Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information for:

Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device

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Reagents and materials

Polydimethylsioxane (PDMS) and curing agent were purchased from Dow Corning (Sylgard 184, Midland, MI, USA). Negative SU-8 photoresist (SU-8 2050) and developer were obtained from MicroChem (Newton, MA). FITC-Phalloidin and Lucifer Yellow (LY) CH dipotassium salt (Molecule weight 521.57) was purchased from Sigma Chemical Co. (St. Louis, MO). Hoechst 33342 and propidium iodide (PI) were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Curcumin, was obtained from Merck (Whitehouse Station, NJ, USA). Polycarbonate (PC) porous semipermeable membrane (pore size, 0.4 μm; thickness, 10 μm) was purchased from Whatman Inc. (Florham Park, NJ). Extracellular matrix (ECM) was kindly donated by Dr. Zhao (Life and Health department, the Graduate School at Shenzhen, Tsinghua University).

Microfluidic device design and fabrication

For drug permeability assays, two different functional parts of the microfluidic devices were

designed and fabricated. For cell culture and drug stimulation, a two layered membrane-based microfluidic system was made from PDMS and consisted of upper and bottom microchannels separated by a PC membrane. PDMS and PC are chosen due to their transparent which allowing to be imaged by optical microscope. A single intersection was formed by the cross pattern of the microchannels, so that molecules could transport from one channel to the other through the membrane. The dimensions of the microchannel were 1 cm long, 3 mm wide and 100 μm deep. The bottom PDMS sheet is thin enough (< 500 μm) to image by an inverted microscope. The design is similar to other membrane-integrated microfluidic devices as reported in the literature.[1-5] The microchannel in the upper PDMS sheet served as the apical side as well as a microchamber for cell culture, and the bottom channel in the lower PDMS sheet served as basal side. Another separated microchannel was designed to immobilize SPE beads for desalting and concentrating drugs permeated from the upper channel before ESI-Q-TOF MS detection. The dimensions of the microchannel used for packing pretreatment materials were 22 mm long, 2 mm wide, and 80 μm deep. The end of the channel contained micropillar arrays with 30 μm wide intervals which were connected to the outlet. The micropillar arrays inside microchannel was used to act as a weir for immobilizing pretreatment materials, which had an average diameter larger than 30 μm.

The PDMS sheets containing upper and bottom channel features were fabricated by standard soft photolithography.[6] The connection holes were firstly punched on the upper PDMS sheet. And then, they were bonded to the membrane using the stamping procedure.[2, 7] Briefly, PDMS prepolymer (10:1 weight mixture of PDMS prepolymer and curing agent) was mixed with toluene in equal ratio and spin-coated (500 rpm for 4 s, 1500 rpm for 60 s) onto a glass slide to form a thin PDMS mortar film. PDMS sheets were stamped onto the mortar film for 1 min and then positioned mortar side facing up. Membrane edges were gently dipped onto mortar, and then put in the center of the thin

PDMS sheet. The thick PDMS sheet was finally sealed on the thin PDMS sheet. After assembly of the membrane and PDMS sheets, the device should be left at ambient conditions for 30-40 min before curing to eliminate air bubbles trapped along the edge of the membrane. The device was finally placed in an oven at 80 ºC overnight to cure the mortar. Another functional part for sample pretreatment was made from PDMS and glass as our previously reported.[8]

Cell culture

Caco-2 cells were maintained in Eagle minimum essential medium (MEM) containing nonessential amino acid supplemented with 10% FBS, 100 μg/mL penicillin and 100 μg/mL streptomycin. After being cultured in flasks at 80% confluence, the cells were released from tissue culture flasks by trypsinization with 0.25% Trypsin EDTA.

Quantification of curcumin by high-performance liquid chromatography

Curcumin was quantified by HPLC, using a Waters Alliance 2690/5 HPLC system with Waters 2996 PDA UV-vis absorption detector. A XBring C18 column (5 μm, 3.9×150 mm, Waters) was used. Mobile phase solvents were (A) 2% acetic acid in water, and (B) acetonitrile. Fifty microliter samples were injected into the column. Gradient elution was applied to separate the three curcuminoids: 0-2 min, 90 A and 10% B; 2-30 min, linear gradient from 10 to 65% B; 30-35 min, held at 65% B; 35-36 min, B went back to10% linearly. The flow rate was set at 1 mL/min, and the detection wavelength was 420 nm.

