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PAPER

Polydimethylsiloxane SlipChip for mammalian cell culture applications

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This paper reports a polydimethylsiloxane (PDMS) SlipChip for *in vitro* cell culture applications, multiple-treatment assays, cell co-culture, and cytokine detection assays. The PDMS SlipChip is composed of two PDMS layers with microfluidic channels on each surface that separated by a thin silicone fluid (Si-fluid) layer. The integration of Si-fluid facilitates two PDMS layers slide to different positions; therefore, the channel patterns can be re-arranged for various applications. The SlipChip design significantly reduces the complexity of sample handling, transportation, and treatment processes. To apply the developed SlipChip for cell culture applications, human lung adenocarcinoma epithelial cell line (A549) and lung fibroblasts (MRC-5) are cultured to exam biocompatibility of the developed PDMS SlipChip. Moreover, embryonic pluripotent stem cell line (ES-D3) is also cultured in the device to evaluate its stemness maintenance in the device. The experimental results show that cell morphology, viability and proliferation are not affected when cultured in the SlipChip, indicating the device is highly compatible with mammalian cell culture. In addition, the stemness of the ES-D3 cells is highly maintained after cultured in the device, suggesting feasibility of using the SlipChip for stem cell research. Various cell experiments such as simultaneous triple staining on cells and co-culture of MRC-5 with A549 cells are also performed to demonstrate functionalities of the PDMS SlipChip. Furthermore, we demonstrate an cytokine detection assay to evaluate the effect of endotoxin (lipopolysaccharides, LPS) treatment on cytokines secretion of A549 cells using the SlipChip. The developed PDMS SlipChip provides a straightforward and effective platform for various on-chip *in vitro* cell culture and consequent analysis, which is promising for a number of cell biology studies and biomedical applications.

Introduction

Polydimethylsiloxane (PDMS) microfluidic devices have drawn extensively attractions for a variety of biomedical applications in recent years because of their unique material properties, great manufacturability, and controllability in spatial and temporal domains. High-throughput capability and high surface area to volume ratio property provided by microfluidics make it possible to precisely and accurately manipulate large-scale biological samples in a simple but effective manner. Moreover, engineering microenvironments is essential for various cell culture applications, such as: tissue regeneration, drug screening and cancer metastasis[1-5]. For example: microenvironments of various substrate stiffness, surface porosity, and surface chemistry are extensively studied[6-9]. Consequently, a robust and stable system is highly required to perform long-term mammalian cell culture under the created microenvironments. Microfluidics is a promising tool not only capable of integrating created microenvironments but also being stable for long-term applications. Therefore, a number of microfluidic devices have been developed to create various physical and chemical microenvironments for *in vitro* cell culture studies[10-15].

Multiple analysis and complex experimental process are usually required in biological studies such as immunofluorescent staining, chemical mixing, screening and enzymatic assays which still presents a hurdle for applying microfluidic systems f cell biology studies. Sample handling in microfluidics seriously limits its applicability for multiple analyses due to the difficul of controlling small-volume sample transportations microfluidic channels effectively with simple instrumentation. For instance, sample transportation for enzymatic assays requi multiple steps involving several anti-body capture and coupli with analytes. However, while plasma treatment strengthens the bonding between substrates and micro-patterned PDMS layers of microfluidic devices, the fixed channel patterns are usu disabled from subsequent analyses and make multiple treatment more tedious and challenging. Although several flow contr methods have been developed to facilitate tiny sample transportation, tedious and complex design of interconnections, valves, and pumps is usually involved in the microfluidic system For example, the setup to conduct cell co-culture in microfluidic devices is accompanied with valves and syringe pumps to control the flow direction for generating desired conditioned mediu , which makes it difficult to conduct experiments in a conventiona incubator. Therefore, it is essential to develop microfluidi devices capable of performing cell culture and consequen analyses conveniently and effectively for multiple assays without using sophisticated actuation mechanisms for samp

manipulation.

SlipChip, a microfluidic device performing multiplexed liquid-phase experiments by simply slipping two contacted substrates without controls of pumps and valves, has been exploited for protein crystallization, polymerase chain reaction (PCR) and drying process[16-20]. SlipChip shows great efficiency for manipulating nanoliter-to-microliter aqueous samples by sliding two contacted plates[20]. Moreover, a series of parallel of analysis can be conducted simultaneously, which is powerful for various screening applications. In addition, the controllability of surface chemistry and straightforward operation make SlipChip more applicable for various fields. Recently, SlipChip devices used for culturing bacteria and investigating bacterial chemotaxis is reported[21,22]; however, the potential to use SlipChip for mammalian cell culture applications has not been carefully studied. In the conventional SlipChip devices, gas cannot freely diffuse through glass substrates. As a result, additional channel designs and operation procedures are required to achieve cell culture inside the SlipChip devices, which makes the devices difficult to be directly used in conventional cell incubators for mammalian cell culture applications[22].

In this paper, we develop a PDMS SlipChip, a PDMS microfluidic device capable of sliding the microfluidic channel patterns to other positions for subsequent processes and analysis in a simple manner, to investigate its potential possibility for *in vitro* mammalian cell culture applications. PDMS is one of the most broadly adopted materials to fabricate microfluidic devices due to its excellent mechanical property, gas permeability, thermal stability, manufacturability and optical transparency. Moreover, the surface chemistry of PDMS-based microfluidic devices can be easily modified by coating extracellular matrix (ECM) proteins, or grafting desired functional groups. The PDMS SlipChip is composed of two micro-patterned PDMS layers sandwiching a silicone fluid (Si-fluid) thin layer. The incorporation of Si-fluid facilitates two PDMS layers to slide to desire positions for sample treatments, which significantly reduce the number of treatment processes for analysis and alleviate complexity of operation. The re-arrangeable channel patterns can be effectively utilized for various operations by simply sliding chips.

In the experiments, the biocompatibility of PDMS SlipChip is evaluated by culturing human lung fibroblasts and carcinomic human alveolar basal epithelial cells in the devices up to 4 days. In addition, the stemness of embryonic stem cells cultured in the PDMS SlipChip is investigated by observing the appearance of stem cell markers. Furthermore, functionalities of the developed PDMS SlipChip are demonstrated by performing multiple treatments, cell co-culture experiments and on-chip cytokine detection assays on PDMS SlipChip. The results demonstrate the developed PDMS SlipChip has advantages of simple fabrication and operation, easy sample handling, applicable for multiplex multiple assays in a simple but effective manner, which suggests its promising potentials for various biomedical researches based on mammalian cell culture.

Materials and Methods

Design and fabrication of PDMS SlipChip

PDMS SlipChip is composed of two PDMS layers with microfluidic channel patterns and a thin silicone fluid layer sandwiched between the layers as shown in Fig. 1(a). The device is fabricated using the well-developed soft lithography replica molding process[23]. In brief, the master molds with positive relief features are fabricated by patterning negative tone photoresist (SU-8 2100, MicroChem Co., Newton, MA) on silicon wafers using conventional photolithography techniques. The molds are then silanized with 1H,1H,2H,2H-perfluorooctyltrichlorosilane (78560-45-9, Alfa Aesar, Ward Hill, MA) in a desiccator for 40 minutes at room temperature to prevent undesired bonding between PDMS and the molds. PDMS precursor is prepared by mixing base and curing agent with ratio of 10:1 (v/v). PDMS precursor is poured onto the fabricated molds, and then cured in the oven at 60°C overnight. The inlets and outlets of micro-channel with diameters of 2 mm are punched using a biopsy punch. To assemble the device, the bottom PDMS layer is firstly bonded onto a glass slide as a supporting substrate. Si-fluid thin film is prepared by spin-coating Si-fluid (DC 200, Dow Corning, Midland, MI) on a separate glass slide with rotational speed of 6,000 rpm. The Si-fluid is composed of dimethyl siloxane, trimethylsiloxane-terminated silicone, and it has viscosity of 10,000 cSt. The Si-fluid is selected to ensure the cell culture and analysis compatibility, easy sliding and good sealing between two PDMS layers. The resulted thickness of the Si-fluid layer is less than 9 μm as shown in Fig. 1 (b). Afterwards, the PDMS top layer is placed onto the Si-fluid thin film, then directly stamp to the bottom layer. The assembled SlipChip can be directly used for cell culture without further bonding process. To modify the wettability of the device surface, the whole PDMS SlipChip, including Si-fluid layer, can be placed into a plasma chamber for oxygen plasma treatment while its sliding ability is still maintained. In the SlipChip ammalian cell culture experiments conducted in this paper, we design different channels for different applications.

