Alveolus Lung-on-a-Chip Platform

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Lung-on-a-chip platforms have emerged as a potential candidate to replace animal experiments because they can successfully simulate human physiology.

lung disease microfluidic organ-on-a-chip

1. Introduction

To understand the clinical applications of the lung-on-a-chip platform, the main respiratory diseases and their pathophysiology, the available approaches to lung cell culture, modelling of the lung microenvironment and microfluidic systems should be overviewed.

Respiratory diseases are top-ranked causes of deaths and disabilities, with a high burden on health service costs across the globe. In the European Union, the total cost of respiratory disease accounts to more than €380 billion annually, including the costs of primary health care, hospitalizations, and lost production due to disability [1]. In 2017, the Forum of International Respiratory Societies identified the most common global causes of severe illness and death, the Big Five: chronic obstructive pulmonary disease (COPD), asthma, acute lower respiratory tract infections, tuberculosis and lung cancer [2].

Among all types of cancer, lung cancer remains a major concern worldwide, with 2.2 million cases registered, being the most fatal one, with 1.8 million deaths in 2020 [3]. Although the pathophysiology of lung cancer is not yet fully understood, it is hypothesized that dysplasia of lung epithelium is triggered by repeated exposure to carcinogens, predominantly cigarette smoke, but also environmental pollutants (e.g., asbestos, arsenic, and chromium) associated with genetic susceptibility [4]. Lung cancer is divided into two major categories: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). The latter is more prevalent and represents 85% of all cases of lung cancer. Depending on the type of cells involved, NSCLC is further divided into the major categories of adenocarcinoma, squamous- and large-cell carcinoma [4]. Effective treatment approaches are available depending on the type of lung cancer and how far it has spread. However, even after the treatment, individuals may present long-term impairments in several outcomes including lung function, exercise capacity, quality of life, and depressive symptoms [5].

2. Lung Cell Culture

The lung cell sources can be divided into primary cells, immortalized cell lines and stem cells. There are advantages and disadvantages to all cell sources. Primary cells are extracted directly from patient's tissue and they include lung fibroblasts [6], small airway epithelial cells [7], bronchial epithelial cells [8] and alveolar type II cells/pneumocytes (AT2) [9]. However, primary cells are highly specialized and lose their proliferation abilities after a while, which is a problem if we desire to keep these cells in a microfluidic device for a prolonged period.

Adenocarcinomic alveolar A549 and NCI-H441 cell lines have commonly been used as a model for alveolar epithelium in different studies and seem to have a good ability to model lung injury and repair [10][11][12][13]. Furthermore, bronchial epithelial cell lines such as BEAS-2B are usually co-cultured with the epithelial cell line A549 to model airway epithelial cell injury in a cigarette smoke model [14], allergic airway inflammation [15], acute respiratory distress syndrome [16] and lung cancer [17]. However, it is unclear how the cell lines maintain the normal physiology of airway cells, which can be a limitation regarding in vitro models and microfluidic devices.

When using stem cells for in vitro models, it is desirable to differentiate them along pulmonary lineages by providing an environment where it is possible to mimic the lung microenvironment. For this reason, we believe that microfluidic devices could be an ideal candidate for this source of cell. Embryonic stem cells (ESCs) have been demonstrated to be able to generate both airway and alveolar cell types in vitro, and could eventually be used in therapeutic and regenerative applications [18]. However, there is a lot of ethical controversy about the use of ESCs and, for this reason, induced pluripotent stem cells (iPSCs) can be used as an alternative resource. Recent data demonstrated that iPSCs can generate type II alveolar cells (iAEC2s) by directed differentiation, and may provide a platform for disease modeling [19]. Unfortunately, deriving mature lung lineages from iPSC precursors is challenging, even with the expertise from researchers regarding the differentiation protocols for producing ectodermal and mesodermal lineages [20]. We must remember that the lung alveolus and airways are composed of multiple epithelial, endothelial and mesenchymal cell types. Therefore, the use of a combination of cells provides physiological relevance and should be considered during applications of regenerative medicine and engineering systems.

