

MICROFLUIDIC MODEL OF CYSTIC FIBROSIS BRONCHI

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ABSTRACT

In this paper we report a microfluidic model to simulate the bronchi of a cystic fibrosis (CF) patient. The biochip is comprised of two cell culture chambers separated by a membrane. On top of the membrane an alginate hydrogel is formed in order to simulate the thick mucus layer spotted in a CF bronchi. In the bottom chamber a monolayer of epithelial cells are cultured to simulate the bronchi tissue. By inoculating the *Pseudomonas aeruginosa* PAO1 strain to the hydrogel layer one can simulate bacterial infections commonly subjected to the CF patient, and the system can be applied for the studies on antibiotic treatment of bacterial infection related to CF.

KEYWORDS: cystic fibrosis, bronchi, model, microfluidic, microfabrication

INTRODUCTION

Here we report work towards a microfluidic system that simulates the cystic fibrosis (CF) bronchi and the impact of the mucus layer on the treatment of bacterial infections. The classical way of studying CF related bacterial infections, primarily *Pseudomonas aeruginosa*, is by growing them in flow-cell systems [1,2]. In these flow cells bacteria are capable of forming biofilm, as in the airways, and can then be monitored using confocal microscopy [3]. However, the bacteria in CF patient bronchi are not subjected to a constant flow of nutrients as in flow-cell based systems. Instead they embed in the mucus that covers the bronchi epithelia through which the nutrients and metabolites are diffusing (Figure 1A). Moreover, the content of the mucus highly affects bacterial attachment and biofilm growth. Consequently, the biological response for biofilm drug treatments can be altered by changes in the mucus [4].

THEORY

While the human primary bronchi have a relatively large lumen diameter (order of magnitude of centimetres), the respiratory bronchioles are about 1 mm in diameter. Therefore, in order to be able to mimic it as closely as possible to the *in vivo* conditions, a microfluidic system is required. The presented microfluidic model of the CF bronchi consists of two chambers separated by a microporous membrane (Figure 1B). The membrane is the underlying support for a hydrogel, which mimics the mucus layer in the CF bronchi. The chamber below the membrane simulates the artery and supplies the media with nutrients and transports the metabolites. These compounds are provided further to the top chamber by diffusion through the membrane and hydrogel. On the bottom side of the membrane epithelial cells are cultured while on the top part of the hydrogel the bacteria cells are inoculated. In order to simulate antibiotic treatment, the media in the bottom channel can be supplemented with drugs. This supplementation can be performed in cycles, which would mimic drug dosage to CF patients. The introduction of the human sub-bronchial gland cell line (Calu-3) without the layer of hydrogel can simulate the normal bronchi while the same system but with the hydrogel can simulate the bronchi of CF patients.

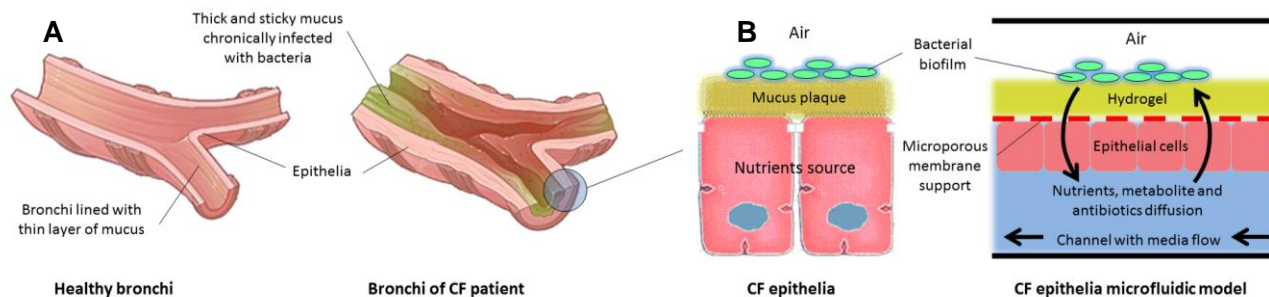


Figure 1: (A) Bronchi of the healthy individual and CF patient. (B) The microfluidic model of CF epithelia.

