A BRONCHIAL EPITHELIUM-MIMETIC MICROFLUIDIC CHIP SYSTEM FOR INVESTIGATING MICROENVIRONMENTAL CHANGE-INDUCED INFLAMMATORY PROCESS

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ABSTRACT

We report a 3D biomimetic microsystem which mimics lung microenvironment for monitoring the role of chemokine induced lung inflammation. The extravasation of fibrocytes in response to eosinophil cationic protein (ECP)-stimulated bronchial epithelial cells induced lung inflammation was mimicked on the micro-fluidic chip system for monitoring the microenvironmental changes.

KEYWORDS

Bronchial asthma, biomimetics, microfluidic chip, eosinophil cationic protein, fibrocytes

INTRODUCTION

Inflammatory processes related to lung involve multiple factors which may lead to pulmonary fibrosis and lung scarring. Asthma causes subepithelial fibrosis in the airway, where the inflammation process is induced by human eosinophil cationic protein (hECP) [1]. It is observed that circulating cells (*i.e.*, the fibrocytes) play a major role in the evolution of pulmonary fibrosis where the fibrocytes are induced by increased expression of certain cytokines as illustrated in Fig. 1 [2].

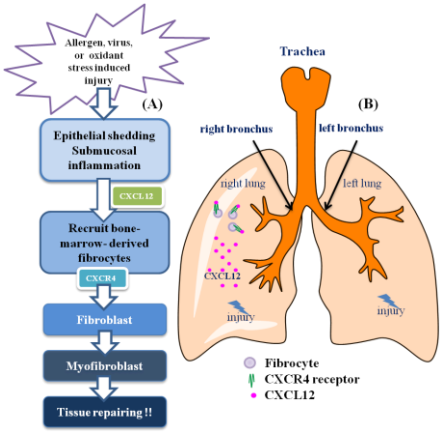

Figure 1. (A) Predicted mechanism of cytokine induced pulmonary fibrosis (B) The role of CXCL12-CXCR4 pathway in fibrocyte extravasation contributing to pulmonary fibrosis.

Due to development of novel microfabrication and microfluidic technology, recently it has been possible to mimic organ level function on microchips for studying the effect of inflammatory cytokines [3].

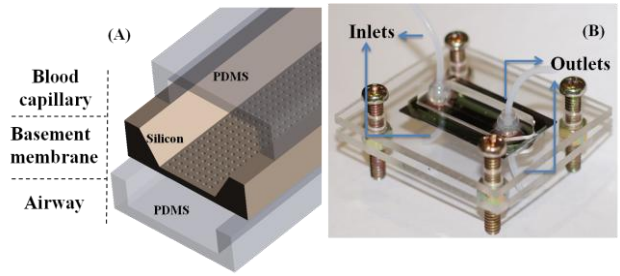

Figure 2. (A) Porous silicon membrane is sandwiched between two PDMS micro channels. The upper layer represents blood capillary whereas the bottom layer represents airway. (B) Chip staged on a chip holder with inlet and outlet.

In this paper, we report a two step 3D microfluidic chip which mimics bronchial epithelial lining for studying chemokine induced expression of cytokine, leading to extravasation of fibrocytes. Microfluidic chip is fabricated by sandwiching a micropore array silicon chip between two polydimethylsiloxane (PDMS) channels as elucidated in Fig. 2. The silicon chip was fabricated by etching 5 μm x 5 μm size holes. The upper channel represented blood circulatory system. In the lower compartment, the micropores were coated with fibronectin to enhance the adhesion of bronchial epithelial

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Beas-2B cells and further culture, therefore mimicking bronchial environment.

EXPERIMENT

Beas-2B cells were cultured for 2 days in the lower channel (Fig. 3A) and stimulated with ECP separately for 12 h and 24 h (Fig. 3B) in a continuous flow system. As illustrated in Fig. 3D, fibrocytes were injected in the upper channel and after 2 h the extravasation behavior was assessed for treatment with ECP (Fig. 3E). The result demonstrated that *in vitro* extravasation behavior of fibrocytes mimicked *in vivo* lung inflammation.

