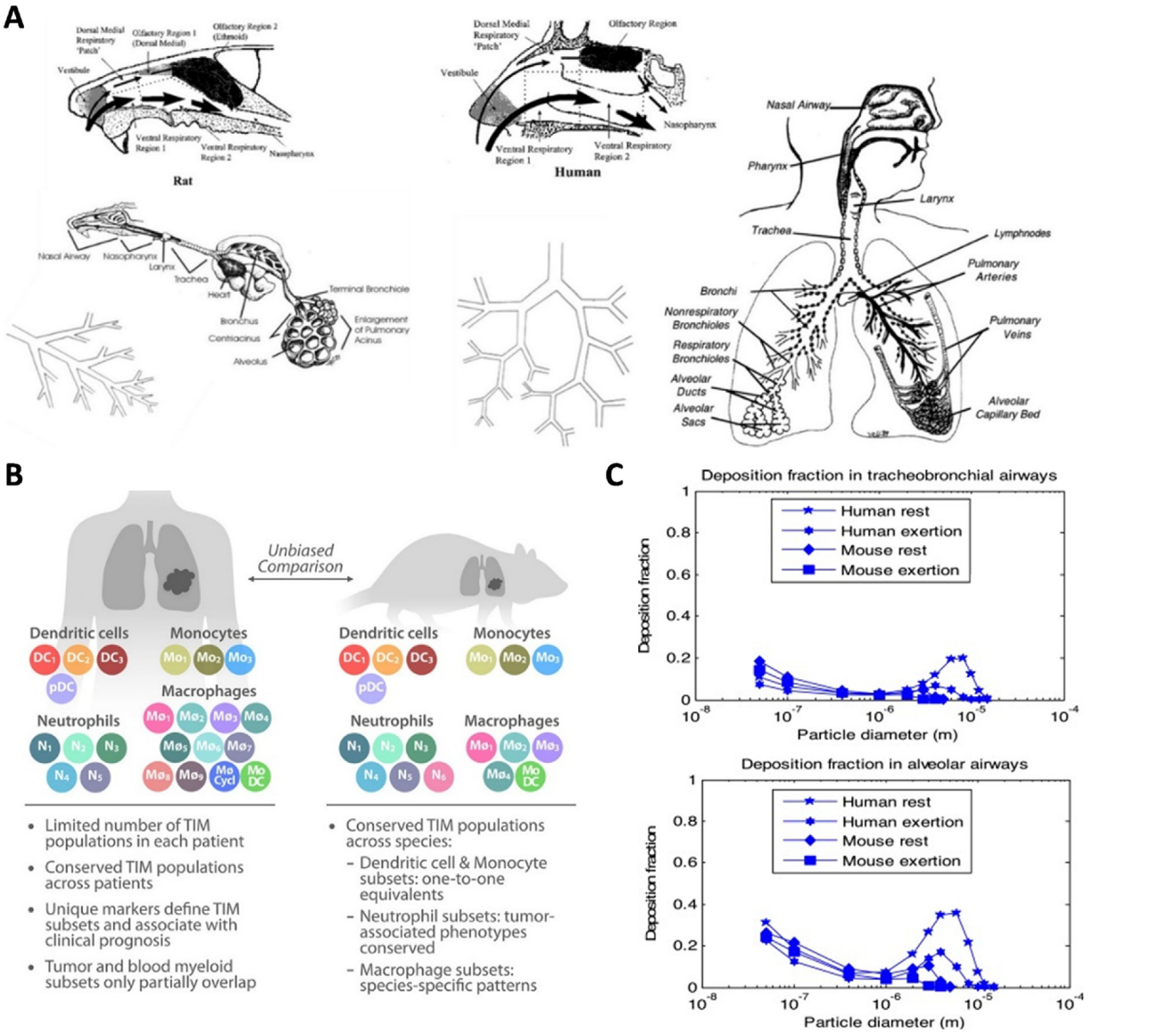


Fig. 2. Differences in important characteristics between humans and animals. A: Interspecies differences in the structure of the upper respiratory tract in humans and animals and the resulting differences in airflow mechanics [7]. B: Single-cell transcriptomic differences in conserved myeloid populations across individuals and species [16]. C: Comparison of predicted deposition between tracheobronchial and alveolar trachea in mice and humans [15].

lung damage accompanied by lung diseases such as COPD and acute lung injury.

The establishment of the barrier function has been one of the main research topics on OoC systems. As shown by in vitro dynamic culture experiments on human bronchi, cells cultured over a long time under ALI and dynamic environments demonstrated a higher expression of the tight junctional proteins than those cultured under a static environment [46]. The shear stress generated by the fluid could provide a stronger barrier function to the epithelial and endothelial tissues. This phenomenon is related to the increase in the intracellular calcium ions when the membrane ion channel, TRPV4, is subject to the shear stress. These features further confirm that the permeability of lung tissues can be regulated by the shear stress in a dynamic culture environment [47]. In summary, a dynamic culture environment and the regulation of lung tissue function are of major importance. These two factors provide new insights into the establishment of a stable in vitro model of lung tissues.