EXPERIMENTAL

The biochip (Figure 2) was fabricated in polycarbonate (PC) by micromilling (Mini-Mill/3PRO, Minitech Machinery Corp., USA). The PC membrane with 0.45 μm pores was inserted between the milled parts and bonded (tetrahydrofuran vapours assisted bonding, 3.5 MPa, 50°C).

The hydrogel layer was formed by introduction of 0.3% sodium alginate to the top chamber and 0.1 M CaCl₂ solution to the bottom chamber. The thickness of the hydrogel was controlled by focusing the sodium alginate stream with PBS (Figure 3). In order to visualise the hydrogel thickness, the sodium alginate was stained with 6-aminofluorescein according to the receipt by Strand *et al.* [5].

The biochip was thoroughly washed with PBS followed by cell culture media (DMEM, 10% FBS). Calu-3 cells with a density of $5 \cdot 10^5$ cells/ml were seeded on the membrane in the bottom chamber.

The inoculum (OD₆₀₀ = 0.01) of the *P. aeruginosa* PAO1 strain tagged with GFP was introduced into the upper chamber to mimic the bronchi infection (Figure 5). The culture was performed for 3 days in the incubator (5% CO₂, 37°C) (HERAcell incubator, Heraeus, Germany) with perfusion of culture media through the lower chamber with flow rate 0.3 ml·h⁻¹.

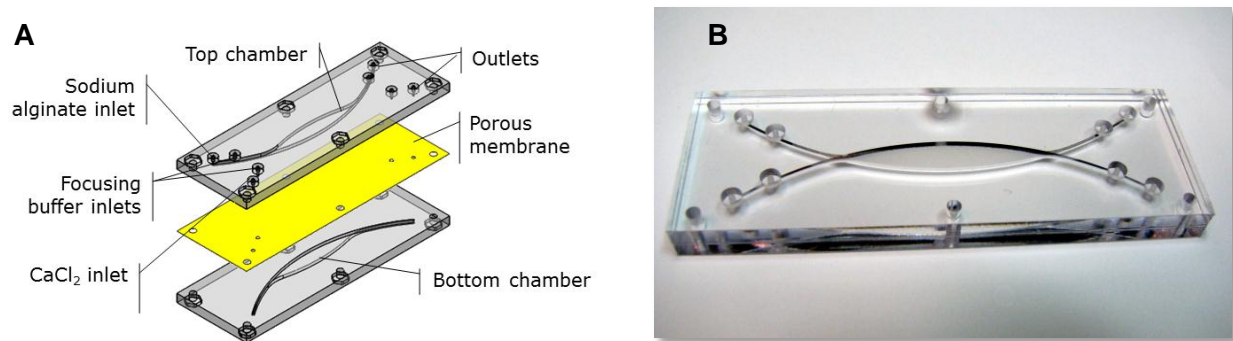


Figure 2: (A) The 3D model of the biochip consist from 3 layers. (B) Fabricated and bonded biochip.

RESULTS AND DISCUSSION

The obtained thickness of the hydrogel layer in the top chamber was 270 ± 20 μm (Figure 4). The focusing buffer was actively pushing the sodium alginate through the membrane, therefore a thin (below 100 μm) layer of hydrogel was formed on the other side of the membrane. The vertical transport of the calcium ions through the membrane was purely diffusional.

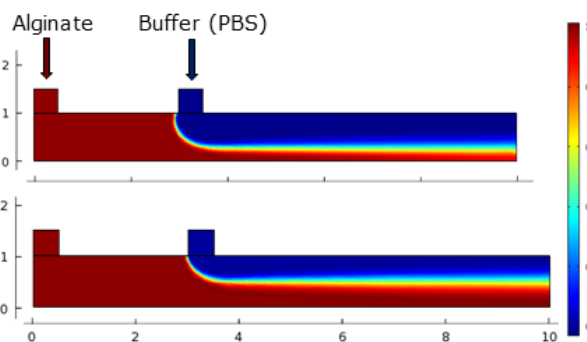


Figure 3: Numerical simulation of the formation of hydrogel layer in the top chamber. The thickness of the layer can be controlled by changing the ratio between the sodium alginate and focusing buffer flow rates.

