Supporting Data for:

A simple PDMS-based microfluidic channel design that removes bubbles for long-term on-chip culture of mammalian cells

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Supplementary Data

Fig. S1. In situ time-lapse imaging of -bubble-induced cytotoxicity. A) T1 shows the intact rMSCs cultured in microfluidic channel in the presence of a fluid flow. T2 and T3 show that a big bubble flowed over the cells. T4 shows that all the well-adhered cells were washed away. The white arrows in T1 and T2 point to the well-adhered cells before and at the early stage of bubble appearance. B) T1 shows the beginning of fluid flow in another rMSCs culture channel. T2 shows that a big bubble was flowing over the cells. T3 shows the rough membrane of the cells immediately after the departure of the bubble. T4 shows the contraction of the cells induced by the bubble. Black arrows indicate the directions of the fluid flow.
Fig. S2. Schematic diagram of the *in situ* time-lapse imaging and cell culture system. Some part of bypass tubing and bypass channel are represented as dotted lines (red). The liquid level is represented as dotted line (blue).
Fig. S3. A) 24-h in situ time-lapse images of MC 3T3 E1 cells at the corners of the curved channel in the presence of fluid flow shear (3 μL/min). B) 6-h in situ time-lapse images of MC 3T3 E1 cells at the corners of the curved channel in the presence of fluid flow shear (70 μL/min). The curved arrows indicate the directions of the fluid flow at every corner of the channel.

Text S1. PDMS-on-Glass Microfluidic Channel Chip Fabrication

The master for microfluidic channel molding was produced by precise mechanical process on a nylon wafer with sunken channels (Beijing TongRui Science and Technology Co. Ltd., China). The wafer were poured with PDMS and allowed to be cured for 1 h at 80°C. The replicas of PDMS were peeled off the nylon master and served as a template with protruding features for the second molding of PDMS replicas with channels. Before the use of the PDMS template, 1H, 1H, 2H, 2H-perfluorodecyltriethoxysilane was deposited on it by vacuum evaporation. The PDMS template was poured with uncured PDMS and cured at 80°C for 1 hour. The replica of PDMS was peeled off the PDMS template and punched holes for the inlets and outlets of the microfluidic channel. The PDMS mold with channels and a piece of clean cover glass were exposed to an air plasma for 5 minutes and sealed irreversibly together to form a closed channel between the glass and PDMS mold. The inlet and outlet of the PDMS channels were connected to polyethylene (PE) tubes. The microfluidic channel were then treated with air plasma again and sterilized with 75 % ethanol (V/V) before use.

Text S2. In situ Time-lapse Imaging of Dynamic Behavior of Cells in the Channel

To realize real-time imaging of cell behaviors in the presence of fluid flow shear, an in situ cell culture and observation system was constructed based mainly on a microfluidic channel, a Leica fluorescence microscopy, a peristaltic pump, and a medium reservoir and collector. As shown in figure S2, the microfluidic channel containing cells were mounted onto the stage of the microscopy equipped with 5 %
CO₂ generator and heating plate. The inlet and outlet of the channels were connected to PE tubing which coupled with peristaltic pump and cell culture medium reservoir and collector respectively. The microfluidic channel was tightly fixed to the stage to avoid shift of the sample during image recording. The fluid flow shear was produced by a peristaltic pump. Since PDMS is gas-permeable, the microfluidic channels fixed in the 5 % CO₂ generator provide adequate gas environment for cell culture. Dynamic behaviors of cells under fluid flow shear were recorded by phase contrast microscopy at 1-min intervals. The green and blue fluorescence images were collected via corresponding fluorescence channels respectively and analysed by Leica Application Suite software package.

**Text S3. Comprehensive Measures Preventing Bubble Generation**

There are a range of factors including medium temperature and components, hydrophilicity/ hydrophobicity of the channels, and operation processes contributing to the generation of bubbles in the microfluidic system.