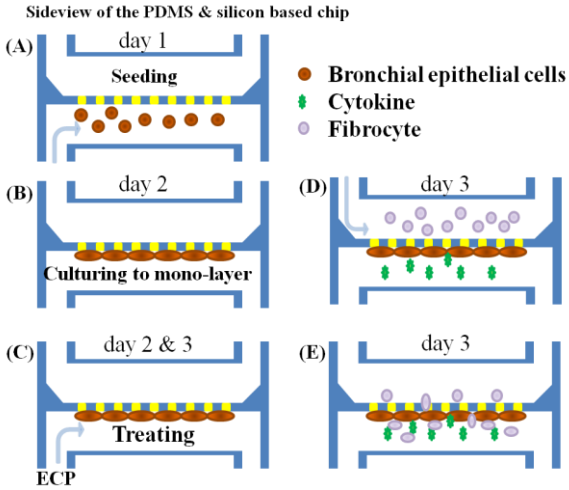

Figure 3. Experimental process for studying fibrocyte extravsation on PDMS & silicon based chip (A) Cell loading and culture (B) Treat with ECP (C) Release of cytokine (D) Load fibrocytes (E) Fibrocyte extravasation

In this study, we employed enzyme-linked immunosorbent assay (ELISA) to determine variations in protein expression of cytokines in Beas-2B cells upon stimulation with ECP. Fig. 4 showed that there was considerable difference in expression of CXCL12 when stimulated by ECP for 12 h and 24 h.

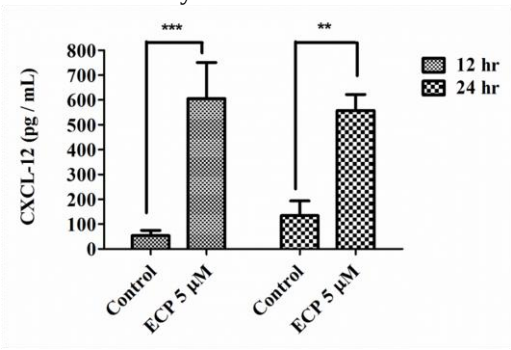

*Figure 4. ECP effect on protein expression of CXCL-12 (**p<0.05, ***p<0.001)*

Upon treatment with ECP, fibrocytes were recruited towards Beas-2B cells in the microfluidic lab on chip system. After extravasation of fibrocytes, the chip was flipped to take bright field and fluorescence images.

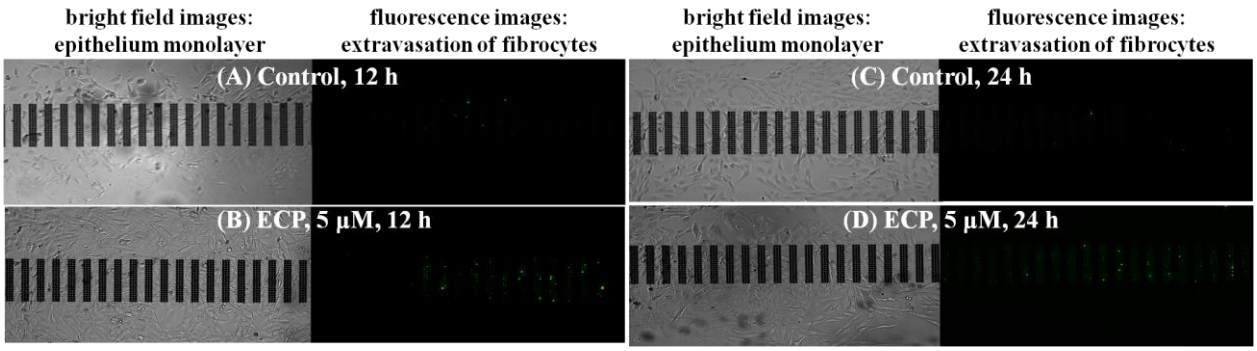

Figure 5. Fibrocyte adhesion and extravasation with cultured Beas-2B cells for 12 h and 24 h in the presence of serum free medium as control and 5 μM ECP.

As observed in Fig. 5B and 5D, fibrocytes migrated to the bottom channel in response to the cytokine *i.e.* CXCL12 released by Beas-2B cells. The migrated fibrocytes were counted upon treatment with ECP for 12 h and 24 h. Fig. 6 showed that ECP was responsible for recruiting the fibrocytes toward Beas-2B cells, leading to inflammation.

ECP plays a critical role in pulmonary fibrosis and is highly involved in acute inflammation in Beas-2B cells. This 3D biomimetic microfluidic system proves a better tool for investigating molecular mechanisms of inflammatory process.

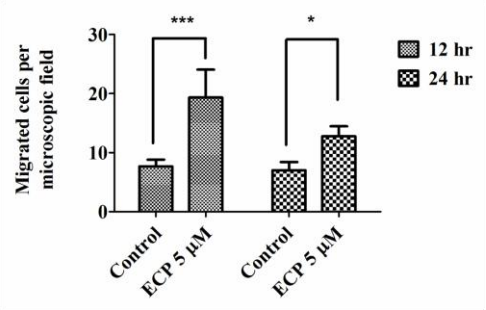

*Figure 6. Number of fibrocytes migrated per microscopic field at 5µM ECP concentration (*p<0.01, ***p<0.001)*

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