MTT cytotoxicity assay

Caco-2 cells were harvested and seeded in 96-well plates at the concentration of 7000 cells per well. An initial stock solution of 10 mM curcumin was prepared in methanol. Curcumin solutions were prepared with the concentration ranged from 25 to 300 μM, and then incubated at 37 ºC in a 5% CO_2 incubator for 1, 2 and 24 h. Medium containing the same concentration of methanol was used

as a control. 10 μL MTT solution (5 mg/mL in PBS) was then added to each well and incubated for 4 h at 37 ºC. 100 μL DMSO was added to dissolve the formazan precipitate and the absorbance at 495 nm was determined using Multimode Detector DTX880 (Beckman Coulter).

Stability study in HBSS

The test was carried out by keeping curcumin in HBSS buffer at pH 6.5 and 7.4 at 37 ºC for 30, 60, 90, and 120 min. The concentration of curcumin used was 10 μM. At the appointed time, 8 μL of the curcumin solution was immediately injected into the micro-SPE column for desalting and concentration and detected online by ESI-Q-TOF MS. The on-chip extraction procedures were the same as the experimental details described in the curcumin extraction section.

Evaluation of Caco-2 cell monolayer integrity and cell viability

At the end of the permeability experiment, cell viability was stained by Hoechst 33342 and PI for 20 min at 37 ºC. The nuclei of viable and dead cells generated blue and red fluorescences under an inverted fluorescence microscope. The monolayer integrity was tested by evaluating the penetrating amount of the fluorescent dye LY in the bottom channel which permeated from the upper channel. LY, a permeation maker, was determined in the direction of A-B to evaluate the monolayer integrity. 5 μM LY solution was prepared in HBSS buffer (pH 7.4) and used for the permeability assay of the differentiated monolayer. The LY solution was injected into the upper channel at a flow rate of 1 μL/min, while the bottom channel was filled with HBSS buffer. After infusion for 1 h, the intersection part of the membrane-based channels were imaged under an inverted fluorescence microscope equipped with a cooled CCD camera at an excitation wavelength of 485 nm and an emission wavelength of 540 nm. Image analyses were performed using public domain image analysis software (ImageJ). Cell layers with less than 1% transfer of the dye from the upper channel to the bottom channel were considered intact.[9]

The Caco-2 cells were also stained with FITC-phalloidin to ensure a single uniform layer of cells. Cells were washed with PBS three times, and then 4% paraformaldehyde was injected into the upper channel to fix the cells for 10 min. PBS was then introduced to wash the cells, treated with 1% Triton X-100 for 5 min in an ice-bath to increase membrane permeability. Cells were blocked with 1% BSA for 20 min, and then incubated with 5 μg/mL FITC-phalloidin for 20 min at 37 ºC. Finally, cells were washed with PBS and imaged. The monolayers were viewed using an inverted fluorescence microscope with the excitation and emission wavelengths of 488 nm and 525 nm, respectively.

Mass spectrometry

The experiments were carried out on a quadrupole time-of-flight mass spectrometry equipped with an electrospray ion source and operated in the negative ion mode. Capillary voltage was set at 2.5 kV and cone voltage at 25 V. Cone gas flow was set at 50 L/h with an ion source temperature of 100 ºC. Desolvation gas flow was set to 550 L/h with the corresponding temperature of 250 ºC. All mass spectra were externally calibrated by sodium formate in the negative ion mode with the mass range of *m/z* 50-1000. For MS/MS analysis, the collision energy was set at 15 eV.