4.3. Cilia and mucus

The lung trachea includes an airway, small airways, and alveolar tissues. The airway is composed of four main types of cells: basal, ciliated, goblet, and club cells. These cells form a physical barrier that ensures the continuity, secretion, and regulation of the epithelium. In addition, this physical barrier protects the airway and the lung from inhaled pathogens and environmental pollutants (Fig. 4C) [42]. Among all different types of cells, ciliated and goblet cells in the airways are the most important because they form the first line of defense in lungs. Goblet cells can produce mucin, forming a gel layer on the surfaces of the airways. Subsequently multiciliated cells drive the gel layer to move by their power pulses, which results in directional mucociliary clearance. Acetylated α-tubulin (α-Ac-Tub) has been used as the main marker of ciliated cells, which can remove particles and pathogens in the mucus from the airways through the movement of the cilia on a coordinated plane. Accordingly, the inhaled pollutants that are captured by the

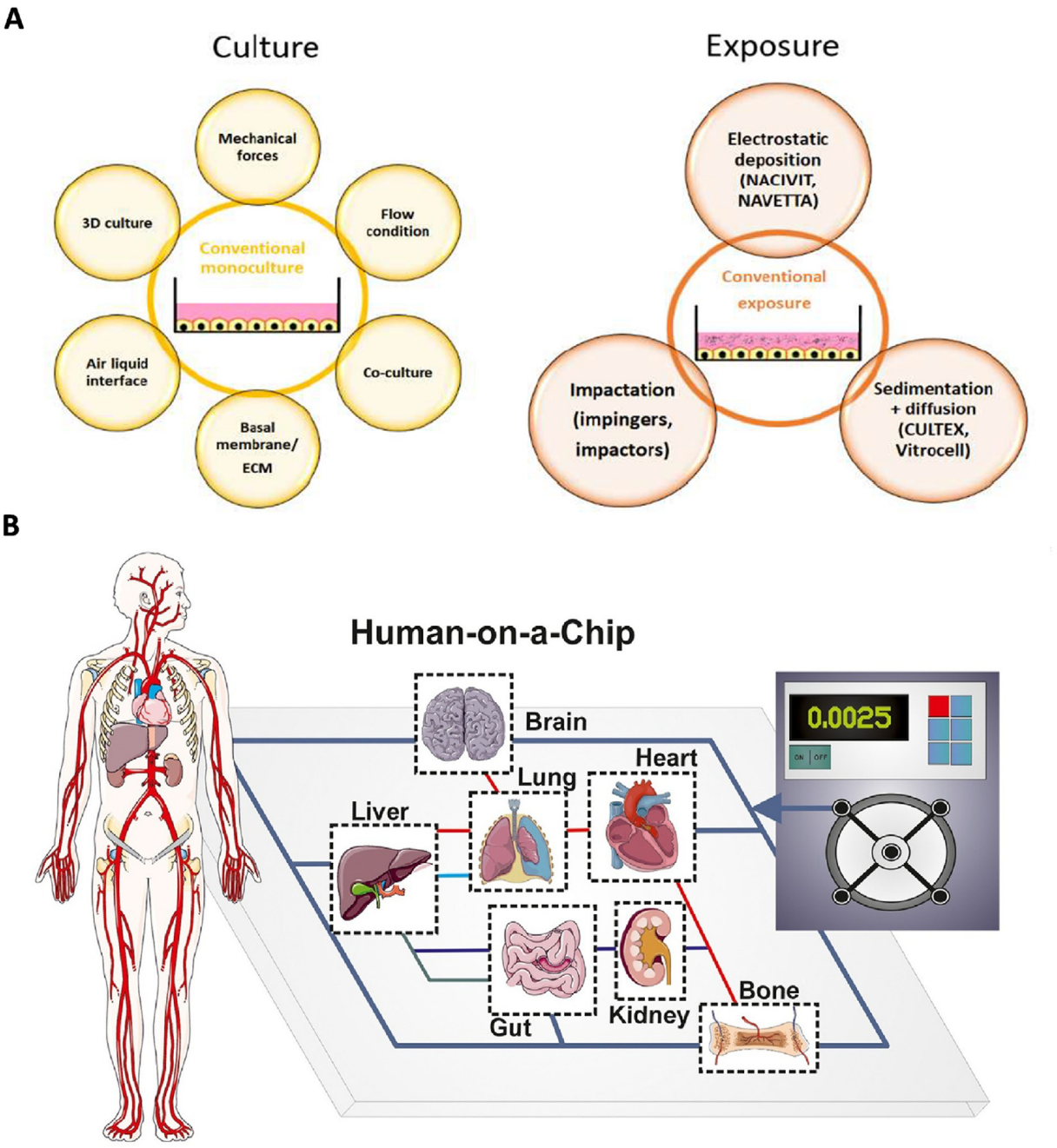


Fig. 3. Current in vitro environmental toxicity models. A: Advancement of in cell culture and exposure technologies that result in physiologically more relevant in vitro models [24]. B: Human-on-a-chip philosophy is by integrating different organ-on-a-chip devices in a single plate [25].

mucus layer on the surfaces of the airways can be discharged from the lungs [48,49].

As shown in the past studies, exposure to environmental pollutants can lead to structural and functional abnormalities of the cilia in the airways along with abnormal mucus clearance and excessive mucus accumulation. These abnormalities cause various lung diseases [50]. Furthermore, in vitro toxicology studies showed that exposure to DEPs reduces the beat frequency of cilia cultured in both healthy and diseased test groups. A physiological microtissue of the lung epithelium was used as the basis of the toxicity analysis [51].