Numerical Simulation

In order to understand the diffusion process between wells located on different layers after contacting in the PDMS SlipChip, a numerical simulation is performed using a commercially available finite element analysis (FEA) software, COMSOL Multiphysics (Ver. 4.3b, COMSOL Inc., Burlington, MA). In the simulation, a three-dimensional model is constructed based on the designed dimensions for PDMS SlipChip: the length, width and depth of the culture well are 4.0, 1.0, and 0.3 mm, respectively. The diffusion coefficient of fluorescein in water (4.9×10^{-10} m^2/s) is used for the numerical calculation.

Cell Culture

To demonstrate the potential applications of PDMS SlipChip for mammalian cell culture, carcinomic human alveolar basal epithelial cell (A549, ATCC, Manassas, VA), human lung fibroblast (MRC-5, ATCC), and murine embryonic pluripotent stem cell (ES-D3, 60205, Bioresource Collection and Research Center, Hsinchu, Taiwan) are utilized for cell culture experiments in this study.

The stocks of A549 cells are cultured in F-12K medium (Gibco 21127, Invitrogen Co., Carlsbad, CA) with 10% v/v fetal bovine serum (FBS) (Gibco 10082, Invitrogen) and 1% v/v antibiotic–antimycotic (Gibco 15240, Invitrogen). The stocks of MRC-5 cells are cultured in MEM medium (Gibco 41090, Invitrogen). The cells are cultured in T-75 flasks (Nunc 156367, Thermo Scientific Inc., Rochester, NY) placed in a humidified incubator with 5% CO_2 at 37°C, and passaged by dissociation with 0.25% trypsin-EDTA (Gibco 25200, Invitrogen) every 3 to 4

days.

The ES-D3 cells are cultured using growth medium composed of Dulbecco's Modified Eagle Medium (DMEM) (Gibco 10566, Invitrogen, Calrsbad, CA) with 15% v/v fetal bovine serum (FBS) (Gibco 10082, Invitrogen), 1% v/v antibiotic/antimycotic (Gibco 15240, Invitrogen), 0.1 mM 2-mercaptoethanol (M7522, Sigma-Aldrich), 0.02% v/v Sodium Pyruvate (Gibco 11360, Invitrogen), 1% v/v Non-Essential Amino Acid (Gibco 11140, Invitrogen) and 1,000 U ml^{-1} ESGRO (Chemicon ESG1106, Millipore). ES-D3 cells are passaged by dissociation with Trypsin every other day. Cell culture petri dishes are coated with 2% wt gelatin (G1890, Sigma-Aldrich) solution prior to cell seeding, and the ES-D3 cells are transfected with Oct4-GFP.

Biocompatibility Evaluation

To examine cell compatibility of the developed SlipChip, both A549 and MRC-5 cells are cultured in the microfluidic devices for four days. The device is first treated by oxygen plasma to make the microfluidic channel surfaces hydrophilic. The extracellular matrix (ECM) protein, fibronectin (F2006, Sigma-Aldrich Co., St Louis, MO), at a concentration of 100 μg/ml is then introduced into the device for four hours inside an incubator before introducing cell suspension into the SlipChip. The cell suspension solution is prepared by centrifuging dissociated cells at 950 rpm for 4 minutes at room temperature. The cell density for seeding is adjusting to 1.0 x 10^6 cells/ml and 1.2 x 10^6 cells/ml for A549 and MRC-5 cells, respectively. To evaluate the cell viability, Calcein AM and Ethidium homodimer-1 (EthD-1) (L3224, Invitrogen) dissolved in the D-PBS (Gibco 14190, Invitrogen) are utilized for staining live (green color) and dead (red color) cells after four-day culture in the device, correspondingly. The fluorescent images of the stained cells are captured using an inverted fluorescence microscope (AF70000, Leica Microsystems, Wetzlar, Germany) equipped with a motorized stage, and a CCD camera (ORCA-R2, Hamamatsu Photonics, Shizuoka, Japan).

To further investigate whether stemness of stem cells can be maintained in the PDMS SlipChip, ES-D3 cells transfected with Oct4-GFP (hOct4-GFP, Plasmid 21153, Addgene, Cambridge, MA) are utilized in the experiments. To prevent ES-D3 cells from attaching and differentiating on the PDMS surface, the SlipChip is coated with 5 wt % bovine serum albumin (BSA) (UR BSA-001, UR) solution for 2 hours in the incubator prior to cell seeding. Cell suspension is prepared to 1.2 x 10^6 cells/ml for introducing stem cells into device.

The stemness of the cells is confirmed by Oct-4, Sox-2, and stage-specific embryonic antigen-1 (SSEA-1) stem cells markers. For Sox-2 transcription factor, ES-D3 cells are fixed in 4% paraformaldehyde (158127, Sigma-Aldrich) in D-PBS (Gibco 14190, Invitrogen) for 30 minutes, and then permeated with 0.1% wt Triton-X 100 for 20 minutes. The permeated cells are blocked in 1% wt BSA solution overnight. Alexa Fluor® 647 Mouse anti-Sox2 antibody (560322, BD Pharmingen, San Jose, CA) is directly stained for 2 hours after blocking. For SSEA-1, ES-D3 cells are fixed in 4% paraformaldehyde in D-PBS for 30 minutes, then blocked in fresh non-permeable-blocking solution composed of 5% normal donkey serum (017-000-001, Jackson ImmunoResearch, West Grove, PA) in 1 X D-PBS for 20 minutes at room temperature. The cells are then incubated with mouse anti-SSEA-1 (1:500) antibody (Chemicon MAB4301, Millipore) for overnight at 4°C, washed three times, and incubated with DyLight-549-labeled donkey anti-mouse IgM (1:500) (715-505-140, Jackson ImmunoResearch) for 2 hours in the dark. The fluorescent images of the stained cell are also captured using the inverted fluorescence microscope.

Multiple Treatment Assay

To further demonstrate the applications of our developed PDMS based SlipChip for multiple assays, we simultaneously stained MRC-5 cells grown within the microfluidic wells with three different kinds of dyes by sliding the chip. MRC-5 cell suspension with cell density of 1.5 x10^6 cells/mL is seeded into three independent wells by introducing the cell suspension into the microfluidic channel. To enable cells to be well adhered on surface, the cell are cultured in the devices one day before the staining. Afterwards, 10 μl of Calcein AM (L3224, Invitrogen) (2 μM), Hoechest (B2261, Sigma) (1 μg/ml), and Cell Tracker Red (C34552, Invitrogen) (5 μM) solutions are filled into three separately channels next to the cell culture wells. By simply sliding the top layer, the microfluidic channels filled with dyes are covered onto the cell culture wells one-by-one correspondingly. As a result, the MRC-5 cells can be simultaneously stained with three different dyes on a single chip. In the experiments, the cells are stained for 30 minutes and then the staining solutions are washed out using D-PBS for three times. The fluorescent images are observed using the inverted fluorescence microscope.