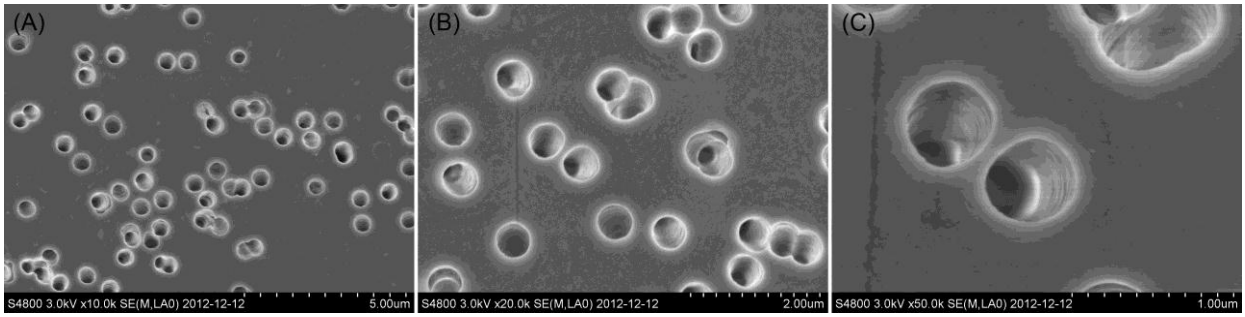

Fig. S1 Scanning electron microscope images of PC membrane coated with 100 μg/mL matrigel for 30 min at 37 ºC.

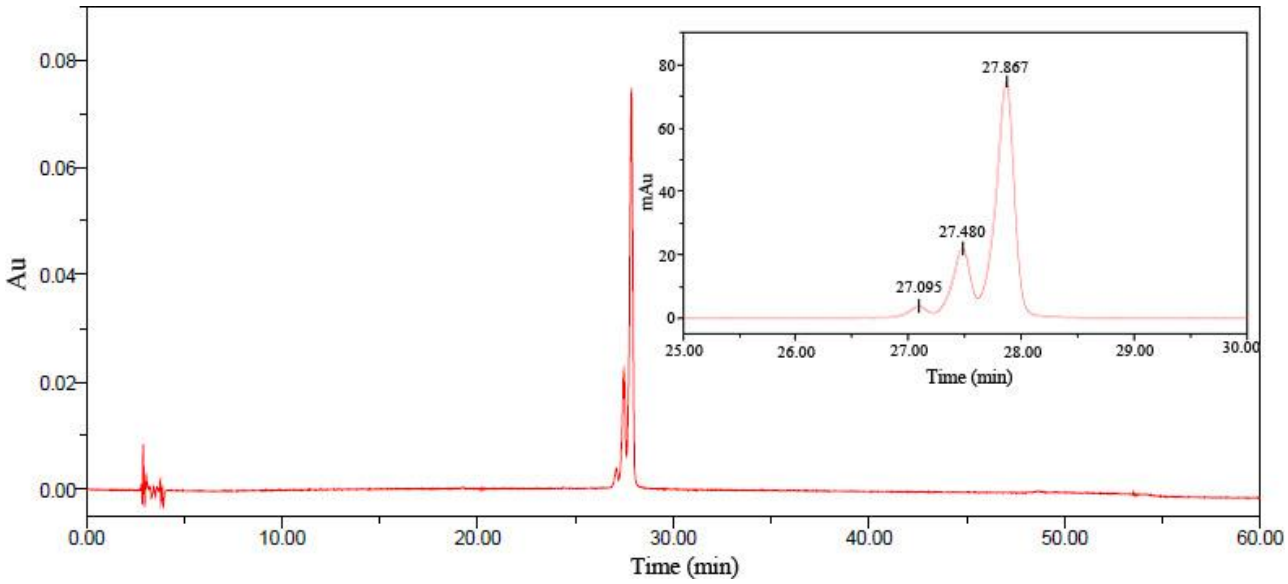

Fig. S2 Typical chromatogram of three cucuminoids analyzed by HPLC. Fify microliter injections of 5 μM total curcuminoids are shown here.

