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1 The past years have witnessed concerted efforts across the respiratory community to establish more
2 relevant *in vitro* preclinical tools for pulmonary research [1–3]. A strong incentive for such
3 endeavors stems from the need to deliver novel therapies for respiratory diseases; a point sharply
4 underlined at the 2014 European Respiratory Society Presidential Summit [4]. Very few new
5 classes of safe and effective drugs have been introduced over the past half century. In contrast to
6 other diseases areas (e.g. cardiovascular, neurological), pulmonary medicine has witnessed
7 substantially fewer drugs approved; a situation coinciding with comparatively less drug candidates
8 and a higher failure rate. Such facts are concurrent to the observation that pulmonary diseases
9 represent a vast and growing healthcare and financial burden worldwide, associated with high
10 morbidity and mortality [5]. Yet, there has been little impact in therapies treating for example
11 chronic obstructive pulmonary disease (COPD), the fourth leading cause of death globally [4]. In
12 parallel, asthma continues to be among the most prevalent worldwide diseases, underscoring the
13 need for effective treatments in pediatric [6] and severe asthmatic populations [7]. Meanwhile,
14 infectious diseases such as tuberculosis (TB) represent an increasing risk due to lack of effective
15 therapies resulting from low efficacy and high toxicity in the face of multidrug-resistant TB [8,9].
16 The same is true for pulmonary infections by biofilm-forming bacteria (as a consequence of cystic
17 fibrosis), when the increasing occurrence of antimicrobial resistance (AMR) will require novel
18 anti-infective therapies, complementary to established antibiotics [10]. **With a dire need to advance
19 available pulmonary therapies, this short editorial underlines bioengineering opportunities for
20 leveraging microfluidic-based *lung-on-chips* in devising novel human relevant *in vitro* models of
21 respiratory disorders and ultimately help accelerate pulmonary preclinical research.**

1. *LUNG-ON-CHIPS* AS ATTRACTIVE *IN VITRO* PLATFORMS

The efforts surrounding the momentum for improved *in vitro* models are closely linked to growing discussions on alternatives to *in vivo* animal experiments [11]. On the one hand, this follows from ethical and political concerns in line with the application of the “3Rs principles” (Refinement, Reduction, Replacement) towards the highest standards for humane experimentation on animals. Alternatively, progress in establishing *in vitro* models comes as a response to the major hurdles faced with animal experiments regarding the extent to which these shed light on human diseases, with some arguing whether findings have a tangible translational impact [12]. Many new drugs have demonstrated good performance in animal models of asthma but failed at the level of safety or efficacy trials in humans, underlining a call for better predictive models [13]. In particular, differences in physiology and pharmacology between animals and humans constitute underlying barriers towards new drug development; a consequence to divergences in airway cell and innate immune responses to injury between humans and prevalent animal models [14].

Human cell-based assays are widely used for preclinical drug development and include profiling compounds in high-throughput *in vitro* studies [4]. Nevertheless, a significant criticism faced with current “gold standard” *in vitro* assays using petri dishes or transwell inserts (Fig. 1) lies in their inability to recapitulate with sufficient accuracy complex interactions between various human cell types and tissues *in vivo* [15]. In turn, the challenge to develop more pertinent *in vitro* models has been a driving force behind the advent of *lung-on-chips*, and more widely *organ-on-chip* systems [16–18]. One of the hallmarks of *lung-on-chips* lies in recapitulating more accurately biological functionality of the human airway barrier at the air-liquid interface (ALI), using e.g. biopsy-driven primary cells. This comes hand in hand with the integration of critical physiological cues (Fig. 1) that are beyond reach with traditional assays [17,19–22]. Broadly speaking, *lung-on-*

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chips hold the ability to (i) reproduce morphometric traits of the lung anatomy (e.g. bifurcating trees, alveolated airways) **potentially** at true scale; (ii) recapitulate stretching motions mimicking cyclic breathing and thereby cellular strains in airways and within the extra-cellular matrix (ECM); (iii) incorporate respiratory airflows along the epithelium as well as (iv) provide flow perfusion associated with physiological shear stresses upon the endothelium (e.g. using setups that feature basal flows of nutrient media beneath a porous membrane).