Cells Co-Culture on PDMS SlipChip

Co-culture of various cells has drawn extensively attractions for investigating cell-cell interactions and mimic physiological condition *in vivo*. Moreover, cytokines secreted by peripheral cells may influence the phenotypes, morphologies, and cellular behaviors of their surrounding cells. For instance, transforming growth factor-β (TGF-β) is considered an essential cytokine for activating fibroblasts to myofibroblasts, which are highly associated to cancer progression.[23] To demonstrate the device capability, the developed PDMS SlipChip is applied for co-culturing carcinomic human alveolar basal epithelial cell (A549) and human lung fibroblasts (MRC-5). First, A549 and MRC-5 cells are seeded in different rows of cell culture wells. The cells are cultured for 1 day to make cells well adhered. For co-culture of the cells, the top PDMS layer is slides to a position that the co-culture chamber can cover both A549 and MRC-5 culture wells that initiates cells co-culture. In this configuration, the secreted cytokines can directly diffuse into co-culture chamber and interacted with cells. After two days, the top PDMS layer is slided to another channel next to the co-culture chamber for immunofluorecence staining. α-smooth muscle actin (α-SMA), a cell marker for myoflibroblast, is employed to evaluate effect of the presence of A549 on activating MRC-5 fibroblasts to myofibroblasts[24]. To stain α-SMA, the MRC-5 cells are first fixed by immersing in 4% wt paraformaldehyde for 30 minutes, Triton X-100permeation for 20 minutes, and blocking in 1% wt BSA solution overnight. MRC-5 cells are than incubated in anti-α-SMA (1:100) antibody (A5228, Sigma-Aldrich Corp., St. Louis, MO) overnight. Afterwards, Alexa Fluor®488 Goat anti-Mouse IgG (1:500) antibody (A-11001, Life Technology, Calrsbad, CA)

is used to conjugated with the α-SMA antibody. The fluorescent images are taken by the inverted fluorescence microscope. Fluorescent intensities of α-SMA are quantified by analysing the captured imaged using ImageJ software (Ver. 1.47, National Institute of Health, Bethesda, MD).

Cytokine Detection Assay

Cytokine detection assays usually involve a series of anti-body captures and several washing steps, which are tedious and increase contamination possibilities. Moreover, a large number of samples are usually required for performing the assays using traditional well-plates methods. Recently, various cytokine detection assays have been extensively performed on the microfluidic devices, which greatly reduce the volume of analytes[25,26]. However, on-chip sample manipulation is still challenging since multiple valves, pumps, and interconnections are required for the operation. In this study, we demonstrate the capability of PDMS SlipChip for on-chip cytokine detection assays directly after cell culture in the same device without additional instrumentation.

In the experiments, the effect of lipopolysaccharides (LPS) stimulation on Interleukin 8 (IL-8) and Tumor Necrosis Factor (TNF-α) expression of A549 cells is performed on the developed SlipChip. The PDMS SlipChip device is composed of two cell culture wells on the bottom layer; and one microfluidic channel for seeding, two chambers for filling the culture medium and four wells for cytokine detection assays on the top layer. In order to promote the anti-body binding efficiency, surface modification is applied to functionalize the surfaces of the wells for the assays. The SlipChip is first underwent oxygen plasma treatment to increase its oxygen functional group and wettability. Afterwards, amine functional group is imported by treating 0.5% wt PEI (181978, Aldrich) solution in the cytokine detection assay wells for one hour. 2% wt glutaraldehyde (3802, Sigma-Aldrich) is injected to cross-link with as-generated amine group for 30 minutes. A549 cell suspension is prepared to 1.0 x 10^6 cells/ml and introduced in two wells on the SlipChip through the microfluidic channel on the top layer. A549 cells are cultured in the devices for 1 day prior to the LPS stimulation. 40 μg/ml LPS (L2630, Sigma) solution is added into medium and injected to one of the chamber, and another chamber is filled with the culture medium as control. The top layer is then slided to the position where the two chambers cover the culture wells one-by-one for 6 hours treatment. IL-8 and TNF-α detection kits (EBS88-8068, EBS88-7346, eBioscience, CA) are used to examine their expression after the LPS stimulation. For the cytokine detection, capture antibody is fixed on surface at 37°C for two hours before the cytokine detection assay. 1 X Assay Diluent is applied to block non-specific adsorption sites at room temperature for one hour. For the reaction, the top layer is further slide to where cytokine detection wells overlaid the cell culture wells. After incubation overnight, the top layer is slide back to original position. Finally, the avidin-conjugated fluorescent dyes are introduced to SlipChip: strepavidin FITC (EBS11-4317, eBioscience) and strepavidin eFluor® 570 (EBS-4317, eBioscience). To examine the expression of IL-8 and TNF-α, IL-8 is labled with red color while TNF-α is labled with green color. The fluorescent images are captured using the inverted fluorescence microscope, and the images are analysed using the ImageJ software.

Results and discussion

Operation of PDMS SlipChip

The schematic three-dimensional illustration is shown as Figure 1(a). First, the micro-channel on top layer is placed to cover two wells on the bottom layer. In this way, sample could be easily injected into two well at bottom layer using the top channel. After sample injection, top layer is sliding so that the two micro-channels on top layer could cover two wells on the bottom layer one-by-one. Two different reagents on the top micro-channel could independently react with samples in two wells on the bottom layer separately in this configuration, which two analysts are simultaneously analysed. Fig. 1(b) shows a fabricated PDMS SlipChip with different channel arrangements after sliding. With channel design of microfluidic devices, the re-arrangeable micron-channel patterns of PDMS SlipChip could be applied for various multiple treatments and large scale analysis. In the experiments, we manually slide the PDMS layers to desired positions with observation under a microscope. In general, the accuracy of 100 μm can be achieved. To improve the accuracy in the device level, the magnet-assisted alignment can be exploited[27].

Time-Dependent Diffusion

Free-interface diffusion is the main mechanism for substances transporting in the SlipChip through concentration gradients. Moreover, free-interface diffusion can be applied for diffusing the nutrients, cytokines and staining dyes from microfluidic channels into cell culture wells in the SlipChip, which is highly suitable for various biomedical applications. The time which nutrients, cytokines and other substances supplied to the targeted cells is essential for cell culture application since these factors are critical for cell survival. To reveal the time for fluorescein diffusion process, numerical simulation software, COMSOL, is employed in this study. Fig. 2(a) shows the concentration profile inside two contact wells with approximate dimensions of 1 mm (W) x 4 mm (L) x 0.26 mm (H) for different periods of time. The initial concentration was 0 and 1 for the wells on the top and bottom layer, respectively. The diffusion process initiates right after the two wells contact each other. After 100 seconds, concentration profile is nearly uniform throughout the top and bottom well. On the 150 second, concentration profile become uniform and both top and bottom layers show equal concentration, indicating that the diffusion can be controlled by designing the volumes of wells. The overall diffusion finished within three minutes, which is fast enough for materials diffusion in the cell culture applications. Mass diffusion in the SlipChip with the wells of similar dimensions is demonstrated by mixing two different food dye solutions of blue and yellow colors in the same dimension wells used in the simulation. First, blue and yellow food due solutions are independently introduced in the wells. When the top layer (filled with blue food dye solution) is slided to the position covering the well in the bottom (filled with yellow food dye), the color becomes green. After three minutes, the well is completely mixed as shown in Fig. 2(b), suggesting the fast diffusion behavior in the SlipChip. The experimental result is similar to that of the simulation, showing that the diffusion can be finish within few minutes. Furthermore, the photos of the well

filled with blue food dye and the mixture of blue and yellow food dyes located in the bottom layer after sliding show the solutions can be well confined in the wells by the Si-fluid during the sliding motion without leakage. The fast diffusion facilitates the mass transportation in PDMS SlipChip for mammalian cell culture applications.

