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PERSPECTIVE

A perspective towards building scalable, integrated, multicellular in vitro airway models with inverted airway-on-a-tube

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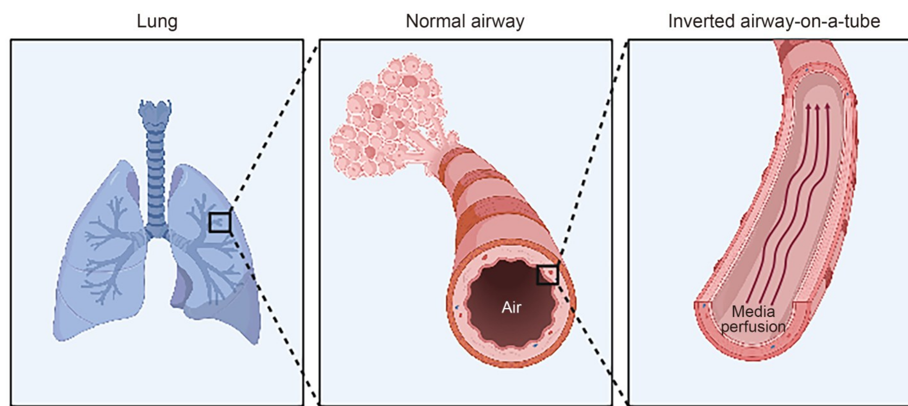
Graphical abstract



1 Current status and challenges

The airway is a structurally and functionally complex system comprising stem and differentiated epithelial cell types that together form the critical airway–blood barrier through interactions between the epithelial and endothelial layers. This system is supported by a diverse array of stromal cells embedded in an extracellular matrix (ECM). Despite the global health burden of respiratory diseases [1], progress in understanding lung disease pathogenesis and developing effective treatments has been hindered by a lack of physiologically relevant in vitro models, highlighting a significant unmet need in bioengineering and biomedical research. The fabrication of improved in vitro models can be realized by leveraging novel biomanufacturing and biofabrication technologies [2].

The tissue microarchitecture of the airway poses unique challenges for in vitro modeling, particularly in replicating the air–liquid interface (ALI) while maintaining accessibility to both luminal and basal surfaces. Established models, such as membrane-supported ALI cultures (e.g., EpiAirway™, MucilAir-HF™, and SmallAir-HF™), lung-on-a-chip platforms, and lung organoids, typically feature discrete epithelial layers or isolated organoids embedded in ECM [3–6]. However, traditional organoid models often lack luminal access, limiting their utility for studying pathogen interactions and drug delivery. Recent advances in biofabrication, microfluidics, and organ-on-a-chip technologies have enabled the development of increasingly sophisticated airway models,

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integrating scaffold or hydrogel-based approaches with stem cell-derived epithelial systems. For example, a new generation of stably inverted apical-out human upper airway organoids [7, 8] has been achieved using ECM removal-induced eversion. These organoids are large (approximately 500 μm), structurally consistent, and maintain their inverted morphology for over 60 days, enabling high-throughput antiviral testing with reduced cell input. A recent breakthrough by Ringquist et al. [9] demonstrated an immune-competent, microvascularized lung-on-a-chip device in a 96-well format that incorporates multiple epithelial, endothelial, and immune cell types. This platform successfully modeled small airway responses to severe H1N1 infection, including cytokine storms, immune activation, and epithelial damage. It therefore offers a promising tool for studying human immune responses in vitro. While these models are promising and provide a proof of concept for curved-surface differentiation, they lack stromal and endothelial components, as well as the option to include biophysical flow.

Despite these advances, the absence of a flowable and circulatory vasculature remains a key limitation, as it is essential for replicating endothelial shear stress and pharmacokinetics. However, achieving full physiological mimicry of a tubular airway with an inner-facing ALI surface is technically demanding and poses scalability challenges. Furthermore, while simple three-dimensional (3D) and on-chip assays can be readily adopted by labs, implementing complex in vitro models requires substantial technical expertise and resources. To facilitate broader adoption and integration into research, clinical, and industrial workflows, we propose a novel complex in vitro airway model. The proposed model incorporates the key features described above and would potentially support biobanking as recently highlighted [10], with the associated benefits for collaborative research, efficient supply chain management, and cryopreservation.

2 Conceptual framework: inverted airway-on-a-tube

Drawing upon recent studies, we propose a theoretical "inverted airway-on-a-tube" system. This design features a tubular, scaffold-reinforced ECM hydrogel structure with a central lumen for endothelial flow culture and an accessible outer surface for epithelial ALI differentiation and ease of aerosol exposure. The ECM also supports co-culture with fibroblasts and other stromal cells, facilitating a more complete recreation of the airway microenvironment.

