FLEXIBLE MICROFLUIDIC DEVICE WITH MICROPOROUS WALLS FOR PERFUSION CELL CULTURE

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Introduction: Conventional state-of-the-art in vitro cell culture models are insufficient in physiological relevance and not predictive of in vivo behavior in animal models and humans. On the contrary, microfluidics has the potential to create an interactive cell microenvironment that mimics the in vivo cell microenvironment because of its user-defined design, relevant length scale and small sample volume. With the recent research focusing on the mimicry of in vivo-like cell microenvironment, perfusion cell culture has demonstrated to be one of the promising solutions to achieve this goal. These perfusion devices were replicated by soft lithography which is based on molding of poly(dimethylsiloxane) (PDMS). However, PDMS absorbs biomolecules non-specifically; thus limiting its applications and compromising the accuracy of many cytotoxicity studies. On the other hand, polystyrene as the device material overcomes the limitation of non-specific absorption of biomolecules in PDMS-based perfusion microfluidic devices. In this study, we present a low-cost, flexible polystyrene-based microfluidic device with microporous walls for long term perfusion cell culture.

Materials and Methods: The device design was based on our previous PDMS-based perfusion microfluidic device design, which consisted of a cell culture channel centered between two side channels (Fig. 1 and 1b). Patterned three-dimensional (3D) interconnected microporous structures were designed around the channels as channel side walls. These microporous structures were created on a polystyrene film by a 20 s treatment using a tetrahydrofuran (THF)/isopropanol (IPA) solvent mixture (40/60 v/v %) at room temperature together with a protective mask as previously reported (Fig. 1c). Unlike soft lithography, a microstructured mold is not required and the whole patterning process only takes few minutes. Custom cut double-sided pressure sensitive adhesive (PSA) tape and a second polystyrene film with inlet and outlet holes were used to assemble the microfluidic devices. The complete process from design concept to working device can be completed quickly and inexpensively in a regular laboratory setting. Combination of simple masking technique and oxygen plasma treatment was used to create selected hydrophilic regions on the patterned microporous structures to control wettability for perfusing media from the two side channels through the hydrophilic microporous structures and into the cell culture channel.

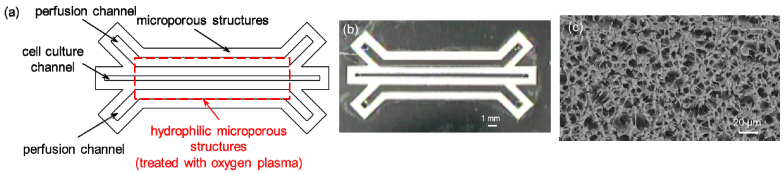

Figure 1. (a) Schematic diagram and (b) image of the flexible microporous polystyrene microfluidic device. (c) Scanning electron microscope (SEM) image of the microporous structures.

Results and Discussion: We first experimentally characterized the perfusion flow dynamics of the flexible microporous polystyrene microfluidic device to confirm the perfusion efficiency. Time lapse images of red colored food dye solution showed good perfusion performance of the device between the two side perfusion channels and the middle cell culture channel (Fig. 2a). We then demonstrate the support for four days perfusion cell culture of a hepatocyte-like cell line (C3A cells) in this flexible microporous polystyrene microfluidic device. The fluorescent images taken after the cell staining clearly indicated that cell viability was much better with the perfusion cell culture comparing to the static cell culture (Fig. 2b and 2c). These results demonstrated that the ability to perfuse media into the cell culture channel is essential for long term cell culture.

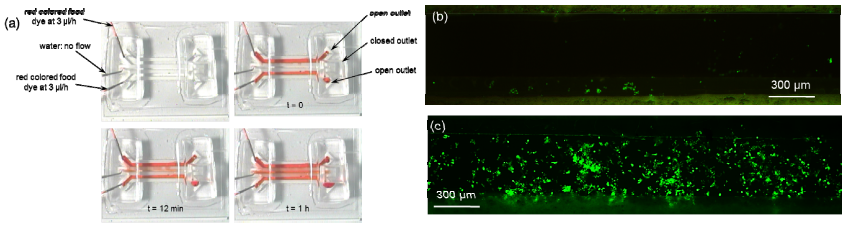

Figure 2. (a) Time lapse images during the perfusion flow characterization experiment. (b) and (c) Live (green) /dead (red) C3A cell staining after four days of cell culture in the flexible microporous polystyrene microfluidic device under (a) static and (b) perfusion cell culture conditions.

Conclusions: We demonstrated the support of four days cell culture of C3A cells in the flexible microporous polystyrene microfluidic device. The microporous structures can be selectively oxygen plasma treated to allow the control of hydrophilicity and hence the perfusion of fluid into the cell culture channel.