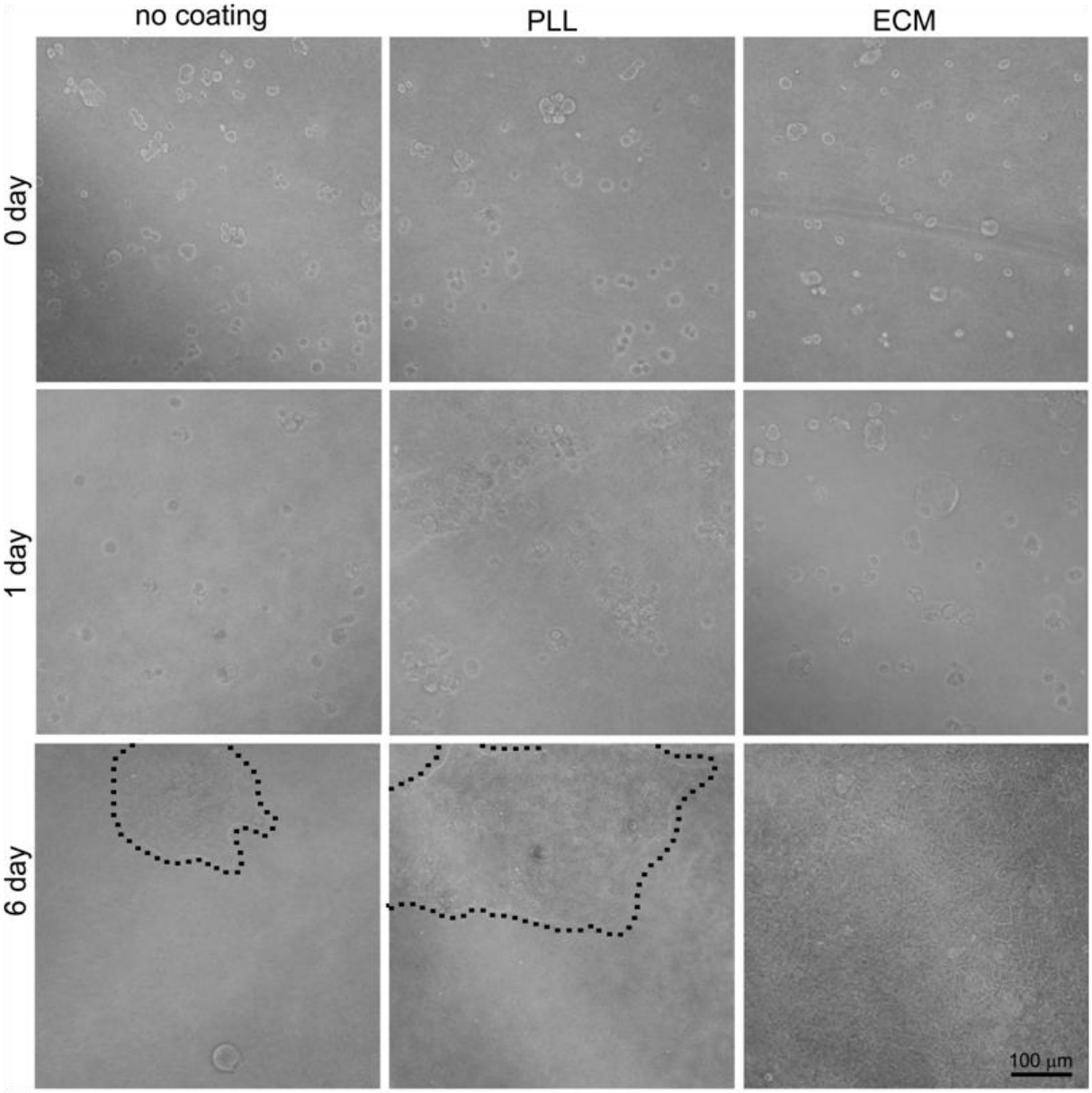

Fig. S3 The effect of different surface modification of the PC membrane methods on cell attachment and proliferation.