Regarding the choice of cell type for culture on the lung-on-chip platform, the specific clinical application should be considered. Asmani and colleagues cultured primary normal human lung fibroblasts (NHLF) or human lung small airway epithelial cells (SAECs) mixed with collagen type-I, and created a fibrotic microtissue system to study idiopathic pulmonary fibrosis [6]. In another study, human airway epithelial cells (Calu-3) were cultured on one side of the microfluidic device and on the other side, to achieve the air–liquid interface (ALI), with human bronchial smooth muscle cells (hBSMCs). Therefore, this model was efficient to study the interaction between the cell matrix in chronic lung diseases [21]. Zhang and colleagues recreated the human alveolar–capillary barrier to evaluate the pulmonary toxicity of nanoparticles [22]. They used a lung-on-a-chip platform in which human alveolar epithelial cells (HPAEpiCs) were cultured on the alveolar side and human umbilical vein endothelial cells (HUVEC) on the capillary side.

3. Modelling Lung Microenvironment

The architecture of an organ is intimately related to its function. The structural design of the lung has been optimized to fulfill its primary function, which is gas exchange. During the branched morphogenesis of the lung, the airway tree is positioned adjacent to the arterial vasculature and ultimately branches into hundreds of millions of alveoli sacs with a diameter ranging between 100 and 200 µm. Given the high number of alveoli sacs and the thin tissue layer that separates air from blood (approx. 500 nm), the lungs offer a large surface area (100 m 2) in a relatively small tissue volume (6 L) to support fast gas exchange. This enables the perfused blood to gather oxygen molecules and release carbon dioxide into the atmosphere in a short time [23].

Given the dynamic nature of lung function, lung cells continuously experience various types of mechanical forces, which are known to deeply impact their function and phenotype [24]. Notably, lung cells undergo biophysical stimulation consisting of: (1) cyclic stretching due to expansion and contraction during respiration; (2) shear stress associated with airflow into the lungs and vascular perfusion; (3) local changes in the composition and hence, stiffness and viscoelastic properties of the ECM; (4) local variations in oxygen partial pressure; (5) the presence of an air–liquid interface in the airway epithelium. Although these stimuli are all of a physiological nature, it is worth noting that lung cells are often exposed to non-physiological stimuli as a result of disease or injury. Several examples of this include ventilation-induced lung injury (VILI), stiffening of the ECM in lung cancer or lung fibrosis and changes in oxygenation levels associated with chronic respiratory diseases including COPD and OSA.

Given the structural complexity of the lungs and the key role that the ECM plays during lung development and adulthood, organ decellularization has been one of the preferred strategies for the development of biomimetic lung 3D models over the past decade [25]. Through this technique, lung donor cells are removed while the acellular 3D scaffold retaining the biochemical components, mechanical properties and the structural integrity of the native lungs (including the original vasculature) prevails.

Despite the current advances, once the 3D structure is bioprinted, the main challenge is to maintain the bioprinted tissue in a proper microenvironment to support its maturation, thus enabling the performance of basic and translational research. At this step, the use of bioreactors to support bioprinted lung tissue maturation through biomimicry is essential, but they must be custom-designed for each bioprinting application. The design, fabrication and performance of the bioreactors required to that goal is quite complex. To our knowledge, there are no commercial bioreactors that are able to provide the growing tissue with the physiological stimulation of the lung (including mechanical stretch and gas and fluid perfusion). However, the use of miniaturized bioreactors to maintain small constructs and organ-on-a-chip systems has been extended along the academic sector and in the industry.

4. Microfluidic Lung Systems

Microfluidic lung systems constitute an effective and affordable small-scale alternative, where single cultures, co-cultures and even 3D cultures can be maintained under tightly controlled conditions. These models allow for the performance of more realistic experiments where specific microenvironmental parameters such as oxygen tension, shear stress and mechanical stretch can be precisely and individually controlled and are compatible with live cell

imaging. Therefore, these platforms have the potential to provide new insights into normal lung function and disease, as well as to more accurately predict the effectiveness and safety of new treatments under a microenvironment biomimicking the lung [26].