2.1 Cell types and architecture

The successful implementation of the inverted airway-on-a-tube design requires careful optimization of both cellular composition and hydrogel properties to recapitulate native airway architecture. At the ALI interface, in a recent bronchioid ("airway-in" model), primary human bronchial epithelial cells could differentiate into a pseudostratified mucociliary epithelium, although without stromal and endothelial compartments [11]. Here, we propose inverting this arrangement. Human umbilical vein endothelial cells (HUVECs) or patient-derived endothelial cells would line the tube lumen, forming the endothelial barrier and expressing the adhesion molecules necessary for tissue function and homeostasis. For the stromal compartment, fibroblasts would be embedded within the engineered ECM. Importantly, the presence of fibroblasts within a 3D model was shown to greatly increase epithelial coverage, differentiation, and viability, regardless of matrix stiffness [12]. This spatial organization has enabled progress towards long-term co-culture systems that are progressively more faithful models of the in vivo system [12, 13].

2.2 Extracellular matrix

To recapitulate the correct microenvironment, the ECM hydrogel must serve as both a mechanical support and a provider of critical biochemical cues, ensuring proper differentiation and maintenance for all resident cell types. It is essential to incorporate decellularized lung ECM or defined recombinant proteins (collagen I/IV, fibronectin, and laminin) to support selective cell adhesion, proliferation, and function [12, 14]. Crucially, the hydrogel's mechanical properties, and in particular the ECM's stiffness, should range from 0.2 to 2 kPa to emulate native airway tissue and yield physiological differentiation and gene expression patterns over prolonged culture, as recently demonstrated by studies using tunable hydrogels and synthetic ECMs. Higher stiffness matrices (2–20 kPa) would instead more closely resemble pathological states such as chronic obstructive pulmonary disease (COPD), fibrosis, and cancer [15]. Finally, a properly engineered ECM also promotes nutrient diffusion while maintaining structural stability for at least 4 weeks of culture, enabling full differentiation of patient-derived airway epithelium.

2.3 Parametric modeling

To explore the effects of biological and physical parameters on the system and to ensure proper nutrient diffusion, a simulation of glucose diffusion from the lumen to the air interface through a three-layer cell–hydrogel–cell sandwich was carried out (Fig. 1a). This simulation varied the thickness of the hydrogel layer as well as the diffusion coefficients and reaction rates of the epithelial and endothelial cell layers (Fig. 1b). These values were simulated at their base values and at 0.1× and 10× to obtain a wide spread and better understand the impact of each variable. A constant glucose level was supplied at the lumen wall, simulating a constant supply

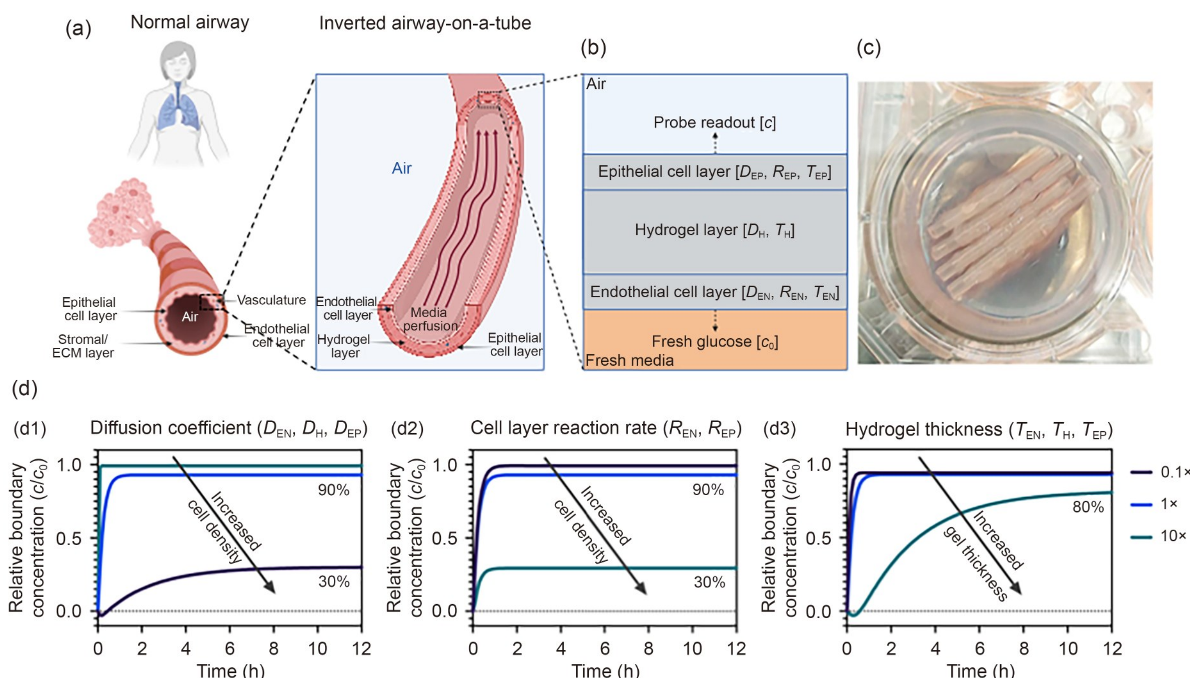

Fig. 1 Glucose diffusion simulation from lumen to air interface. (a) Diagram of the inverted airway-on-a-tube structure. (b) Cross-section of the structure used for the simulation. (c) Fabricated inverted airway-on-a-tube structure. (d) Sweeps of 0.1×, 1×, and 10× variables for (d1) diffusion coefficients (D_{EP}=D_{EN}=5.5×10^{-11} m^2/s [18], D_H=3.35×10^{-10} m^2/s [16]), (d2) cell layer reaction rate (R_{EP}=R_{EN}=3.9×10^{-3} mol/(m^3·s) [18, 19]), and (d3) hydrogel thickness (T_H=200 mm). The thickness of the cell layers was maintained at 100 mm for all simulations (T_{EP}=T_{EN}=100 mm)

of fresh high-glucose culture media, with a boundary probe readout taken at the air interface.