Cell Culture and Biocompatibility

To apply our developed PDMS SlipChip for cell culture, A549 and MRC-5 cell lines are utilized for evaluating biocompatibility of the PDMS SlipChip devices. Both cells are cultured in the channels with approximate dimensions of 0.5 mm (W) x 2 mm (L) x 0.26 mm (H) for four days. Live/dead staining is used to validate the cell viability of SlipChip. Fig. 3 demonstrates the bright field and fluorescent images (Live/Dead staining) of cell cultured for 4 days. Both A549 and MRC-5 cells reach confluent after 4-day culture. Moreover, majorities of the cells are alive after cultured in the SlipChip for four days (cell viability > 99 %), indicating high biocompatibility and low cytotoxicity of PDMS SlipChip. Furthermore, the incorporation of silicone fluid does not have influence on cell viabilities and cell morphologies. We further culture A549 cells in the PDMS SlipChip for 10 days, and the experimental results are shown in the Supportin Information (Fig. S2). The results show most of the A549 cells live (> 98%) after 10-day culture, although the cells are over-confluent. The results suggest that the developed PDMS SlipChip can also be exploited for various long-term cell culture studies.

Stem Cell Culture and Stemness Evaluation

The easily direct differentiation of stem cells prior to apply them for further experiments is one of challenge for using microfluidic system in stem cell research. In addition, the significant decreasing of stemness during operation disables microfluidic device for controlling the differentiation ability. Therefore, it is important to maintain stemness while simultaneously cultured in microenvironments using the microfluidic systems. Oct-4 GFP transfected pluripotent embryonic stem cells, ES-D3, are cultured in the PDMS SlipChip for 2 days to verify the feasibility to perform stem cells culture utilizing the PDMS SlipChip. ES-D3 formed embryoid bodies (EBs) as shown in Fig. 4, and green fluorescent demonstrate the Oct-4 expression of the stem cells cab be maintained after two-day culture inside the PDMS SlipChip. Also, the other stem cell markers to label undifferentiated mouse ES cells, Sox-2 and SSEA-1, are used to confirm the puripotency and stemness of the cultured ES-D3. The fluorescent images shown in Fig. 4 suggest that the ES-D3 cells still express the Sox-2 and SSEA-1, indicating that stemness and puripotency are highly maintained in the SlipChip. Therefore, the developed PDMS SlipChip can be further used in stem cell research.

Multiple Treatment Assay

Multiple treatments are commonly used in cellular analysis for biological studies; however, they are seldom carried out within a single microfluidic device due to the tedious liquid transportation. The re-arrangeable microfluidic channels of the SlipChip can solve this problem by simply sliding the analytes and samples to the desired position for subsequent assays. To demonstrate the application of the developed PDMS SlipChip, the simultaneous staining of three kinds of dyes on the cells grew on independent wells is performed. By simply sliding the dye-filled channels to contact with the cell culture wells, MRC-5 cells cultured within three different wells with approximate dimensions of 0.5 mm (W) x 2 mm (L) x 0.26 mm (H) can be stained by Cell Tracker Red, Calcein AM and Hoechst as shown in Fig. 5 without cross-contamination. Furthermore, it saves time to use the SlipChip to conduct multiple staining. Comparing to conducting experiments independently, this sliding functionality can enable a variety of multiple and high throughput assays to perform on a single PDMS SlipChip much effectively, such as drug screening, protein crystallization and combinatorial chemistry.

Cell Co-Culture

In this study, we utilize the PDMS SlipChip to perform co-culture of cancer cells (A549) and fibroblast cells (MRC-5). Both cells are cultured in the wells with approximate dimensions of 0.5 mm (W) x 2 mm (L) x 0.26 mm (H). Within the same chip, we also examine the expression of α-SMA protein, a cell marker for myofibroblast cells, on the MRC-5 cells by immunofluorescence staining. Furthermore, three different volumes of the culture wells including one well for non-co-culture, have been designed on the SlipChip as shown in Fig. 6(a). It is noted that only the MRC-5 cells are observed after sliding the top layer for co-culture experiments. The volume ratio of three culture wells is 4.3: 2.3: 1 for large, small and non co-culture wells, respectively. Therefore, the device can be used to screen the volume effect of the conditioned medium on myofibroblasts activation in a simple but effective way. In addition, high surface area to volume ratio in microfluidics leads to the conditioned medium with higher concentration of soluble factors secreted by the cells. As a result, the device can show better efficiency for cells co-culture. In the experiments, both A549 and MRC-5 cells are first independently cultured in the SlipChip for one day as shown in Fig. 6(b). By sliding the top layer, co-culture experiment can be easily initiated and co-culture for 2 days to induce the MRC-5 cells becoming myofibroblasts. It is noted that co-culture of A549 and MRC-5 does not have mutual impact on the cell survival, growth and proliferation indicated by the bright field phase images as shown in Fig. 6(b).

After two-day co-culture, MRC-5 cells are fixed and stained α-SMA to verify activation of myofibroblasts. The fluorescent images of α-SMA staining are shown in Fig. 6(c). The fluorescence images show that the MRC-5 cells co-cultured with the A594 cells have higher α-SMA expression level compared to those without co-culture. To qualitatively study the protein expression level difference, average fluorescence intensity within each MRC-5 cell in the entire fields of view are calculated. In addition, a statistical analysis, unpaired, two-tailed Student's t-test, is performed, and the results are shown in Fig. 6(c). According to the analysis, the fluorescence intensities of α-SMA staining of the co-cultured cells are higher than that of the mono-cultured MRC-5 cells indicating the higher α-SMA expression levels in the co-cultured MRC-5 cells. The results suggest that the co-cultured fibroblast cells are successfully transformed to myofibroblast cells in the SlipChip. Furthermore, the average fluorescence intensity within the MRC-5 cells co-cultured in the small wells (i.e. larger surface area to volume ratio) is more than 65% higher than that within the MRC-5 cells co-cultured in

large wells. The larger surface area to volume ratio enhances the effect of the secreted cytokines from the A549 cells, which transform the fibroblast cells to myofibroblast cells with higher efficiency. The α-SMA expression level difference clearly shows the importance of the medium volume when conducting co-culture experiments. The demonstration confirms the high efficiency co-culture capability of the developed SlipChip, and the device can be further scaled up to investigate co-culture with more cell types and volume effect. Using the developed SlipChip for co-culture experiments, various experiments studying interactions between different cells can be realized more easily and effectively for biologist to investigate cell-cell interactions and cell biology study.

Cytokine Detection Assay

On-chip cytokine detection assay is performed using the developed PDMS SlipChip to evaluate the IL-8 and TNF-α expression after the LPS stimulation onto the cells as shown in Fig. 7(a). The bright-field phase image shown in Fig. 7(b) suggests that LPS treatment does not influence the A549 cell survival and morphology. The IL-8 and TNF-α expression levels are further evaluated by analysing the fluorescent intensity of the captured images as shown in Fig. 7(c). The fluorescent images show the antibodies are successfully fixed on the PDMS surfaces by the aforementioned surface modification. In addition, the images suggest the fluorescence intensities in the wells for IL-8 and TNF-α cytokine measurements of the LPS treated cells are higher than that of the untreated cells.

In order to quantitatively study the protein expression level differences, the average fluorescence intensities across the entire captured images are calculated. Also, statistical analyses, unpaired, two-tailed Student's t-test, are performed to study the differences between the samples as shown in Fig. 7(d). The results suggest that the LPS stimulated A549 cells secret about two times more IL-8 comparing to the untreated A549 cells ($p \leq 0.01$). In contrast, the stimulated cells express slightly more TNF-α (~ 16%) than the untreated one; however, there is no statistical difference between two cases. The promotion of IL-8 and TNF-α expression due to the LPS stimulation has been discussed in the previous literatures, in which suggest that IL-8 and TNF-α secretions range in pM scale[28]. In the experiments, an inverted fluorescence microscope equipped with a CCD camera is exploited for the fluorescence intensity quantification. The setup is sensitive to the ambient light comparing to the sophisticated well plate reader. Therefore, the intensity measurement results may not be as precise as described in the existing literature[28].