2. FROM TOXICITY ASSAYS TO DISEASE MODELS

The main efforts to date witnessed with microfluidic-based *organ-on-chips* have focused on toxicity models [21,23–27]. In respiratory research, this follows the seminal work of Huh *et al.* [28]. The premise of such assays lies in triggering an inflammatory response of an initially healthy airway barrier model (i.e. using mono- or co-cultures of lung cells) upon exposure to foreign threats via the inhalation route [29,30]. Exposure assays include most prominently inhaled particulate matter (PM), noxious gases, viruses and bacteria. Quantitative assessment is then conducted via characterization of well-established biological endpoints including cytokine secretion (e.g. interleukins), cell viability and gene expression, as well as measurements of lung tissue barrier properties using permeability assays (e.g. apparent permeability coefficient) and Trans-Epithelial Electrical Resistance (TEER). **In parallel, there have been recent discussions on leveraging *organ-on-chips* to model various cancers [31,32]; in practice yet, few have tackled lung specific cancers. A recent *organ-on-chip* explored the potential of modelling orthotopic lung cancer growth in helping explain the resistance to therapy in patients [33].**

One of the strongholds of *lung-on-chips* is their potential as research tools to deliver much more realistic, *in situ*-like, human inhalation assays [20], thereby mimicking physiological and

mechanistic determinants surrounding the journey of airborne aerosols to deposition at the ALI. This represents an important departure from traditional *in vitro* assays, in particular those conducted with liquid instillations on cell cultures under submerged conditions or via direct spraying at an ALI (Fig. 1). Recalling that *in vivo* exposure models comprise animals such as rodents whose anatomy differs quite significantly from humans [4], the prospect of more realistic *in situ*-like assays becomes even more critical when aiming to reproduce *in vivo* deposition outcomes (e.g. localized concentration, hot spots, etc.) in human airways [34–37]. Despite progress and recent discussions [38,39], few *lung-on-chip* studies have gone beyond cytotoxicity assays and instead been geared at investigating respiratory diseases (e.g. using patient cells) or conversely eliciting a respiratory disease state. Noticeable examples (Fig. 2) include an asthma model [27], pulmonary edema [40] and a COPD model [41]. Recently, an *airway-on-chip* study has explored epithelial and smooth muscle cell interactions in the pathogenesis of chronic lung diseases [42].

3. EXPERT OPINION

Voiced concern on the lack of therapies for respiratory disorders is calling for accrued efforts in establishing improved *in vitro* disease models of COPD, idiopathic pulmonary fibrosis (IPF), lung infections, acute lung injury (ALI) and pulmonary hypertension amongst other. Ultimately no single *in vitro* platform is likely to reflect the overall complexity of such diseases, in particular chronic ones. Yet the prospect of advanced *lung-on-chips* that successfully integrate relevant physiological cues of the human pulmonary milieu is extremely promising to explore specific clinical phenotypes, and thereby identify clinically-relevant endpoints. In this context, *in vitro* models are still widely seen as complimentary to *in vivo* animal experiments, but in most instances they do not yet offer viable alternative approaches on their own [11]. Whether *lung-on-chips* can

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ultimately reverse this situation remains an open question. Due to their complex designs, characteristics and functions, *lung-on-chips* are yet to establish themselves as standardized preclinical tools at the hands of end users within respiratory research and medicine [43].