4.4. Polar proteins and directional mucociliary clearance

The cilia of the bronchial epithelial cells in the human respiratory tract pulsate toward the direction of the throat. Thus, air pollutants can be removed from lungs once they are captured by the mucus layer on the surface of the airways (Fig. 4D) [43]. Therefore, the directional pulsation of cilia is of major importance for the health of the respiratory system [52]. The cilia in the airway tissues exhibit a collective and directional beating behavior. The exchange of information between different cells is achieved mainly by the planar cell polarity (PCP). There are two primary core PCP proteins—frizzled 6 (FZD6) and Vang-like protein 1 (Vangl1)—which are at the front and back of the cell polarity, respectively. These

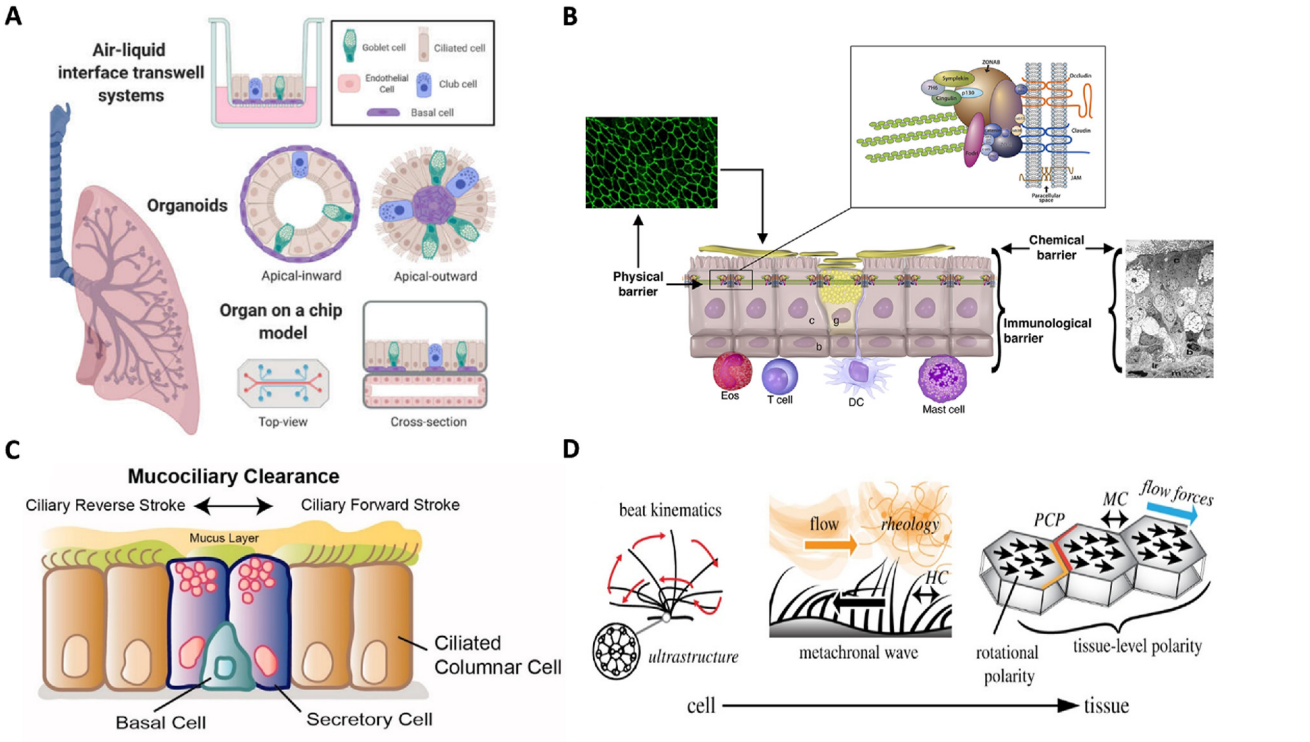


Fig. 4. Characteristic of Lung-on-a-chip. A: Organ-on-a-chip systems of the human respiratory tract with dynamic air-liquid interface properties [40]. B: Schematic diagram of the human lung barrier function [41]. C: An illustration of human mucociliary clearance and ciliary beating movement [42]. D: Relationships between important structures (ranging from cell to tissue) and functions of airway cells [43].

proteins are located in the interstitial region between the cells, which is related to the barrier function. During the growth of ciliated cells, the cilia undergo cytoskeletal remodeling and are directed to the front position [43,53].

If the Vangl1 polar proteins become missing owing to mutation, then the communication channel between the cells will be blocked. In this case, the cilia will be unable to pulsate directionally, which will result in accumulation of mucus in the airways and potential risk of lung disease [54]. As shown by in vitro cell tests conducted on an airway epithelium with cystic fibrosis and a nasal epithelium with sinusitis, the absence of PCP signaling caused by the lack of Vangl1 expression in the cell tissues affects cilia polarization and generates evident mucociliary defects [55]. However, PCP has received significant attention in the field of tissue engineering. It was found that the orientation of tissue cilia and the flow direction of mucus clearance remained unchanged nine months after inverting the airway in a mouse. Past studies have also used a mixture of cavernous body and collagen to grow human epithelial tracheal tissues differentiated from human-induced pluripotent stem cells (iPSC). After transplanting these tissues into the trachea of mice, the original tissue was cut off at two, six, and nine months to examine the particle clearance rate and the pulsating direction. However, no significant difference was found between the post-operative and preoperative behavior. In addition, the PCP protein was found to remain unchanged using FZD6 and Vangl1 as its markers [56,57].