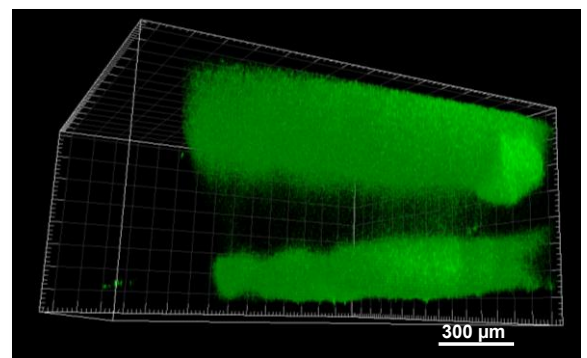


Figure 4: Confocal image of the hydrogel formed in the biochip. Green fluorescence represents calcium alginate and the gap between is the porous PC membrane. The alginate was stained according to [5].

The Calu-3 cells were cultured in the bottom chamber of the system. After reaching the 70% of confluency (3rd day of culture) the *P. aeruginosa* PAO1 strain was inoculated to the upper chamber. The bacteria were allow to form biofilm in the hydrogel for 3 days (Figure 5A). The Calu-3 cells were stained with live/dead stain (Calcein AM/PI) in order to visualise the viable and necrotic epithelia (Figure 5B).

The future application of the presented CF bronchi model lies in simulation of antibiotic treatment of CF related bacterial infection. The simulation of the treatment can be performed by supplementation of the culture media with drugs. The results expected from this should be a better understanding of the problems in the treatment of the chronically infected CF patient.

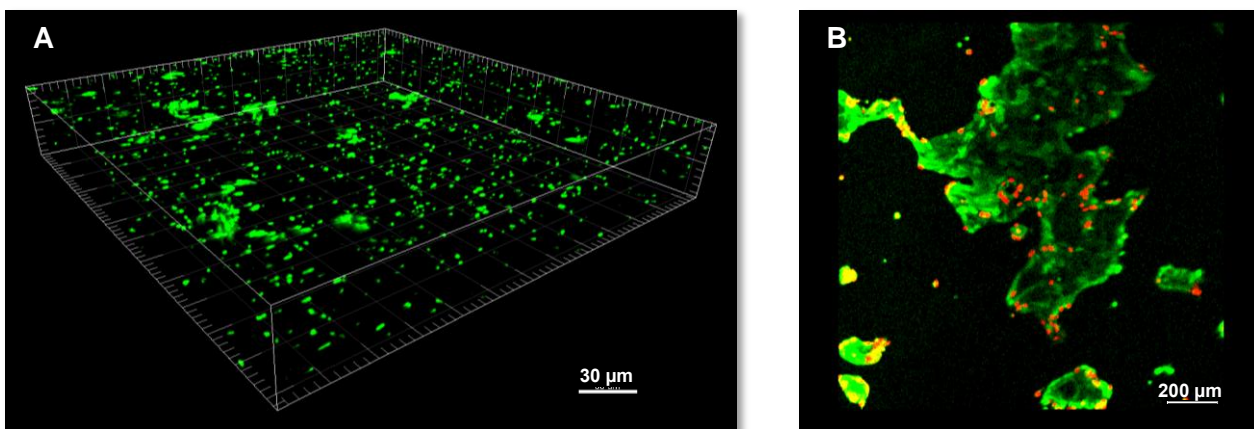


Figure 5: (A) Confocal image of the *P. aeruginosa* PAO1 strain tagged with the GFP growing in the hydrogel. (B) Fluorescence image of Calu-3 cells stained with live/dead stain (Calcein AM/PI) growing below the hydrogel.

CONCLUSION

We have successfully designed and fabricated a microfluidic biochip which can be used as a model of the CF bronchi. The co-culture of the Calu-3 cells and *P. aeruginosa* PAO1 strain using the constructed biochip was shown. By alternating the thickness of the hydrogel layer, the presented model can be used in the future for comparative studies of the antibiotic treatment of bacterial infections in normal and CF patients. This system is a significant advancement in the mimicking of the airways function on the chip reported earlier [6].

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