In the case of pulmonary infections (e.g. pneumonia, TB), there is a significant need to advance *in vitro* models of pathogen-host interactions, whereby a major challenge lies in faithfully recapitulating interactions between recruited cells, as well as local airway cells, and a given pathogen [44]. To date, few *lung-on-chip* studies on lung infections are available [25,27]; these have been mostly based on simulating conditions using e.g. bacterial wall-derived components that stimulate cytokine secretion (e.g. lipopolysaccharides). Looking ahead, however, future *lung-on-chips* should aim to model chronic infections by biofilm-forming bacteria, ideally allowing the repeated administration of anti-infectives [45]. With an aim towards clinical relevance in patient populations, exploring new therapeutic approaches (e.g. pathoblockers) calls for purposeful and standardized endpoints with meaningful readouts not only for antibacterial efficacy but also regarding pathophysiological changes at the host end [46].

Not unlike the challenges faced with *in vivo* animal models, though, the relevance and success of *lung-on-chip* models will lie in their validity to mirror major hallmarks of respiratory conditions, including foremost chronic progressive and irreversible changes in the lungs. Such endeavors are extremely challenging, in particular when etiological factors (e.g. occupational exposure, cigarette smoke) and natural history of the disease are poorly understood (e.g. COPD, IPF) or conversely in the absence of unique molecular signatures (e.g. ARDS). Nevertheless, and in contrast to human tissue *in vivo*, the relatively simple cell composition of such platforms offers advantages. The use of primary (and immune) cells originating from different donors holds the potential to explore disease diversity across populations, towards personalized medicine applications, and as such a

path towards understanding basic mechanisms of disease initiation. As a final remark, we briefly recall that *in vitro* studies should be distinguished between investigations aimed at determining underlying mechanisms of injury or disease and those focused on the mechanisms of resolution, in particular when concerned with therapeutic action. In the latter case, one open research avenue with *lung-on-chips* lies for example in administering target therapeutics in the hope of observing symptoms returning to normal conditions following the elicitation of a disease state.

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CONFLICT OF INTEREST

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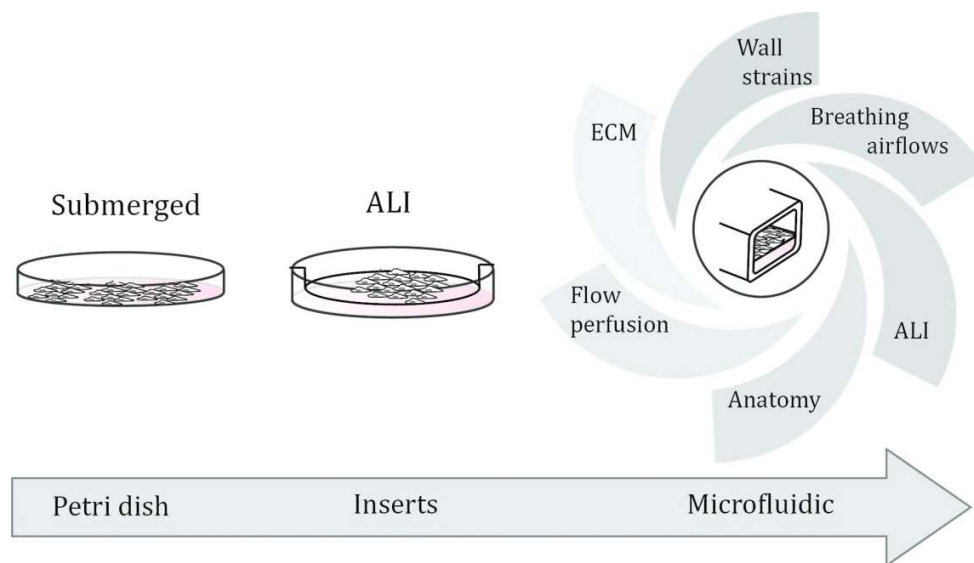
CAPTIONS

Figure 1

Schematic of preclinical *in vitro* research tools in respiratory medicine. Traditional assays include the use of petri dishes where cell cultures are grown under submerged conditions. The introduction of filter inserts permits cell cultures (e.g. epithelium, co-cultures, etc.) at the air-liquid interface (ALI), as well as growing endothelial cells on the basal side of the membrane. With the introduction of *lung-on-chip* platforms, ALI-based cultures can now include an array of physiological cues more closely in line with the innate pulmonary milieu.