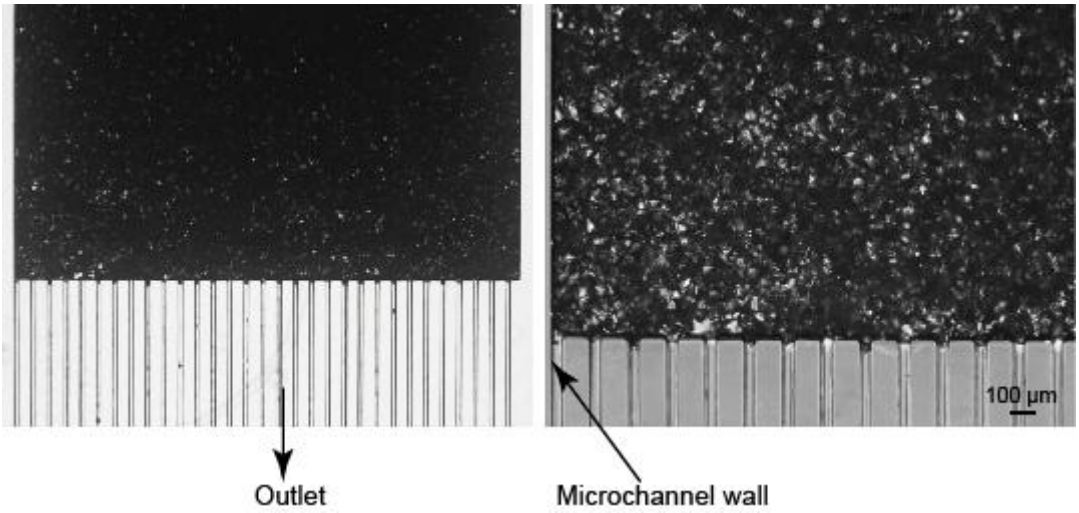

Fig. S4 Micorscopic images of the pretreatment microchannel. (A) The image shows the end of the sample pretreatment microchannel, where the C18 silica packing materials were firmly trapped by the micropillar arrays. (B) The enlarged image is to show the close packing of the materials.

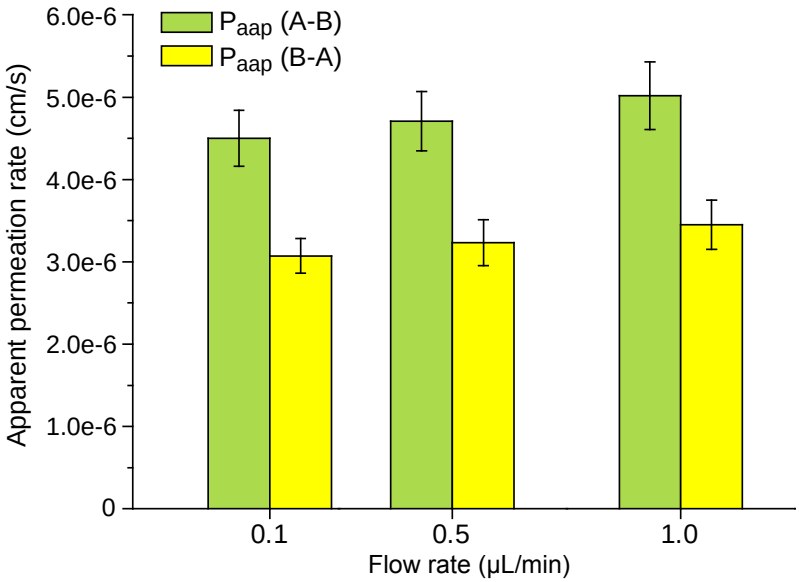

Fig. S5 The effect of flow rates on permeability of curcumin. The standard error bars mean the variation of three individual experiments.

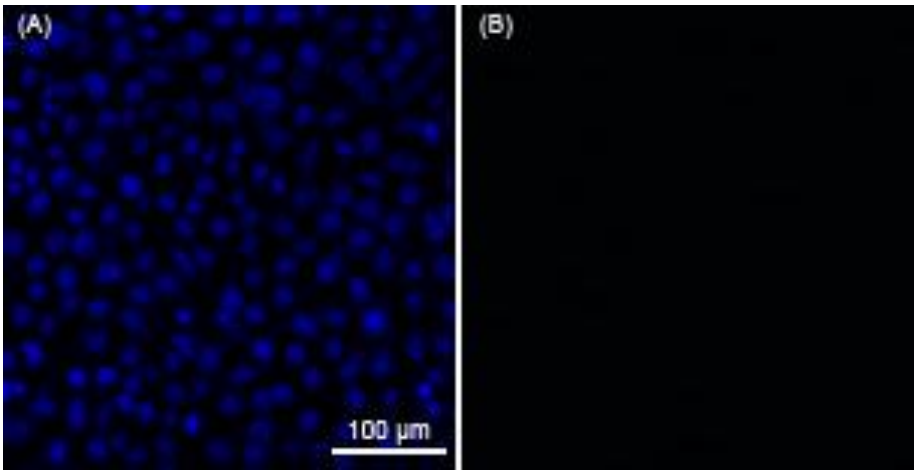

Fig. S6 Images of Caco-2 cells on PC membrane were stained with Hoechst 33342 and PI for cell viability assay at the end of the transport experiment. (A) Viable cells were stained light blue by Hoechst 33342. (B) Dead cells were stained red by PI.