Based on the past literature, the formation of PCP protein during epithelial differentiation is the key to the distinctive behavior of cilia after differentiation. Studies have shown that the growth and function of respiratory organs rely on an efficient and directional fluid flow such that the cilia can swing in a directional manner [58]. To allow a differentiated airway to be used in tissue engineering, it is critical to understand the correlation between PCP and the orientation of the cilia beating. In recent years, the development of iPSC airway-on-a-chip has allowed control of the PCP direction of multiciliated cells using the shear stress of the fluid [13]. This technology can achieve a unidirectional mucociliary flow and induce the differentiation of multiciliated cells, thereby providing new breakthroughs and insights in airway reconstruction. Furthermore, mechanical stress can be applied to adjust the PCP and establish a directional cilia swing, enabling an LoC to remove foreign bodies [59,60]. By introducing this capability, a more comprehensive bionic model of airway mucus cilia with a clearance function can be developed for air pollution toxicology detection and screening.

5. Application of lung-on-a-chip for air pollution toxicity assessment

With the rapid industrial development and urbanization in recent years, human hazards caused by air pollution from factories and vehicles have become a major threat to the environment and health safety. According to the statistics collected by the World Health Organization, approximately 7 million premature deaths are due to air pollution every year [61]. Therefore, there is an urgent need to evaluate the negative impact of air pollution on human health. To evaluate and detect pollutant particles in air, several in vitro LoC models have been developed and used in past studies. These models allow assessing the impact of air pollution on bionic lung tissues, which can provide new insights into the potential harm of air pollution on human health.

With the rapid development of microfluidic processing and production technology and the availability of lung cells, new microfluidic systems that can evaluate the biological toxicity of environmental substances more accurately and reliably by combining substance concentration gradient generators and

bronchial epithelial culture have been proposed. A concentration gradient of benzopyrene (BaP), a polycyclic aromatic hydrocarbon, was generated stably in experiments. A high concentration of BaP was found to stimulate pronounced apoptosis. The type and structure of the cells during the stimulation process were observed via optical and fluorescent imaging. As shown by the results, a high concentration of pollutants leads to a small cell size [62]. The image analysis provided an in-depth understanding of the actin decomposition process, contributing to the application and development of microfluidic systems for dynamic monitoring of environmental pollutants.

Microfluidic technology can strongly control substance concentration and generate bionic shear stress when integrated into a comprehensive micro system. Accordingly, it can provide a dynamic and mechanical microenvironment to lung tissues and reproduce complex lung physiology and pathological reactions. A stretchable micro diaphragm can be produced using PDMS. Subsequently, lung cells can form a tight lung barrier tissue on this porous membrane. Such a membrane will experience cyclic stretching generated by the micro system, simulating the respiratory mechanical stress in a real lung. As shown by experimental results, the stretching behavior can affect the permeability of lung tissues significantly [31]. No significant up-regulation of the ROS occurred when SiO_2 nanoparticles were added to upper lung tissues without cyclic stretching. However, when the cells were subjected to 10% tensile deformation and mechanical strain at a pseudo-respiration frequency of 0.2 Hz, the SiO_2 nanoparticles were found to increase the ROS by four times within 2 h (Fig. 5A) [32]. By applying a bionic mechanical strain to in vitro lung tissues and analyzing the effect of the mechanical stress on the physiological and pathological lung function, we can develop new understanding of the specific function of nanoparticle toxicology in lung tissues.