During the cytokine detection assay, the device is rearranged for four times through sliding the PDMS layers. During the operation, no obvious abrasion or leakage is observed, and the measurement results demonstrate the SlipChip can be well functioned without failure. Furthermore, our developed PDMS SlipChip combines cell culture and subsequent *in situ* cytokine detection assays on the same chip, which reduce the steps for sample handling and transportation from wells to wells. Therefore, sample contamination possibilities can be minimized using the SlipChip, which helps to reduce the amount of samples required for experiments. Moreover, with microfluidic channel design, a large number of cytokines can be screened simultaneously, which is highly desired for various biomedical studies.

Conclusion

This paper reports a PDMS SlipChip capable of performing mammalian cell culture and multiple treatments and analysis within single devices without sophisticated pumps, valves, and interconnections for flow control. The free-interface diffusion is main mechanism for transporting substances to the targeted samples. The re-arrangeable channel patterns make various on-chip operations feasible, and the numerical simulation results suggest that the diffusion is fast enough to supply nutrients and cytokines for cell cultured in the SlipChip. In the experiments, the epithelial and fibroblast cells (A549 and MRC-5) are cultured in the device, and the results show the excellent biocompatibility and low cytotoxicity of the developed PDMS SlipChip, indicating the device is suitable for mammalian cell culture applications. Furthermore, the murine embryonic stem cells (ES-D3) are also tested in the device, and the stemness of the cells can be highly maintained after cultured in the SlipChip, which makes the device promising for potential stem cell research. In addition, multiple consequent experiments can be easily performed within the same SlipChip, including: staining, co-culture and cytokine detection assays demonstrated in the experiments. The experimental results show that the cell culture and consequent analysis within the device can be operated without tedious interconnections, and sophisticated pumps and valves. Consequently, the PDMS SlipChip can be extensively utilized in biological labs with minimal instrumentation and training. In conclusion, the versatile PDMS SlipChip not only provides great potential applications for *in vitro* cell biology study and biomedical sciences and but also pave a way to advance microfluidic research.

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References

1. Y. Sapir, O. Kryukov and S. Cohen, *Biomaterials*, 2011, 32, 1838-1847.
2. M.-C. Liu, H.-C. Shih, J.-G. Wu, T.-W. Weng, C.-Y. Wu, J.-C. Lu and Y.-C. Tung, *Lab on a Chip*, 2013, 13, 1743-1753.

3. C.-C. Peng, W.-H. Liao, Y.-H. Chen, C.-Y. Wu and Y.-C. Tung, *Lab on a Chip*, 2013, 13, 3239-3245.
4. I. K. Zervantonakis, S. K. Hughes-Alford, J. L. Charest, J. S. Condeelis, F. B. Gertler and R. D. Kamm, *Proceedings of the National Academy of Sciences*, 2012, 109, 13515-13520.
5. M. B. Chen, J. A. Whisler, J. S. Jeon and R. D. Kamm, *Integrative Biology*, 2013, 5, 1262-1271.
6. K. Y. Baik, S. Y. Park, K. Heo, K.-B. Lee and S. Hong, *Small*, 2011, 7, 741-745.
7. C.-W. Chang and M.-J. Wang, *ACS Sustainable Chemistry & Engineering*, 2013, 1, 1129-1134.
8. C. J. Bettinger, J. P. Bruggeman, A. Misra, J. T. Borenstein and R. Langer, *Biomaterials*, 2009, 30, 3050-3057.
9. D. W. Lin, C. J. Bettinger, J. P. Ferreira, C. L. Wang and Z. Bao, *ACS Nano*, 2011, 5, 10026-10032.
10. C.-W. Chang, Y.-J. Cheng, M. Tu, Y.-H. Chen, C.-C. Peng, W.-H. Liao and Y.-C. Tung, *Lab on a Chip*, 2014, DOI: 10.1039/C4LC00732H.
11. Y.-A. Chen, A. D. King, H.-C. Shih, C.-C. Peng, C.-Y. Wu, W.-H. Liao and Y.-C. Tung, *Lab on a Chip*, 2011, 11, 3626-3633.
12. Y.-H. Chen, C.-C. Peng, Y.-J. Cheng, J.-G. Wu and Y.-C. Tung, *Biomicrofluidics*, 2013, 7, 064104.
13. L. J. Millet, M. E. Stewart, R. G. Nuzzo and M. U. Gillette, *Lab on a Chip*, 2010, 10, 1525-1535.
14. M. Hosokawa, T. Hayashi, T. Mori, T. Yoshino, S. Nakasono and T. Matsunaga, *Analytical Chemistry*, 2011, 83, 3648-3654.
15. X. Shi, S. Ostrovidov, Y. Shu, X. Liang, K. Nakajima, H. Wu and A. Khademhosseini, *Langmuir*, 2013, 30, 832-838.
16. W. Du, L. Li, K. P. Nichols and R. F. Ismagilov, *Lab on a Chip*, 2009, 9, 2286-2292.
17. F. Shen, W. Du, E. K. Davydova, M. A. Karymov, J. Pandey and R. F. Ismagilov, *Analytical Chemistry*, 2010, 82, 4606-4612.
18. L. Li, W. Du and R. F. Ismagilov, *Journal of the American Chemical Society*, 2009, 132, 112-119.
19. S. Begolo, F. Shen and R. F. Ismagilov, *Lab on a Chip*, 2013, 13, 4331-4342.
20. F. Shen, W. Du, J. E. Kreutz, A. Fok and R. F. Ismagilov, *Lab on a Chip*, 2010, 10, 2666-2672.
21. C. Shen, P. Xu, Z. Huang, D. Cai, S. Liu and W. Du, *Lab on a Chip*, 2014, DOI: 10.1039/C4LC00213J.
22. L. Ma, S. S. Datta, M. A. Karymov, Q. Pan, S. Begolo, and R. F. Ismagilov, *Integr Biol*, 2014, 6, 796-805.
23. Y. Xia and G. M. Whitesides, *Annual Review of Materials Science*, 1998, 28, 153-184.
24. T.-H. Hsu, J.-L. Xiao, Y.-W. Tsao, Y.-L. Kao, S.-H. Huang, W.-Y. Liao and C.-H. Lee, *Lab on a Chip*, 2011, 11, 1808-1814.
25. T. Wang, M. Zhang, D. D. Dreher and Y. Zeng, *Lab on a Chip*, 2013, 13, 4190-4197.
26. S. Sun, M. Yang, Y. Kostov and A. Rasooly, *Lab on a Chip*, 2010, 10, 2093-2100.
27. J.-C. Lu, W.-H. Liao and Y.-C. Tung, *J Micromech Microeng*, 2012, 22, 075006.
28. K. Zhang, P. Wang, S. Huang, X. Wang, T. Li, Y. Jin, M. Hehir and C. Xu, *Mol Biol Rep*, 2014, 41, 4253-4259.

