Breathing life into in vitro models

Chapter 8.

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The simple act of breathing can be taken for granted by many, however, this changed in the past two years when society was confronted with an airborne virus, SARS-CoV-2, responsible for the latest worldwide pandemic. Of all the internal organs, the lungs are one of the most prone to infection and injury due to being continuously exposed to external agents such as airborne particles, chemicals and infectious organisms [1].

According to the World Health Organization (WHO), respiratory diseases are one of the top leading causes of death and morbidity in the world [2]. In the latest report from the Forum of International Respiratory Societies (FIRS), it was stated that roughly 65 million people suffer from chronic obstructive pulmonary disease (COPD). Of those, annually 3 million are estimated to develop fatal consequences, making COPD the third leading cause of death worldwide. Additionally, asthma affects 334 million people and 14% of it are children globally, making it the most common chronic disease during childhood. Annually, millions of people die from pneumonia, which is also the leading cause of death among children under 5 years old [3]. In regards to tuberculosis, yearly, at least 10 million people develop it and 1.4 million die from it, making it the most common lethal infectious disease. Lung cancer prevails as the most deadly cancer killing 1.6 million people each year. Globally, 4 million people die prematurely per year, i.e. before the average age of death, from a chronic respiratory disease.

Society is not familiarized with these numbers, and in addition to the scenarios described above, there is increasing evidence showing that novel diseases have been and will continue emerging. Data shows that more than five new diseases are registered every year, some of which have the potential to grow to pandemic proportions [4]. FIRS states that diagnosis, control, and treatment of these diseases should be among the top priorities in global decision-making in the health sector.

Early diagnosis and correct treatment are crucial steps in improving long-term prognosis for patients with diseases such as COPD and asthma, and might even prevent irreversible loss of pulmonary function [5]. Many misdiagnoses (under- or overdiagnoses) and subsequent mistreatment cases could be solved if health practitioners had disease-specific detection tools, since symptomatology is often

shared between multiple respiratory disorders. Biomarkers are quantitative indicators of the presence, severity, or type of a disease. They hold the promise of personalized medicine, which aims at tailoring treatments to the needs of individual patients [6]. However, the search for clinically useful biomarkers is challenging, and the vast majority of biomarkers are failing at the initial verification and validation stages. Similar trends are also seen for newly developed drugs and therapies, which fail mainly due to a lack of efficacy and safety [7]. Increasing the effectiveness during preclinical stages is critical to reducing costly failures later on, during clinical trials. Current advances in cell biology, tissue engineering, microfabrication, and microfluidics have enabled the development of micro-engineered models that represent tissues or even organs, namely microphysiological (*in vitro*) systems (MPSs), also known as organs on chips (OoCs). These systems could increase the predictive power of preclinical assays and reduce costs[8], and decrease the need for animal testing that could be used not only in pharma but also in other fields such as cosmetics, food additives, or environmental contaminants[9]. Particularly, OoC technologies hold great potential in creating relevant and customizable research platforms as they offer controlled physical, chemical and biological cellular environments on a microscale. Additionally, they allow for high-throughput and high-content research, which helps to increase experimental output yields and reduce statistical variability. Moreover, in the future, organ-on-chip technology could offer standardized research systems that can still be made patient-specific.

This thesis presents a set of incremental steps that aid in increasing realism in lung microanatomical models and that are easily combined with microfluidic OoC technology, with an example for this integration. Firstly, we began by focusing on the microanatomy of alveolar sacs and by reverse engineering of bioinspired membranes for the air-liquid culture of alveolar epithelial cells in the form of arrays of circular spherical porous microwells created by a combination of ion track etching and microthermoforming. The same concept was also applied to bronchioles, developing corresponding biomimetic cell culture membranes with differently sized and shaped microwells. Secondly, we integrated the membrane for alveolar cell culture in a microfluidic platform. Thereby, in addition to the culture of lung epithelial cells lining the microwells on the front side of the membrane, we cultured lung microvascular endothelial cells on the membrane's

backside there lining the network-type interalveolar septum-like space between the microwells. Thirdly, we set up a platform for long-term culture, monitoring and imaging, and micromanipulation of bronchial organoids using (dense) polymer film-based thermoformed microwell arrays. The platform proved advantageous for following the growth of individual organoids, as well as for their manipulation by microinjection.

Our work paves the way for more innovative, anatomically more correct, and hopefully clinically more relevant on-chip *in vitro* platforms. These can be used for disease modeling, regenerative studies, and the development of therapeutics for lung, as well as for other organs. We showed that it is possible to "bend the conventions" and create curved lung *in vitro* models. We proved that combining microthermoformed biomimetically shaped membranes with microfluidic organs on chips is feasible. We additionally showed that microthermoformed film-based microwell arrays can enable long-term culture and support organoid research. Altogether, this thesis demonstrates that it is possible and promising to move away from conventional flat, 'two-dimensional' cell culture models and towards more complex and clinically relevant three-dimensional ones. By doing all the above-mentioned, we hope to motivate researchers to be creative and continue developing bio-inspired research platforms, where striking aspects, such as the micro-anatomical features, take center stage. Moreover, increasing complexity and realism in *in vitro* models of human tissues/organs can help unveil critical knowledge of physiological and pathological aspects of these tissues that would not be accessible otherwise. When designing new *in vitro* models, researchers should look at the relationship between form and function present in the respective tissues, and how it can inspire improved human models. Bioinspired features such as curvature of tissues should continue to be investigated, firstly still in an academic setting, as an important feature for an accurate representation of the organ *in vitro*. Unfortunately, we are still far from the day when such platforms would be largely understood, validated and reach the clinical application. Excluding exceptional cases where OoC data has been incorporated as part of an application package, no OoC system has been qualified by a regulatory body, and therefore, none managed to reach the market [10]. The acceptance of such models will require still-to-be-defined qualification/validation criteria, which will be highly dependent on the context of use and the purpose of the specific device.

Nevertheless, given the high incidence of lung-related diseases and the underlying economic burden, it seems relevant and advisable to redirect funds and efforts to this field. Lung-on-chip technology could be tailored to be used in diagnostics, enabling early, rapid, and cost-effective detection of diseases. This can be exemplified, in a technically simplified manner, by the antigen self-test created for SARS-Cov2, which became indispensable in managing the spread of the disease. The havoc created by the second wave of the infection led scientists to pursue the topic and new microfluidic platforms have been idealized for the detection of SARS-CoV-2 [11]. Additionally, as already mentioned, OoC models can be the first step in screening drug candidates. Compared to animal models and experimentation, OoCs can benefit the development of drugs for existing and emerging diseases by reducing the translational limitations and at the same time increasing the throughput of drug testing. Also, in contrast to animal models, OoCs have the capacity to be patient-specific.

In summary, despite the arduous road ahead, OoC technology, if harnessed correctly, can greatly impact society. Ultimately, they can improve society's quality of life by contributing to more accessible, cost-efficient and faster healthcare solutions.

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