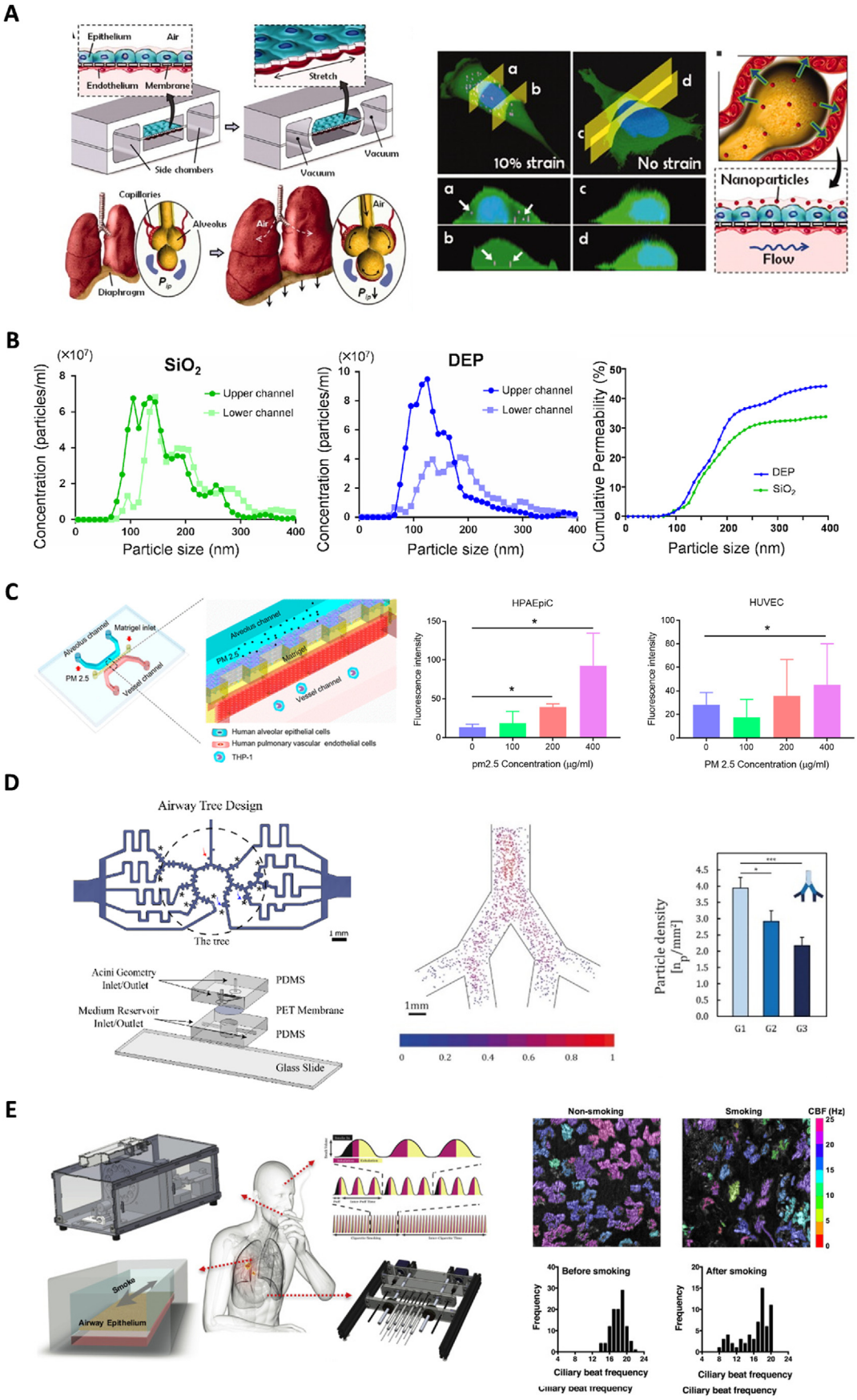
Furthermore, an in vitro biomimetic alveolar system was established with three-dimensional multiple tissues, air-liquid interface, and dynamic circulation functions to analyze the harmful effects of SiO_2 and diesel exhaust particles (DEP). It can not only explore the damage of harmful substances from fine PM to physiological tissues, but can also in-depth analysis of specific harmful substances that can penetrate through alveolar tissue and cause health effects. This system led to efficiently identify the main particle size (size between 0 and 200 nm) and composition that are harmful to the alveoli, which are very close to the phenomenon in human alveoli (Fig. 5B) [63]. Another example is that 3D lung-on-a-chip model has been shown to examine the adverse effects of PM2.5 in the respiratory system. This model, lined by human alveolar epithelial cell lines and human umbilical vein endothelial cells, reproduced the organotypic structure and function of the alveolar−capillary barrier. Using this chip, it was discovered that the PM2.5 permeability alteration under various exposure levels in a dynamic way. In addition, immunocytes could be introduced to form a triple co-culture system to investigate the interaction of injured tissue and immunocytes. Thus, it was found that a low concentration of PM2.5 causes limited cytotoxicity to the epithelial and endothelial cells, but a higher concentration of PM2.5 (>200 μg/mL) could significantly increase the ROS generation, apoptosis, inflammation responses of epithelial cells and endothelial cells and attachments of monocytes to the vessels (Fig. 5C) [64]. Taken together, these methods showed great potential in analyzing the extent of hazardous substances towards respiratory health to overcome the technical bottleneck of traditional in vitro assays and standard animal models.

Moreover, exposure to air pollutants is related to the pathogenesis of pulmonary fibrosis, a human lung micro-tissue array device has been developed to evaluate the biological toxicity of multi-walled carbon nanotubes (MWCNTs) and the corresponding potential of pulmonary fibrosis. In this micro-tissue array device, the heads of a pair of micro-pillars were arranged in a dog-bone pattern, and epithelial micro-tissues were formed between the micro-pillars. The contractile strength of a fibrotic tissue was measured in situ between the micro-pillars. Stimulating a lung epithelial tissue with a high concentration of MWCNTs led to a high level of cytotoxicity. However, stimulating the tissue with a low concentration of MWCNTs produced mechanical forces found in the natural fibrosis process. Apart from demonstrating the potential of MWCNTs to induce pulmonary fibrosis, this study showed the capability of the lung micro-tissue array system for the detection and toxicant screening of air pollutants [67].

The importance of an ALI exposure environment has already been demonstrated in past inhalation toxicology tests conducted on ALI cultures. An in vitro model was stimulated by real exposure technology (gas, vapor, aerosol), and the characteristics of the exposed particles were examined carefully, including the particle size distribution and the actual deposition concentration [17]. However, the development of LoC technology in recent years has led to highly realistic inhalation detection that mimics the physiology and mechanism of aerosol deposition on lung tissues from air. These studies aim to reproduce the results of in vivo deposition (local concentration, area) and achieve highly realistic in situ detection, which have now become important breakthroughs in the research field compared to traditional in vivo and in vitro exposure tests.

A past study had also proposed an in vitro exposure platform of the human lung bronchial airway epithelium to simulate the deposition of exposed particles in the airway tree structure. The experiments revealed a characteristic local deposition of 2-μm particles, which was related to the geometric structure of the airway tree and the gravity-induced sedimentation of the particles when transported by air. Compared with the extensive in vitro models in the past, an LoC that uses the airflow to transport particles can better reflect the gravity-induced sedimentation behavior of particles under different flow rates. Consequently, it can reconstruct the characteristic local toxicological reactions in the geometric structure of a lung (Fig. 5D) [65]. In addition, as shown by the computer simulation of fine particle deposition on an in vitro LoC, the deposition of fine particles is affected by numerous different factors including surface adsorption and desorption kinetics, respiration mode, Brownian motion of the fine particles, and gravitational sedimentation of the large particles. It was found that the particle deposition rate during human movement is thrice faster than that when at rest. This drastic increase was attributed to the increase in the airflow and respiratory frequency during exercise. Therefore, different respiratory exercises will also cause different levels of particle toxicity [68].