The diffusion coefficients affect how rapidly fresh glucose can perfuse throughout the system and, over the medium–long term (>8 h), both the diffusion coefficient (Fig. 1d1) and reaction rate (Fig. 1d2) plots plateau at only 30% of the initial glucose reaching the air interface for the high cell density condition (Figs. 1d1 and 1d2). The similarity of their results is understandable, as glucose uptake by a cell layer is a function of both time and cell number. While they plateau at the same steady-state condition, in the short–medium term (<8 h), the increased reaction rate has more immediate effects while the decreased diffusion rate takes longer to stabilize. The hydrogel thickness has minimal long-term impact on the glucose output, with >80% glucose reaching the air barrier after 12 h, even with a 10× thickness (2 mm) (Fig. 1d3). These results show that the correct balance of ECM composition and cell density plays a much larger role in maintaining a nutrient-rich system than does hydrogel thickness. Since long-term culture is of primary importance for allowing cell differentiation in this system, it is crucial to ensure the ECM hydrogel has physiological diffusion properties and appropriate cell density. If the cell density makes it infeasible for nutrients such as glucose to fully reach the air interface, perfusable channels could be added to the hydrogel to deliver nutrient-rich media between the layers and facilitate cell maintenance.

The diffusion coefficient in a hydrogel (D_H=3.35×10^{-10} m^2/s) was estimated from the Stokes–Einstein–Sutherland equation [16]. The diffusion coefficient in tissue layers (D_{EP}=D_{EN}=5.5×10^{-11} m^2/s) was estimated from previously published experimental data on multicellular tumor spheroids [17, 18]. The cell layer reaction rate (R_{EP}=R_{EN}=3.9×10^{-3} mol/(m^3·s)) was calculated by multiplying the maximum glucose concentration per cell (3.9×10^{-17} mol/(s·cell) [18]) by the cell density found in the organoids (1×10^{14} cells/m^3 [19]).

2.4 Potential limitations

We recognize that the proposed model faces several technical challenges, including the long-term maintenance of a differentiated air–liquid interface epithelium on a curved surface, sustaining endothelial barrier function under continuous perfusion, and ensuring media compatibility in long-term tri-culture. Although each element has been demonstrated individually, integrating them into a single platform remains an open challenge. However, airway cells readily form broncho or tracheospheres [20] and “airway-out” organoids [8, 21], providing reassurance that the curved surface will not be a significant barrier. Similarly, although culture media selection can be challenging for complex model systems, once the cell–cell crosstalk is established, as in vivo, it can potentially offer support and biological insights [22, 23]. In this perspective, we present the model as a conceptual framework that identifies key areas for future optimization rather than a fully realized system.

Importantly, this design could address the challenges of cryopreservation. Self-assembled airway organoids without a scaffold support are highly susceptible to mechanical disturbances during freezing and thawing, which can compromise structural integrity and lead to epithelial layer fracture. Similarly, microfluidic-based devices often suffer from mismatches in thermal, physical, and mechanical properties between rigid enclosure materials (e.g., glass, thermoplastics, and silicone elastomers) and the soft, hydrated biological systems they contain. Phase changes at the material interface can lead to hydrogel distortion, delamination, and cellular damage.

3 Concluding remarks

An inverted airway-on-a-tube structure comprising scaffold-reinforced hydrogel presents a promising design for addressing the conflicting requirements of model complexity and culture accessibility. This design offers flow input, supports multicellular tissue architecture, and ensures the structural integrity of engineered airway constructs during storage and transport. By enabling both ALI differentiation and potential compatibility with cryopreservation protocols, the inverted airway-on-a-tube satisfies key functional and logistical criteria for advanced in vitro modeling. Its modularity and scalability also position it as a promising platform for model standardization and integration with bioelectronics for in situ monitoring [24], aligning with evolving regulatory frameworks such as the Food and Drug Administration (FDA)'s guidelines for therapeutic testing.

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Author contributions JFM: methodology, software, and writing—original draft; CM: writing—original draft; AC: writing—review & editing; FM: writing—review & editing and supervision; YYSH: conceptualization, writing—review & editing, and supervision.

Declarations

Conflict of interest YYSH is an associate editor of *Bio-Design and Manufacturing* and was not involved in the editorial review or the decision to publish this article. The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

Data availability Data and parametric modeling files are available upon request from the corresponding authors.

Use of generative AI tools No generative AI tools were used in this study.

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