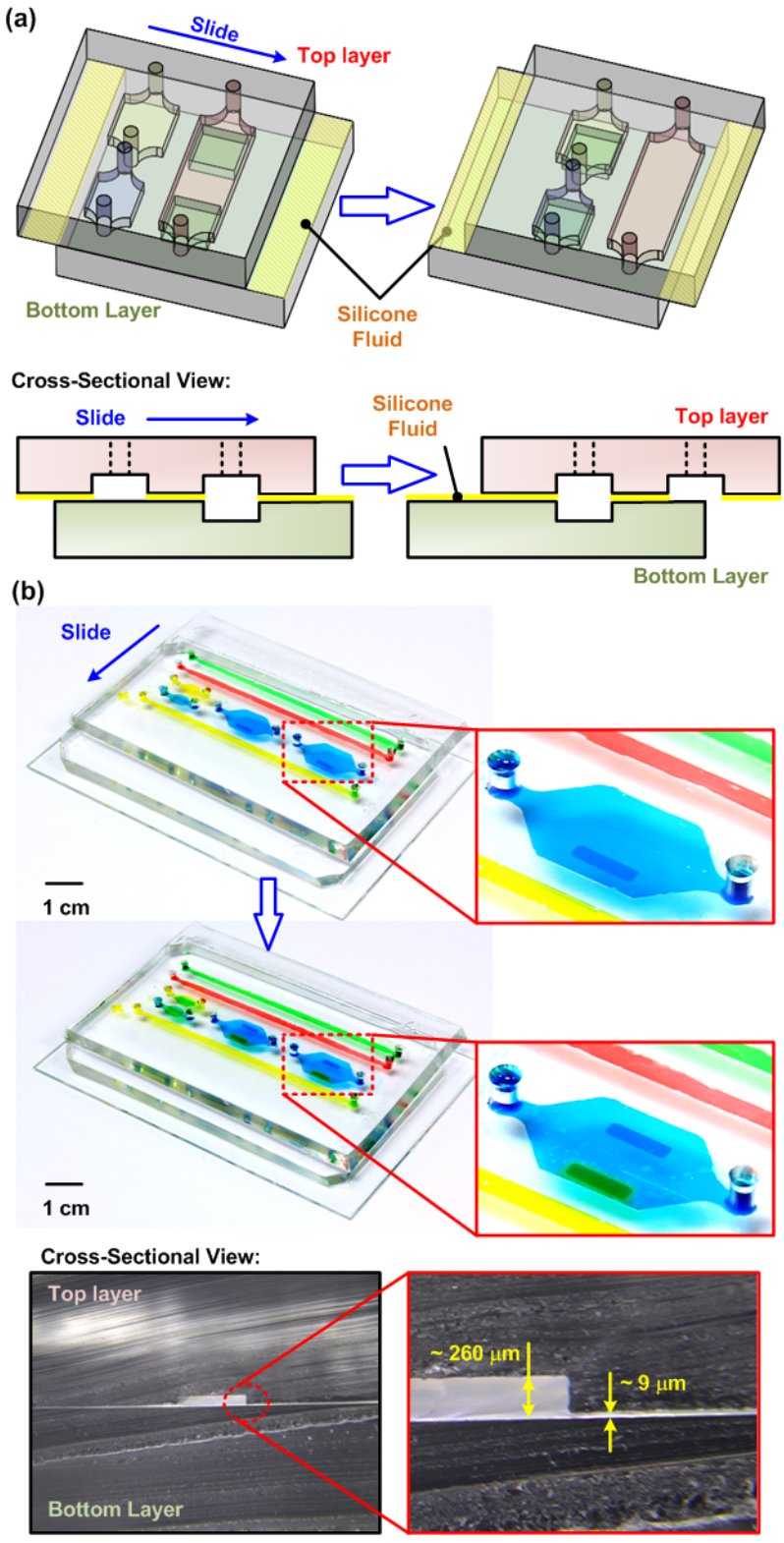

Fig. 1. (a) Schematic and operation of the PDMS SlipChip. (b) Photos of the fabricated PDMS SlipChip with different microfluidic channel arrangements after sliding. The cross-sectional microscopic images show the thickness of the sandwiched Si-Fluid layer is approximately 9 μm.

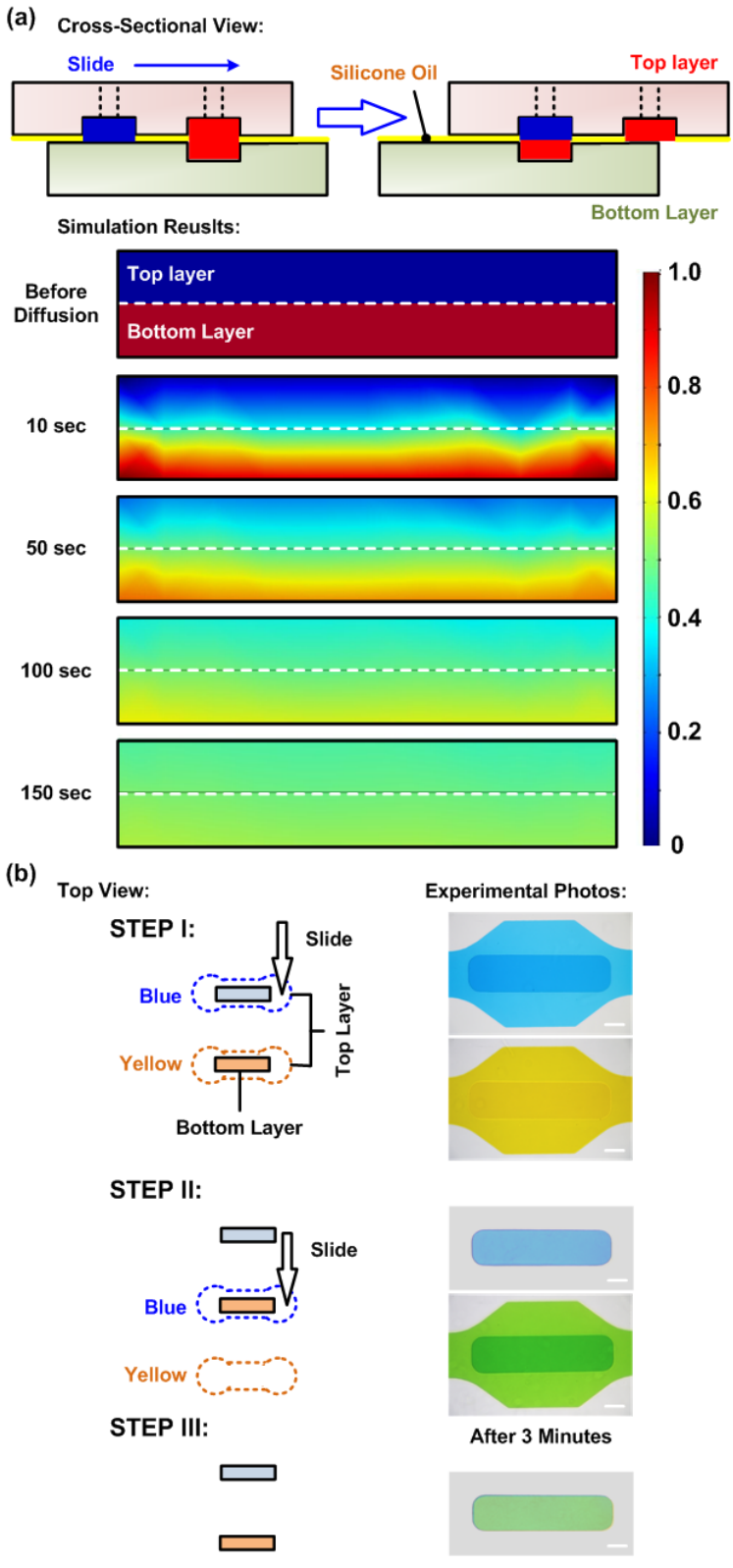

Fig. 2. (a) Schematic and the numerical simulation results of the diffusion between the wells located on the top and bottom layers after contact. (b) The experimental photos of the diffusion between the wells after sliding the top layer for contact. Scale bars are 500 μm

