FABRICATION OF HUMAN RESPIRATORY CONSTRUCT FOR IN VITRO DRUG DEVELOPMENT
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ABSTRACT
We report a new approach that overcomes current limitations of closely simulating the complex lung physiology by integrating both bronchiolar and alveolar lung compartments into a single platform. An ideal lung platform combines air-liquid interface, branching of bronchi, and mechanical deformation of alveolar sac. To capture all these features in a 3D respiratory platform, we have developed a rapid fabrication method that uniquely harnesses lamination of laser patterned substrates and adhesives. Tubular and porous, mixed cellulose ester hollow fibers (to mimic the bronchioles) or, poly-L-lactide (PLLA) membrane (to mimic the alveoli) was integrated between the layers and stacked into a 3D tissue culturing environment.

KEYWORDS: Organ on a chip, pulmonary system, rapid fabrication, drug toxicity

INTRODUCTION
In vitro human organ bio-assessment platforms are envisioned to replace expensive and time-consuming animal testing models for biomedical research and drug discovery [1]. However, a major challenge in the development of a human organ construct is to reconstitute physiologically realistic microenvironments that are capable of maintaining cell differentiation and tissue-specific functions [2]. For instance, primary human bronchiolar and alveolar epithelial cells do not differentiate into respiratory epithelium when submerged in culture medium. Here, we report a new approach that integrates hollow fibers and polymeric membranes to recapitulate the physiological complexity of both the lung bronchiole and alveolus. The two-phase flow system can simulate a dynamic liquid layer in both the luminal surface of the hollow fiber and the apical side of the membrane to promote growth and differentiation of primary human bronchiolar and alveolar epithelial cells. By alternatively changing the air and the liquid flow rates, we can create a stable and reproducible air-liquid culture environment for long-term studies on human lung tissues in vitro.

Figure 1: Construction of bronchiolar and alveolar units using rapid stacking approach. (a) Stacking of (a) bronchiolar unit. (b alveolar unit. (c)Assembled bronchiolar unit. (d) Assembled alveolar unit. Porous hollow fiber and PLLA membrane can be integrated to create 3D microenvironment. Scale bar = 5 mm.
EXPERIMENTAL

The 3D respiratory platform was constructed using a rapid fabrication method that uniquely harnesses lamination of laser patterned substrates and adhesive transfer tape tapes (Fig. 1). This fabrication method enables the construction of a 3D lung platform incorporating the necessary microenvironment and an air-liquid interface with biocompatible materials. Our fabrication process utilizes a CO₂ laser cutter to accurately create layers of mini/micro patterns on biocompatible polymeric substrates (e.g. acrylic, polycarbonates, etc.) followed by alignment and bonding using silicone based adhesive transfer tapes [3]. Tubular mixed cellulose ester based hollow fibers with 0.4 µm pores (to mimic the bronchioles) or, poly-L-lactide (PLLA) based membranes with 800 µm thickness (to mimic the alveoli) can be integrated between the layers and stacked into a 3D tissue culturing platform. Modulating the volume and location of the flow chamber allows flow patterns to be precisely controlled, facilitating optimal shear stress for cell growth. The membranes can be inflated and deflated to mimic breathing mechanism by the alveoli. The platform can also be easily integrated into any existing circulation and pumping systems for long term culture.

RESULTS AND DISCUSSION

We have systematically investigated different parameters to identify optimal conditions for cell seeding and tissue culture. We have observed that cells were damaged if the liquid flow surrounded by hollow fiber has higher pressure acting inwards with lower internal pressure in the follow fiber (Fig. 2a). By maintaining low flow rate (10 µL/min) on the outside of the hollow fiber and injecting air inside the hollow fiber, the liquid flux can be dramatically reduced to prevent the formation of the liquid plugs (Fig. 2b). In the alveolar platform, we observed that the PLLA membrane could be inflated and deflated with the air pressures at ±5 kPa.

We also demonstrate that human bronchial epithelial cells (BEAS-2B) can be seeded and grown in the lumen of the hollow fibers, while human alveolar epithelial cells (AT1) and human lung microvascular endothelial (HLMVE) cells can be co-cultured on both side of the PLLA membranes (Fig. 3). After 5 days of cell culturing in the alveolar device, both cell lines showed high viability and confluence covered inside the hollow fibers and on both sides of the PLLA membrane.
Figure 3: Human lung cell cultured in bronchiolar and alveolar platforms. (a) BEAS-2B cells were cultured in hollow fiber for 5 days. (b) Alveolar-capillary interface can be established by co-culture of human ATI (green) and HLMVE (red) cells for 6 days. Scale bar = 100 µm.

The bronchiolar section of the platform was then investigated to explore its potential for toxicological testing of drugs. Solution of fluorescent microbeads were passed through the lumen of the hollow fiber to verify the coverage of bronchiolar epithelium (Fig. 4a). Due to the roughness of the hollow fiber lumen, empty hollow fibers trapped more beads than the hollow fibers covered with cells. The coverage of the lumen after 5 days were further verified using live and dead staining. Furthermore, a toxicity model was established by introducing camptothecin, a pro-apoptotic anti-cancer drug (Fig. 4b). Dose-dependent increase in apoptosis was observed after exposure to camptothecin.

Figure 4: Validation of lung organ platform: (a) Deposition of microbeads inside lumen of hollow fiber shows cell layer was formed to prevent the adhesion of microbeads. Scale bar = 100 µm. (b) Cytotoxicity of camptothecin was established in bronchiolar platform.

CONCLUSION
This 3D artificial pulmonary system will mimic complex lung organ physiology suitable for systemic absorption, distribution, metabolism, excretion, and toxicology (ADMET) studies and the recapitulation of human pulmonary diseases in vitro. This constitutes an important step towards high throughput analysis and screening drug toxicity.

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REFERENCES

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