Supplementary Information

Biologically inspired lung-on-a-chip for studying protein-induced lung inflammation

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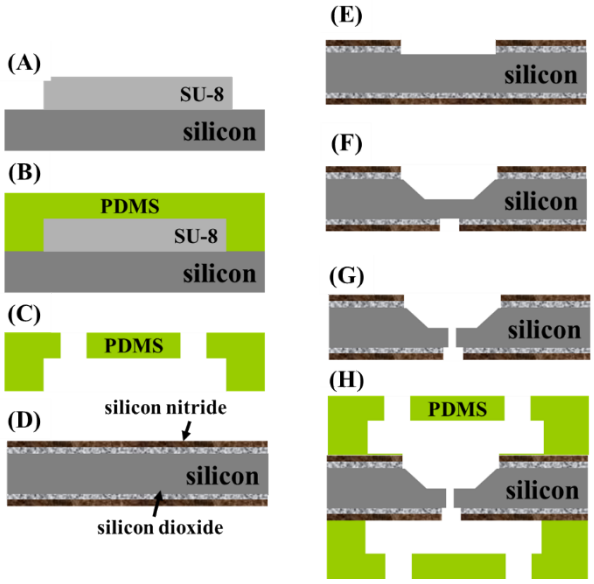
Microdevice design and fabrication

Microfluidic chip was fabricated by sandwiching a micro pore array silicon chip between two polydimethylsiloxane (PDMS) chips. As illustrated in Fig.1 the microfluidic chip was fabricated by using photolithography process. PDMS microchannel of 800μm in depth and 70μm in width was fabricated by soft lithography process. The silicon wafer was coated with SU-8 negative photoresist (SU8-2035, Microchem) and the dimensions of the microchannel were defined by photoresist patterning [Fig. S1(A)]. The mixture of elastomeric base and hardening agent of PDMS (Sylgard 184, Dow Corning, USA) of 10:1 proportion was poured on the top of silicon-PR mold and baked at 90 °C for one hour [Fig. S1(B)]. Holes of 1 mm were mechanically drilled on the top and the bottom cover of PDMS for making fluidic connections to the outside tubing [Fig. S1(C)]. Fig. S1(D, E, F, G) depicts the process for micropore array fabrication. In the first step, silicon dioxide of 1μm thickness was thermally grown on the silicon surface in order to make the surface hydrophilic. Later, 5000 Å nitride was deposited on the silicon wafer by using low pressure chemical vapor deposition (LPCVD). Etching pattern was defined on the top side of the wafer for wet etching in KOH and the uncovered nitride and dioxide was etched by reactive

ion etching (RIE). The wet etching depth is 450µm and 50µm was left unetched for further processing (wafer thickness is 500µm). In the next step the micropore array position was defined on the bottom surface and further etched by using RIE for removing the exposed nitride and dioxide. Using deep reactive ion etching (DRIE) technique further etching of the remaining silicon was carried out to obtain a micropore array [Fig. S1(G)]. The PDMS microchannels (top and the bottom part) and the micropore array silicon chip were exposed to oxygen plasma and were bonded together after alignment as depicted in Fig. S1(H). Finally, silicon tubing's were fitted into the holes to gain access to the microfluidic channels. Syringes connected with poly-tetrafluoroethylene (PTFE) tubes (0.5 mm in diameter) were used for injecting cells or medium into the microfluidic channels.

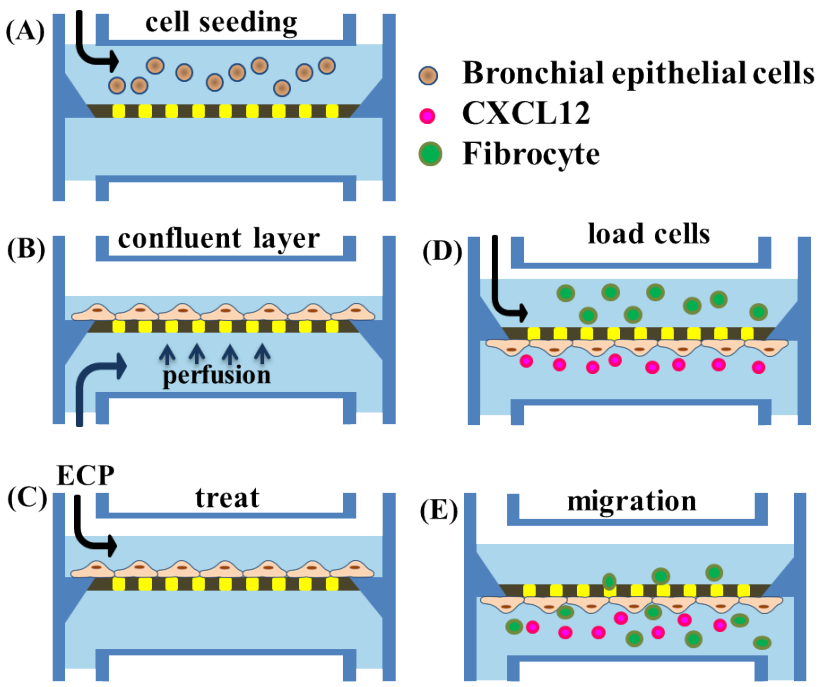


Supplementary Figure S1. The microfabrication process of microfludic chip. (A) SU-8 negative photoresist was used to define the microchannels (~ 70 µm high). (B) PDMS was moulded for fabricating the top and the bottom microchannels. (C) Fluidic connections to outside tubing were mechanically drilled through the PDMS. (D) The siliconoxide was thermally grown and nitride was deposited by using LPCVD. (E) On the top side of the wafer the etching pattern was defined and the exposed nitride and dioxide was etched by RIE. (F) The exposed silicon on the top side was etched by KOH and on the bottom side micropore array was defined and etched by RIE to etch the nitride and the oxide. (G) Using DRIE micropores were obtained. (H) The

PDMS microchannel and micropore array chip were exposed to oxygen plasma and bonded together.

Experimental Process

After microdevice fabrication and assembly on the chip holder, the microfluidic device and syringes with teflon tubings were sterilized by fluxing 75% (v/v) ethanol and exposure to ultraviolet light for 30 min. Microfluidic channels were further fluxed with PBS solution to remove the traces of ethanol if any. In the upper channel, the micropores were coated with 100μg/mL fibronectin from human plasma (Sigma) to enhance the adhesion of Beas-2B cells and further culture by medium perfusion from the lower channel. The lower channel continuously provides fresh medium to the cells through the micropore membrane which assists physiological mass transport. Beas-2B cells were cultured at 37 °C for 24 hr in the lower channel, Fig. S3(A,B) and stimulated with 5 μM ECP-6His at 37 °C for 12 hr and 24 hr separately under continuous flow , Fig. S3C. As illustrated in Fig. S3D, fibrocytes were injected in the upper channel and after intervals of 3 hr, 6 hr, 12 hr and 24 hr the extravasation behavior was assessed in the presence of ECP, Fig. S3E. Upon treatment with ECP-6His, the fibrocytes were recruited towards Beas-2B cells in the microfluidic lab on chip system. After the extravasation of fibrocytes, the chip was flipped to take bright field and fluorescence images.



Supplementary Figure 2: Experimental process for studying fibrocyte extravsation on PDMS & silicon based chip (A) Beas-2B cell loading and culture by medium perfusion (B) further culture to form a confluent monolayer (C) ECP treatment and subsequent cytokine release (D) Fibrocyte loading (E) Fibrocyte extravasation