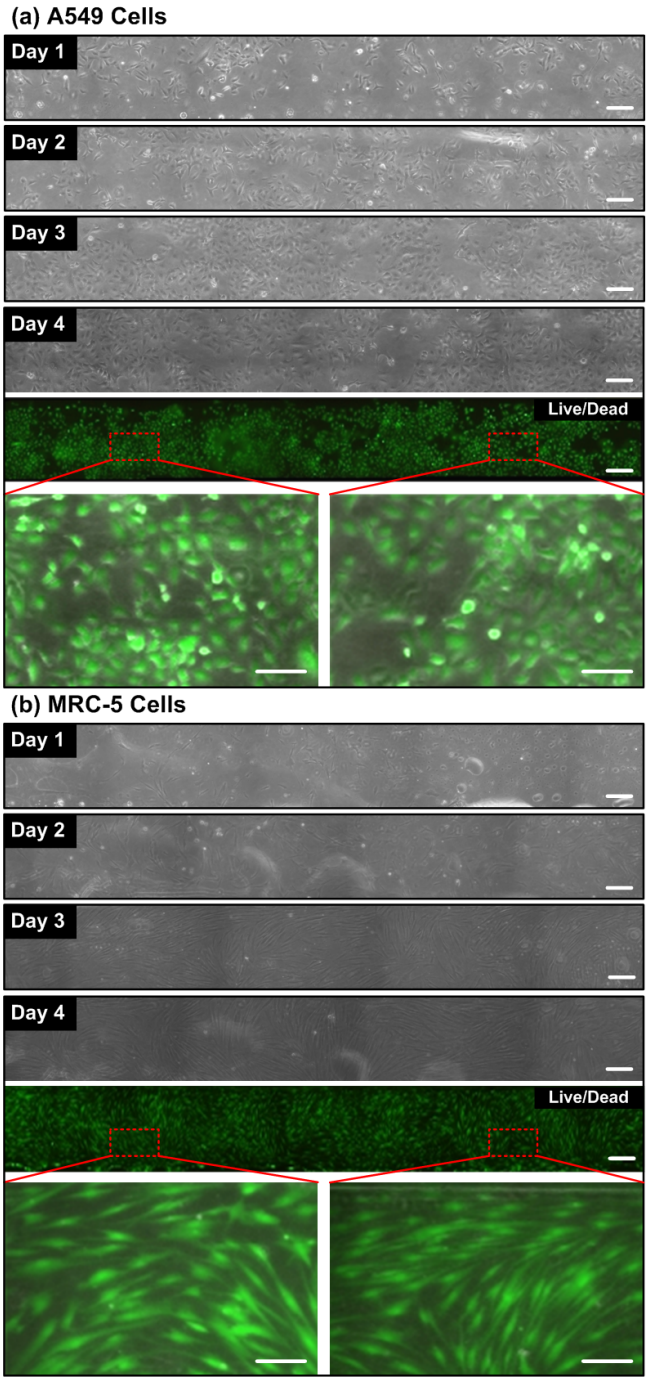

Fig. 3. (a) Bright field images of A549 cells cultured in a PDMS SlipChip from day 1 to 4. Fluorescence live (green)/dead (red) image of the cultured cells on day 4, and bright field and fluorescence overlaid images of the cells within different areas. (b) Bright field phase images of MRC-5 cells cultured in a PDMS SlipChip from day 1 to 4. Fluorescence live (green)/dead (red) image of the cultured cells on day 4, and bright field and fluorescence overlaid images of the cells within different areas. Scale bars are 100 μm. The zoomed-in brightfield phase images of the cells are shown in Supporting Information (Fig. S1).

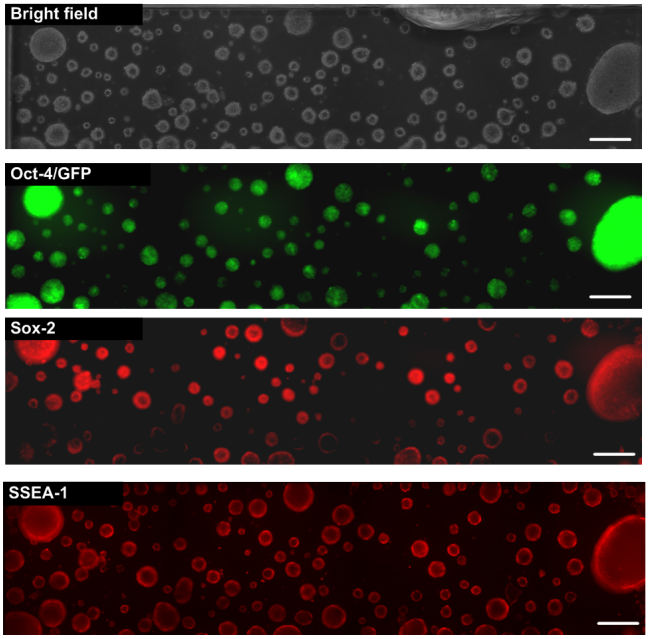

Fig. 4. Bright field and fluorescence (Oct-4/GFP, Sox-2, and SSEA-1) images of ES-D3 cells after two-day culture in the PDMS SlipChip. Scale bars are 200 μm.

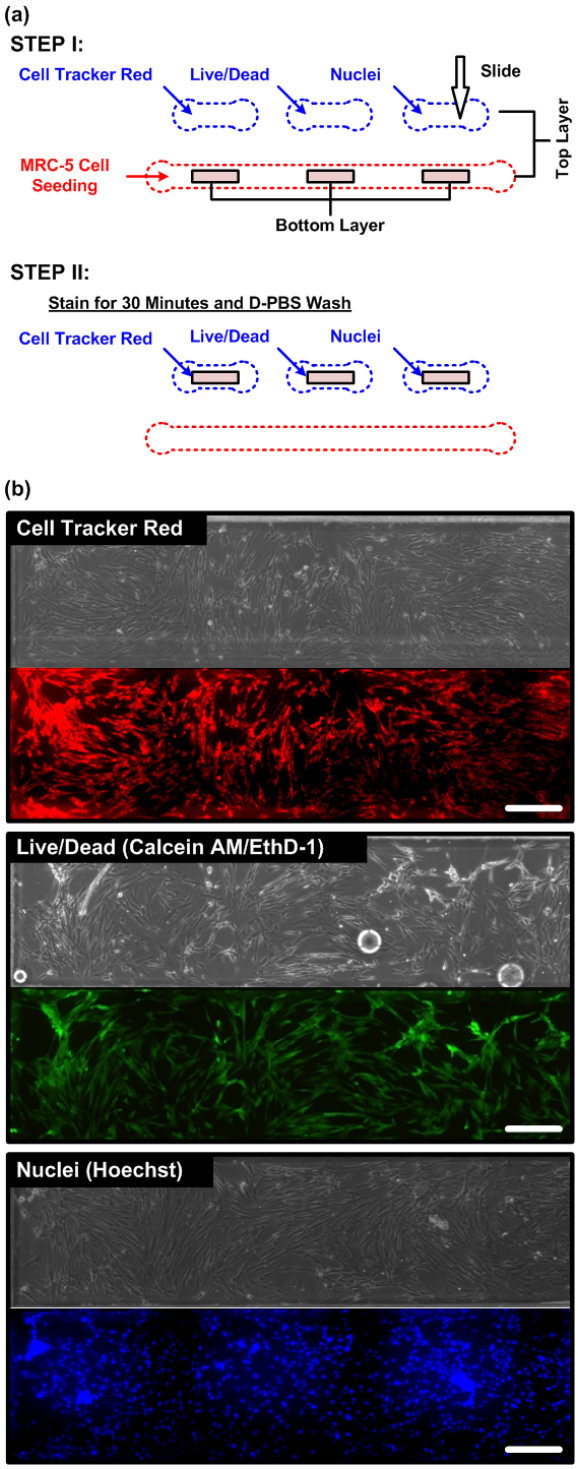

Fig. 5. (a) Operation procedure and the device layout for multiple treatment assays using the PDMS SlipChip. (b) MRC-5 cells located in three different wells are simultaneously stained by Cell Tracker Red, Live/Dead (Calcein AM/EthD-1) and nuclei (Hoechst), respectively. Scale bars are 200 μm. The zoomed-in brightfield phase images of the cells are shown in Supporting Information (Fig. S3).