Figure 2

Recent examples of *lung-on-chips* modeling respiratory diseases include an asthma model [27], pulmonary edema [40] and a COPD model [41], as well as most an *airway-on-chip* study on epithelial and smooth muscle cell interactions in the pathogenesis of chronic lung diseases [42]. By and large, most microfluidic *in vitro* designs still consist of simple, straight channels featuring an alveolar capillary barrier (ACB) model made of co-cultures of epithelial and endothelial cells grown above and below a porous membrane, respectively.

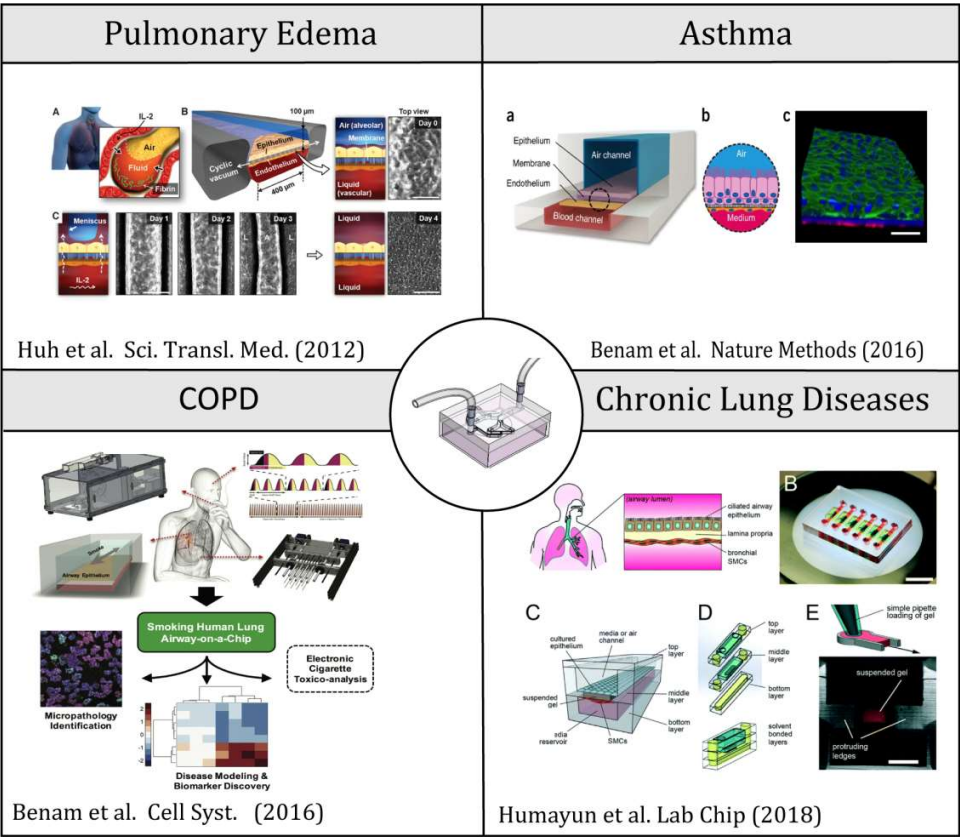


Evolution of *in vitro* lung models

Schematic of preclinical *in vitro* research tools in respiratory medicine. Traditional assays include the use of petri dishes where cell cultures are grown under submerged conditions. The introduction of filter inserts permits cell cultures (e.g. epithelium, co-cultures, etc.) at the air-liquid interface (ALI), as well as growing endothelial cells on the basal side of the membrane. With the introduction of lung-on-chip platforms, ALI-based cultures can now include an array of physiological cues more closely in line with the innate pulmonary milieu.

155x112mm (300 x 300 DPI)

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