To date, research efforts have mainly been conducted to recreate alveoli, bronchi and bronchioles. The first lung-on-a-chip device was fabricated by Hugh and coauthors (2010), who recreated the blood–air barrier [27]. Through the use of soft lithography, they generated a PDMS device consisting of two microchannels, separated by a 10 µm thick, porous and stretchable membrane coated with proteins of the ECM. The upper side of the membrane was seeded with human alveolar epithelial cells, while the lower side was seeded with lung capillary endothelial cells. By depleting the cell culture media of the upper channel and maintaining a continuous media flow in the lower channel (vascular), researchers generated an air–liquid interface similar to the alveolar–capillary barrier. The simulation of physiological breathing was also feasible by cyclically stretching cells through the application of vacuum, which deforms the elastic membrane to which cells are adhered. Using this model, Hugh et al. studied lung physiological processes such as the differentiation potential of epithelial progenitor cells, carried out toxicological studies and modeled the pulmonary edema and thrombosis [28].

Lung-on-a-chip models providing mechanical stimuli, aiming to study mechanical injury caused by a cyclic stretch in VILI or by movement of liquid plugs mimicking those diseases in which pulmonary surfactant production is compromised (asthma, pneumonia, cystic fibrosis, etc.), have been developed [29][30]. Regarding VILI , Tas et al. (2021) recently developed a lung-on-a-chip model using 3D printable moulds and a commercial nanofibrous poly(caprolactone) membrane. The thin (~20 µm) membrane was cast between two PDMS channels fabricated from 3D printed molds [29]. Proof-of-concept experiments using murine lung epithelial cells showed the good performance of the model, which recreates the air–liquid interface while providing mechanical stretch at relevant magnitudes (25%) without the need to employ complex biofabrication techniques. On the other hand, Nonaka et al. (2020) designed a computer-controlled PDMS chip to expose rat lung mesenchymal stem cells (MSCs) cultured in lung ECM to realistic biochemical and stiffness substrate cues while applying cyclical stretch simulating ventilation (20% amplitude, 12 cycles/min), aiming to determine whether biophysical preconditioning could potentiate the immunomodulatory properties of MSC [31]. Dynamic stretch was generated by the application of cyclical positive pressure underneath a flexible membrane where 3D cultures were attached. After biophysical preconditioning for 7 days, MSCs were applied through femoral venous injection to treat mild VILI in Sprague Dawley rats. Amongst the other results, only preconditioned MSCs were found to induce a significant recovery in elastance. Although this is the first study to demonstrate that biophysically preconditioned MSCs were more effective than non-preconditioned MSCs in reducing mild VILI in a rodent model, more detailed studies highlighting the importance of biophysical stimuli to enhance translational research are required.

It is well known that oxygen levels have a direct impact on processes such as cell proliferation, cell migration and stem cell differentiation. The prediction of oxygen levels within the microfluidic lung systems is of vital importance to create adequate oxygen tension to study cellular behavior under realistic conditions. The most common oxygen-sensitive indicators employed to measure oxygen levels in microfluidic devices are optical fibers and oxygen-sensitive compounds including ruthenium- and metalloporphyrin-based molecules. A comprehensive review of

these and other oxygen sensor types for applications in microfluidic systems was published by Grist et al. (2010) [32]. In previous works, we and coworkers fabricated simple devices that allowed for the fast diffusion of gases to the cell culture through thin elastic and gas-permeable PDMS membranes [33][34][35]. When connected to commercial gas blenders or to two gas reservoirs providing different oxygen concentrations (i.e., 20% and 1% O 2), through an automated two-way solenoid valve, cells can undergo cyclic changes in PO 2 in short periods of time (approx. 6 s), as determined by an oxygen-sensitive ruthenium dye and optical fiber. In addition, some of these models allowed for the application of cyclic stretch at a frequency and amplitude simulating lung breathing [31][33]. Although these systems did not recreate the complex 3D architecture of the alveolar barrier or incorporate shear stress through microfluidics, because they were out of the scope, their simple fabrication, low cost and ability to apply several biophysical cues to cultured cells made them attractive tools. These models have a special relevance for the study of the molecular mechanisms underlying disorders such as OSA and lung cancer, where changes in oxygen tension play a pivotal role in determining their fate and new treatment approaches. In this line, the increased expression of inflammatory molecules such as prostaglandin E 2 has been observed in macrophages in response to intermittent hypoxia (OSA model), as well as their increased migration towards hypoxic cancer cells, likely due to an increased release of cytokines with chemoattractant properties by the latter [34][35].

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