In the inhalation dose toxicology tests of pollutants, subacute exposure can result in highly clinically significant toxicity. A subacute or long-term drug exposure can simulate the chronic inhalation toxicology of air pollution to lungs more realistically. In a new cell model system, hAELVi and THP-1 were used to simulate alveolar macrophages. Relevant cell organizations in the alveolar area were reconstructed, and an in-situ exposure was provided to the in vitro alveolar model. The lipopolysaccharide was aerosolized to stimulate the cells and induce IL-8 secretion, and the toxicology was monitored during the exposure period. This cell model system provides a highly realistic and in vitro in situ exposure detection platform for determining acute (48−72 h) and subacute (14−28 days) toxicity in the alveolar area. This LoC can provide prolonged exposure and cell culture. The high stability of this new model and the dose exposure tests at different periods provided a new in vitro model concept for inhaled dose toxicology testing methods [69,70].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HPAEpiC
Fluorescence intensity
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HUVEC
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Airway Tree Design
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Acini Geometry
Inlet/Outlet
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Inlet/Outlet
PDMS
PET Membrane
PDMS
Glass Slide
1mm
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G1
G2
G3
E
Smoke
Airway Epithelium
Non-smoking
Smoking
CBF (Hz)
Before smoking
After smoking
Frequency
Ciliary beat frequency
Ciliary beat frequency

In addition to environmental pollutants, smoking can impair the physiological function of lungs and is the primary cause of COPD in humans. However, the distinct characteristics of different patients make it difficult to obtain bionic authenticity of the injury response caused by smoking under physiological respiratory conditions. A research team led by Harvard University developed an integrated microfluidic chip that simulates the small airways of a human lung using micro respirators and smoking machines. This device can simulate the exposure of lung cells in the human body to smoke and allows obtaining the breathing and smoking behavior parameters [71]. As shown by this LoC model, Nrf2, which is induced and activated by the oxidative stress after smoke exposure, can regulate the expression of ARE gene products (the second type of metabolic detoxification enzyme, antioxidant protein, drug efflux protein gene). The above microfluidic chip can further characterize the abnormal ciliary swing and IL-8 as an indicator of the induced COPD exacerbation, thereby demonstrating the toxic effects of smoking on lungs (Fig. 5E) [66]. Furthermore, the use of e-cigarettes has kept increasing in recent years with the objective to reduce the side effects of traditional cigarettes. However, there is no substantive proof that the inhalation during the use of e-cigarettes is safe for human health. Therefore, the lung toxicology of e-cigarette exposure has been investigated in many studies in recent years. These studies have also examined the effect of e-cigarette exposure using microfluidic small airway-on-a-chip. Although no significant difference in genomic expression was observed, the frequency distribution of the cilia beating was changed after e-cigarette exposure. The same finding was obtained from other in vitro models, with which no significant difference was observed. In general, the environmental exposure at the ALI can better represent the complete behavior of the human lung barrier and provide a more comprehensive analysis and quantification of functional proteins than an immersion stimulation in an e-liquid extract.

6. Opportunities and challenges

Many past studies have developed functional LoC models to analyze the toxicity of air pollution in the environment as well as the pathophysiology of lung tissues at the molecular scale. The novel LoC technology has also provided important data on the function of a damaged human lung exposed to air pollution and toxic particles, which allows assessment of in vivo toxicology and human health [72]. However, to develop an LoC for studying an air-contaminated environment, one must emphasize the cultivation conditions in the bionic environment, which include the 3D tissue microenvironment, tissues constructed by the ECM, and biomechanical environment of the tissues [73–76]. It is highly conducive for cells to differentiate into specific functions related to the biological environment when human lung tissues are grown in a bionic environment. In addition, the toxicity of inhaled air pollutants in lungs is caused by a combined effect of the inhaled particles, exhaust gas, and biomechanical environment. It is important to keep a lung epithelial tissue in direct contact with air in an LoC model to study the correlation between the mucociliary clearance and the deposition of airborne particles in the dynamic airflow [77].

Developing models for fibroblasts and macrophages is critical for the analysis of inflammation in lungs caused by air pollution. Immune recruitment and interstitial thickening caused by the inflammation of epithelial tissues can help rebuild the immune blood circulation for removing particulates and provide metabolic functions to an LoC. The features of a model can provide insights into the highly specific interactions between nanoparticles and cells as well as the related molecular mechanisms of inflammation. Such findings facilitate the development of in vitro models and help understand the toxicity of air pollutants in lung tissues. In addition, the intrinsic properties of air pollutants are of high importance in the field of environmental toxicology. On-site exposure can provide an in-depth understanding of the interaction between air pollutants and the surface characteristics of the lung epithelium and show the damage and surface composition of particulates of specific sizes in the lung environment. Moreover, it offers a novel method for screening the toxicity of environmentally hazardous substances and new materials.

An LoC is a highly repeatable, reliable, and compatible microsystem. It has a high application potential in preclinical trials and can reduce the inspection process effectively. In addition, an LoC will significant implications in the field of air pollution monitoring and toxicity assessment owing to its capability of detecting pollution sources and the toxicity of new materials in the early stage. Consequently, relevant regulations and standards can be formulated to restrict the pollutant exposure in human living environments. An LoC can be further used to help provide personalized treatments for different types of diseases including COPD, pulmonary fibrosis, and asthma. The high cost of drugs and the difficulty in rapid detection of substance toxicity are the two main drawbacks of the existing models (animal models, 2D cell models). In contrast, an in vitro LoC model can facilitate the pre-clinical development of different available drugs and new drugs. It can also provide personalized organ models to realize precision medicine. Finally, the application of an LoC in air pollution research aims to provide a standardized inhalation toxicity test that is reproducible and repeatable as well as provides highly relevant data on the functional structure of the lung microenvironment. However, the data should be collected based on a large range of cell exposure conditions and cell models. Using the specific behavior obtained from the model as the toxicity endpoint, an LoC is expected to be used as a substitute for animal-based inhalation toxicity tests to provide more dependent explanations on the interaction between cells and air pollution.