**S 3.1. System Temperature**

Given the small thermal mass and the large surface area/volume ratio of microfluidic systems, temperature is a particularly important consideration for microfluidic cell culture devices. In particular, the temperature of medium is an important factor affecting air bubble generation. Gases tend to dissolve in liquid at relatively low temperature. If the medium temperature is relatively low before the perfusion of the medium into the channel, the pre-dissolved gases molecule in the medium will escape to form macroscopic bubbles in the channel. Therefore, pre-heating the cell suspending medium to 37 °C and keeping the same temperature of the channels during the cell seeding operation are necessary. Besides, the channel, medium reservoir and collector, and tubing should be kept at 37°C during the whole operation time whether for microscopic imaging or cell culture in incubator.

**S 3.2. Surface Properties of PDMS Channel**
Microfluidic channels with hydrophilic surfaces are ideal devices for experiments using water as a fluid, on the contrary, hydrophobic microfluidic channels nucleate air bubbles easily and make it difficult to get rid of air bubbles in the channels.\textsuperscript{1} PDMS has repeating units of $-\text{OSi(CH}_3)_2-$ groups which lead to a hydrophobic surface ($\theta_{\text{H}_2\text{O}} = 108^\circ$).\textsuperscript{2} Exposing this surface to an air or oxygen plasma introduces silanol (Si-OH) groups and makes the surface hydrophilic. PDMS that has been treated with plasma can be kept hydrophilic indefinitely by keeping the surfaces in contact with water or polar organic solvents. In our experiments, usually, the plasma-treated channels are filled with 75 % alcohols and sealed with parafilm to maintain the hydrophilicity of the surface. We also tried the extraction of un-cross-linked PDMS contaminants from the bulk polymer using organic solvents (e.g. diisopropylamine) to decreases the rate of regeneration of the hydrophobic surface.\textsuperscript{3}

\textbf{S 3.3. Medium Composition}

The generation of gas bubbles is closely related to the components of the cell culture medium. In our experiments, bubbles usually appear in the channels containing medium with 15 % FBS as early as 15-20 minutes. However, when the FBS concentration dropped to 5 %, the channels can operate normally without any bubble for at least 1-1.5 hour. The adequate concentration of FBS is proposed to maintain the biochemical activity of the cells in the channel and the optimal bubble-free operation time of the system. Medium with 10 % FBS in the channel without special treatment can work for 1 hour without bubble interference. Our other experience is that $\alpha$-MEM is superior to Dulbecco's modified eagle medium (DMEM) in the bubble-free cell culture in microfluidic channels. The higher calcium ion and phosphate induced increase of hydrophilicity of the $\alpha$-MEM solution than that of DMEM may be the reason for this phenomenon.

\textbf{S 3.4. Driving Force}

There are two kinds of pumps, syringe pump and peristaltic pump, normally used as driving force for the fluid flow in the channel. Due to the limitation of volume and stepping rate, syringe pump is only suitable for short-term experiments. But, based on
the working mechanism of syringe pump, the medium is pushed out of the pump and unlikely to generate bubble. Peristaltic pump, which can provide continuous fluid flow for the microfluidic channel, is an appropriate method for both short and long term experiments. However, we found in our experiments that the relative position of the pump and the microfluidic channel may influence the bubble generation and discharge. If the pump is placed before the channel, like the syringe pump, the liquid in the tubing is pushed into the channel and is not prone to yield bubbles. On the contrary, if the pump is placed after the channel, the liquid is pumped and the dissolved gases tend to escape form the liquid and form bubbles. Moreover, in our experiments, if the peristaltic pump placed after the IBT-B, the gas will be pumped into the channel via the bypass filter and lead to the failure of the experiment.

In addition to aforementioned factors, other details such as avoiding external mechanical forces, degassing medium in vacuum before use, and checking possible bubbles at the joint between PE tubing and channels are all necessary for a long-term cell culture in microfluidic channel.

References