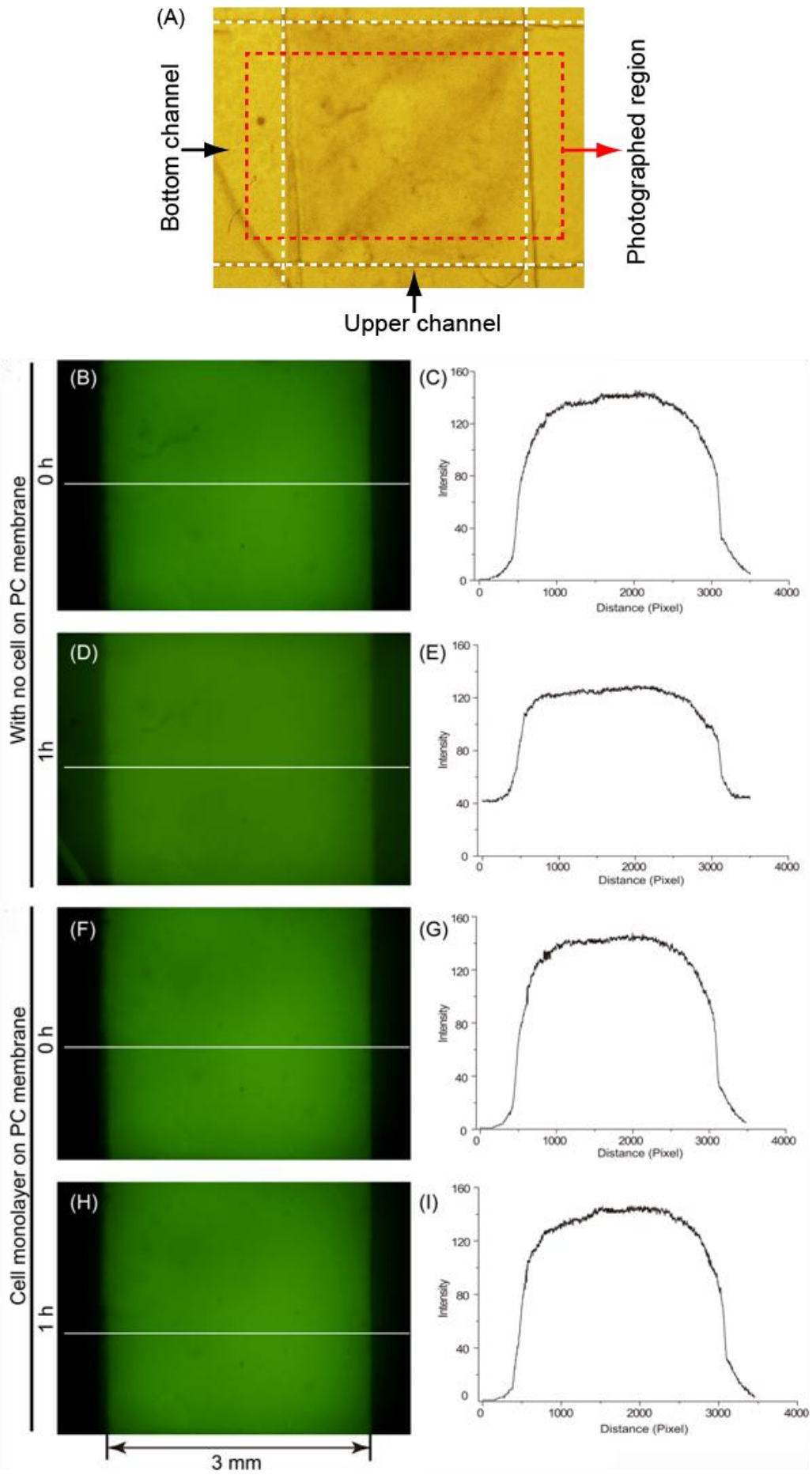
(A)
Bottom channel
Photographed region
Upper channel
With no cell on PC membrane
0 h
1h
Cell monolayer on PC membrane
0 h
1 h
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
Intensity
Distance (Pixel)
3 mm

Fig. S7 Evaluation of the cell monolayer integrity on membrane-based microfluidic device. (A) Micorscopic images of the microchannels at the intersection. The area within the red square was used for imaging Fluorescence. (B, D, F, H) Microscopic fluorescence images obtained from 0 and 1h after 5 μM LY was injected into the upper channel with no cell or cell monolayer on the PC membrane, and the corresponding fluorescence intensity evaluated along the dashed lines (C, E, G, I).

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