7. Prospect

Air pollution is a very urgent global health problem. However, the current in vitro cell assays and animal models still have a bottleneck to explore the effect of fine particulate matter (PM) on human health. In addition, most cell experiments are limited to the analysis of the cytotoxicity of air pollution particles or components on cells. Based on recent advances in human lung-on-a-chip technologies, this review provides new forms of human experiments in vitro for evaluating harmful effects of air pollutants to more physiologically and clinically relevant preclinical models than traditional in vitro assays and standard animal models. Moreover, their greatest novelty lies in their ability to reconstruct dynamic vascular perfusion, tissue-tissue interface (air–liquid interface), and lung-specific mechanical cues (cilia and mucus, polar proteins and directional mucociliary clearance), which are the important in-

Fig. 5. Application of lung-on-a-chip in air pollution research. A: Reproducing physiology of breathing in human body on lung-on-a-chip [32]. B: Size distribution and cumulative permeability of SiO_2 and DEP particles in chip devices. Reprinted with permission from the study by Lin et al. [63] Copyright 2021, the Elsevier. C: ROS generation after PM2.5 exposure of epithelial and endothelial cells on the chip. Reprinted with permission from the study by Xu et al. [64] Copyright 2020, ACS. D: Fluorescence image of particle deposition patterns and quantification of particle deposition density in the airway-on-chip platform following exposure. Reprinted with permission from the study by Shani et al. [65] Copyright 2020, the Frontiers. E: Schematic describing the overall method for analyzing effects of inhaled whole cigarette smoke and physiological recapitulation of cigarette smoke-induced ciliary dysfunction on-chip. Reprinted with permission from the study by Benam et al. [66] Copyright 2016, the Cell systems.

vivo physiological characteristics for the study of respiratory health assessment. Therefore, the lung-on-a-chip technologies allow to construct the artificial models with well-defined physiological characteristics under biomimetic microenvironments. It can better elucidate the relationship between the effects of air pollutants on important cellular and molecular factors for human physiology and pathophysiology compared to traditional experimental methods. Overall, the ability of lung-on-a-chip technologies can recapitulate human lung physiology, it makes possible to explore the interactions of air pollutants with lung organs to assess the acute and chronic adverse health effects of hazardous substances in the future. These innovative biomimetic chip devices will pave the way for reliable and more efficient testing of harmful substances without the need for animal experiments.

Author statement

Ko-chih Lin: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft. Chun-Zai Yen: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft. Jia-Wei Yang: Writing – review & editing. Johnson H.Y Chung: Writing – review & editing. Guan-Yu Chen: Project administration, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

G.Y.C. would like to acknowledge financial support from the Ministry of Science and Technology (MOST-110-2636-E-009-007-), Council of Agriculture, Executive Yuan (110AS-24.2.1-AD-U2) and the Higher Education Sprout Project of the National Yang Ming Chiao Tung University and the Ministry of Education, Taiwan.