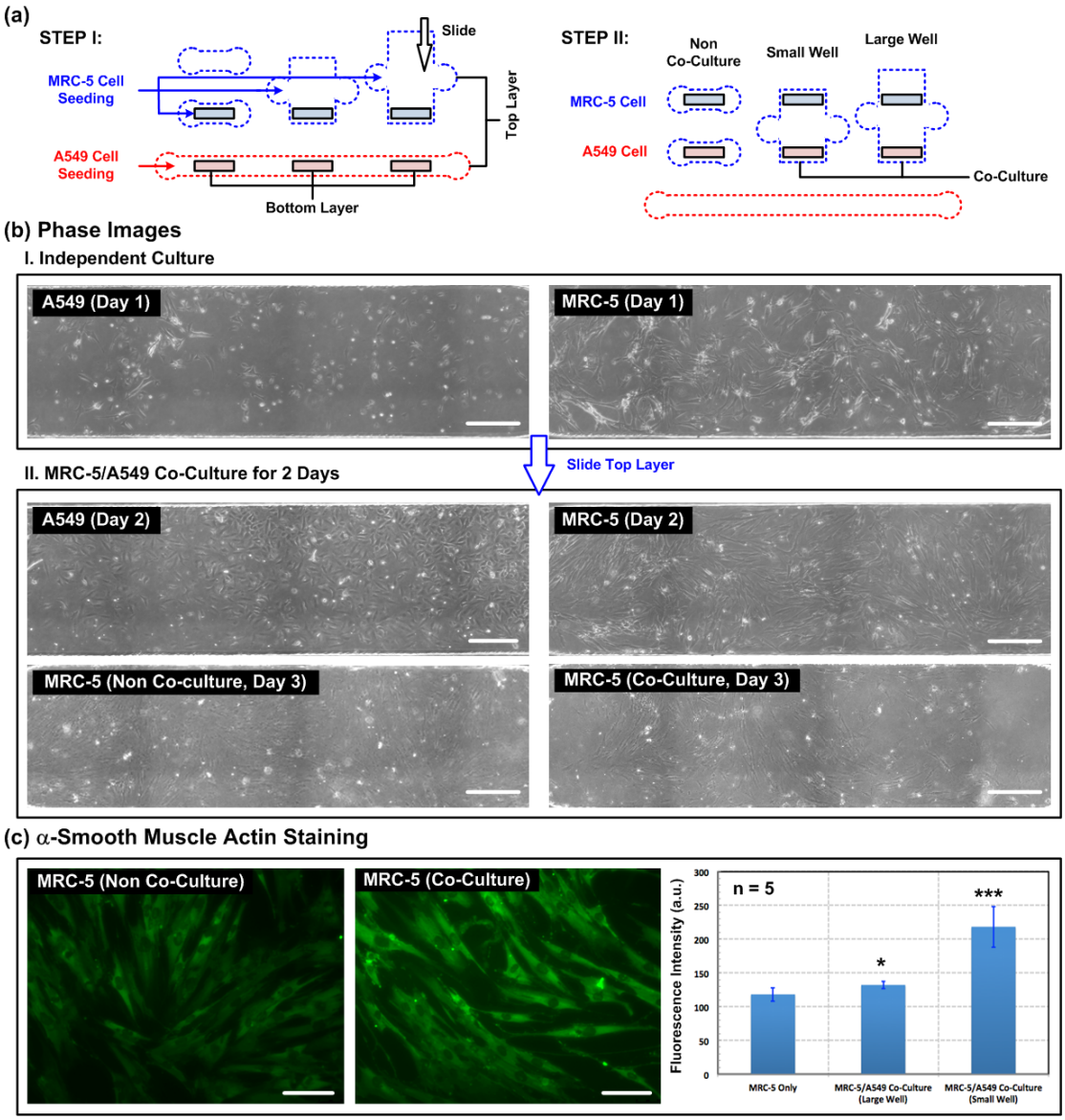

Fig. 6. (a) Operation procedure and the device layout for cell co-culture experiments using the PDMS SlipChip. (b) Bright field phase images of the A549 and MRC-5 cells before and after co-culture. Scale bars are 250 μm. (c) Fluorescence images of the MRC-5 stained with α-SMA with and without co-cultured with A549 cells. Scale bars are 100μm. The comparison and statistical analysis of the experimentally measured α-SMA fluorescence intensities. The data are expressed as the mean ± sd (n = 5). The zoomed-in brightfield phase images of the MRC-5 cells are shown in Supporting Information (Fig. S4).

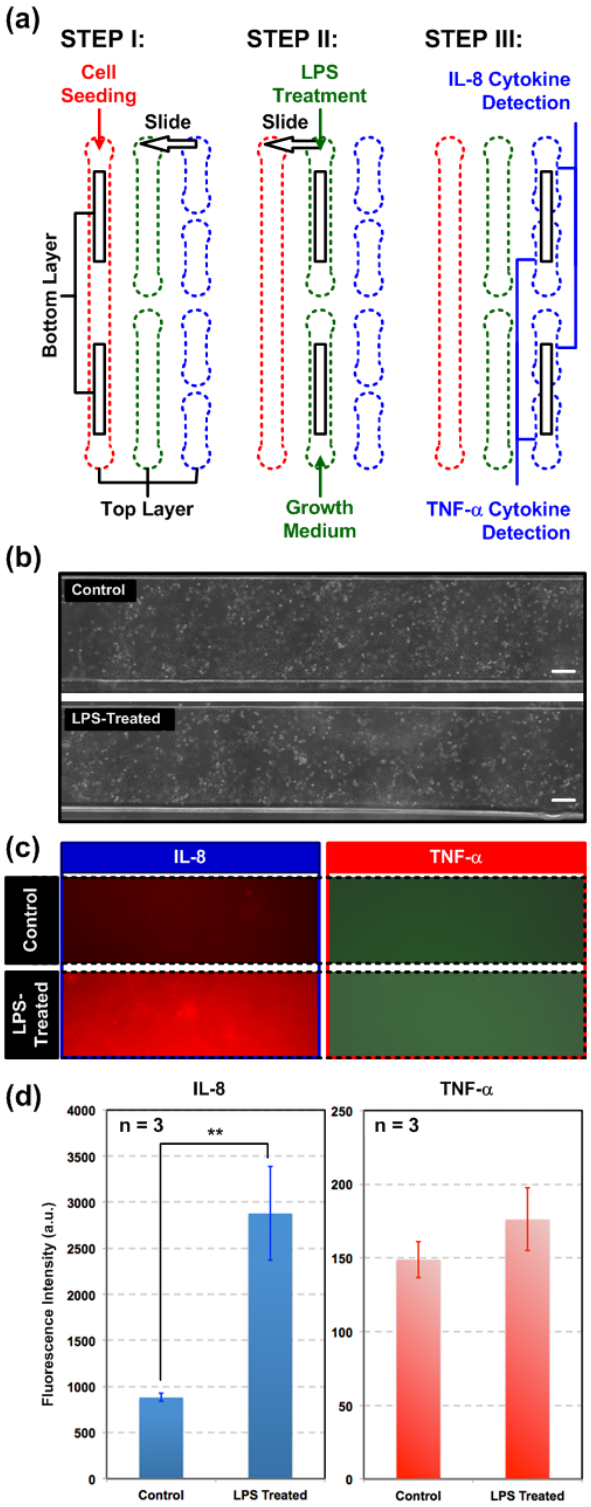

Fig. 7. (a) Operation procedure and the device layout for cytokine detection assay experiments using the PDMS SlipChip. (b) Bright field phase images of the untreated and LPS-treated A549 cells within a PDMS SlipChip. Scale bars are 200 μm. (c) Fluorescence images of the wells for IL-8 and TNF-α cytokine detection. (d) The comparison and statistical analysis of the experimentally measured IL-8 and TNF-α fluorescence intensities. The data are expressed as the mean ± sd (n = 3). The zoomed-in brightfield phase images of the cells are shown in Supporting Information (Fig. S5).