References

[1] K.-H. Kim, et al., Environ. Int. 74 (2015) 136.
[2] P. Petit, et al., Environ. Int. 124 (2019) 109.
[3] Y. Zhang, et al., Front. Physiol. (423) (2021) 12.
[4] Y.-F. Xing, et al., J. Thorac. Dis. 8 (1) (2016) E69.
[5] J.A. Swenberg, et al., Toxicol. Pathol. 41 (2) (2013) 181.
[6] T. Hofmann, et al., Applied In Vitro Toxicology 4 (2) (2018) 220.
[7] A.J. Clippinger, et al., Toxicol. Vitro 48 (2018) 53.
[8] A. Gissi, et al., ALTEX 34 (3) (2017) 353.
[9] J. Zavala, et al., Int. J. Environ. Res. Publ. Health 17 (6) (2020) 2124.
[10] D. Krewski, et al., Arch. Toxicol. 94 (1) (2020) 1.
[11] S. Halappanavar, et al., Small 17 (15) (2021) 2007628.
[12] Q. Wu, et al., Biomed. Eng. Online 19 (1) (2020) 9.
[13] N. Sone, et al., Sci. Transl. Med. 13 (601) (2021), eabb1298.
[14] R.K. Wolff, M.A. Dorato, 3.16 - inhalation toxicology studies*, in: C.A. McQueen (Ed.), Comprehensive Toxicology, second ed., Elsevier, Oxford, 2010, p. 225.
[15] R. Zilionis, et al., Immunity 50 (5) (2019) 1317.
[16] A.V. Kolanjiyil, et al., Respir. Physiol. Neurobiol. 260 (2019) 82.
[17] D. Movia, et al., Front. Bioeng. Biotechnol. 8 (2020) 549.
[18] R. Mohammadpour, et al., Adv. Drug Deliv. Rev. 144 (2019) 112.
[19] Y. Liu, et al., Chemosphere 255 (2020) 126954.
[20] R. Bengalli, et al., Toxicol. Lett. 306 (2019) 13.
[21] W.H. Lee, et al., J. Am. Coll. Cardiol. 73 (21) (2019) 2722.
[22] E. Fröhlich, Replacement strategies for animal studies in inhalation testing, 2021. Preprints.org.
[23] G.R. Jackson Jr., et al., Appl In Vitro Toxicol 4 (2) (2018) 149.
[24] T.J. Bennet, et al., Cells 10 (7) (2021) 1602.
[25] N. Picollet-D'hahan, et al., Trends Biotechnol. 39 (8) (2021) 788.
[26] Agency, U. S. E. P., Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology, NAM), 2018.
[27] Q. Wu, et al., Biomed. Eng. Online 19 (1) (2020) 9.
[28] K. Ronaldson-Bouchard, G. Vunjak-Novakovic, Cell Stem Cell 22 (3) (2018) 310.
[29] J.C. Nawroth, et al., Adv. Drug Deliv. Rev. 140 (2019) 12.
[30] L. Tang, N.Y. Lee, Lab Chip 10 (10) (2010) 1274.
[31] A.O. Stucki, et al., Lab Chip 15 (5) (2015) 1302.
[32] D. Huh, et al., Science 328 (5986) (2010) 1662.
[33] M. Nikolic, et al., Front. Bioeng. Biotechnol. (120) (2018) 6.
[34] Y. Yang, et al., Lab Chip 19 (19) (2019) 3212.
[35] R. Kaunas, APL Bioengineering 4 (1) (2020), 010905.
[36] A. Jain, et al., Clin. Pharmacol. Ther. 103 (2) (2018) 332.
[37] S. Diabaté, et al., Nanomaterials (1) (2021) 11.
[38] S. Upadhyay, L. Palmberg, Toxicol. Sci. 164 (1) (2018) 21.
[39] K. Ohlinger, et al., Toxicol. Vitro 60 (2019) 369.
[40] E. Iverson, et al., Viruses 12 (12) (2020).
[41] E.J. Swindle, et al., J. Allergy Clin. Immunol. 124 (1) (2009) 23.
[42] A.R. Iskandar, et al., Toxicol. Mech. Methods 26 (6) (2016) 392.
[43] J.C. Nawroth, et al., Phil. Trans. Biol. Sci. 375 (1792) (2020) 20190160.
[44] T.K. Sivabakya, G. Srinivas, SN Comprehensive Clinical Medicine 2 (9) (2020) 1299.
[45] Y. Gon, S. Hashimoto, Allergol. Int. 67 (1) (2018) 12.
[46] P. Chandorkar, et al., Sci. Rep. 7 (1) (2017) 11644.
[47] V.K. Sidhaye, et al., Proc. Natl. Acad. Sci. Unit. States Am. 105 (9) (2008) 3345.
[48] M.Z. Nikolic, et al., Development 145 (16) (2018).
[49] J.A. Whitsett, Ann Am Thorac Soc 15 (Suppl 3) (2018) S143.
[50] P. Misiukiewicz-Stepien, M. Paplinska-Goryca, Clin. Immunol. 227 (2021) 108754.
[51] H. Bayram, et al., J. Allergy Clin. Immunol. 102 (5) (1998) 771.
[52] T. Namba, S. Ishihara, PLoS Comput. Biol. 16 (2) (2020), e1007649.
[53] H. Koyama, et al., Biophys Physicobiol 16 (2019) 89.
[54] S. Fuertes-Alvarez, et al., Cell Death Dis. 9 (12) (2018) 1183.
[55] E.K. Vladar, et al., JCI Insight 1 (13) (2016).
[56] T. Tsuji, et al., Respir. Res. 19 (1) (2018) 22.
[57] R. Nakamura, et al., J Tissue Eng Regen Med (2021).
[58] B. Mitchell, et al., Nature 447 (7140) (2007) 97.
[59] J.C. Nawroth, et al., bioRxiv (2021), 2021.05.07.443164.
[60] D. Trieu, et al., Biomicrofluidics 8 (6) (2014), 064104.
[61] P.J. Landrigan, Lancet Public Health 2 (1) (2017) e4.
[62] F. Zhang, et al., ACS Sens. 3 (12) (2018) 2716.
[63] K.C. Lin, et al., Appl Mater Today (2022) 26.
[64] C. Xu, et al., ACS Biomater. Sci. Eng. 6 (5) (2020) 3081.
[65] S. Elias-Kirma, et al., Front. Bioeng. Biotechnol. (91) (2020) 8.
[66] K.H. Benam, et al., Cell Syst 3 (5) (2016) 456.
[67] Z. Chen, et al., Lung microtissue array to screen the fibrogenic potential of carbon nanotubes, Sci. Rep. 6 (2016) 31304.
[68] S.M.A. Arefi, et al., Biomicrofluidics 14 (4) (2020), 044117.
[69] A. Artzy-Schnirman, et al., Eur. J. Pharm. Biopharm. 144 (2019) 11.
[70] A. Artzy-Schnirman, et al., Advanced Biosystems 3 (9) (2019) 1900026.
[71] K.H. Benam, et al., Nat. Protoc. 15 (2) (2020) 183.
[72] J.-W. Yang, et al., Front. Bioeng. Biotechnol. (519) (2020) 8.
[73] J.W. Yang, et al., Front. Cell Dev. Biol. (2019) 7.
[74] P. Zamprogno, et al., Communications Biology 4 (1) (2021) 168.
[75] D. Huang, et al., Proc. Natl. Acad. Sci. Unit. States Am. 118 (19) (2021), e2016146118.
[76] M.J. Mondrinos, et al., Lab Chip 17 (18) (2017) 3146.
[77] E. Frijns, et al., Environ. Sci. Technol. 51 (9) (2017) 5259.