A THERMO-RESPONSIVE PNIPAAm-GRAFTED-PDMS SURFACE USED FOR CELL CULTURE IN MICROFLUIDIC CHANNELS

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ABSTRACT
A thermo-responsive PNIPAAm-grafted-PDMS (PNIPAAm-g-PDMS) surface was used for cell culture. Cos7 cells could adhere to the PNIPAAm-g-PDMS surface and proliferated therein at 37°C. The cultured cells could detach from the surface at room temperature without trypsin digestion. The viability of cells harvested from the PNIPAAm-g-PDMS surface via lowering temperature was higher than that of cells harvested from native PDMS surface via trypsin digestion. A PNIPAAm-g-PDMS microchannel was designed for the in-channel thermo-modulated cell culture/harvest. Cells could be long-term cultivated and be harvested without enzymatic treatment. Successful thermo-modulated cell passage operation was demonstrated in the microfluidic chips with the PNIPAAm-gPDMS channels.

KEYWORDS: Thermo-responsive surface, Microfluidic, Cell passage, PNIPAAm

INTRODUCTION
To perform a complete cell-based assay on a microchip, a series of cell handling operations are expected to be integrated on microfluidic chips [1]. Cell culture and harvest is obviously the most upstream operation unit for a completely integrated cell chip. However, among the recent published papers on manipulation and assay of cells with microfluidic chips, few deals with the operation for on-chip cell culture and harvest.

PNIPAAm is a thermal-responsive polymer that exhibits a reversible phase transition at around 32°C (known as the lower critical solution temperature, LCST) [2]. When the PNIPAAm chains are grafted onto a substrate surface, the surface can reversibly switch between hydrophilic (at a temperature below its LCST) and hydrophobic (at the temperature above the LCST) in response to environmental temperatures. The bulk PNIPAAm grafted silicon, glass, gold and polystyrene have been used for the cell culture/harvest [3].

The present paper reports a PDMS micro chip with channels whose surface was grafted with PNIPAAm for in-channel thermo-modulated cell culture, harvest and passage operations. Deferent form the conventional cell culture and harvest methods where trypsin is usually required to digest the extracellular matrix (ECM) for cell harvest, the developed protocol is based on the thermo-switchable hydrophobic/hydrophilic properties of the PNIPAAm-grafted-PDMS (PNIPAAm-g-PDMS) surface, therefore, excludes the trypsin digestion. Different from the reported works where bulk PNIPAAm grafted silicon, glass, gold and polystyrene have been used for cell culture/harvest in tissue engineering [3], the present work is focused on the investigation into the adhering, proliferating, and detaching behaviors of cells inside microfluidic channels made of PNIPAAm-g-PDMS with the consideration of the fact that PDMS is the most preferential materials for fabrication of microchips for cell assays.

EXPERIMENTAL
The microfluidic device used for cell culture was fabricated by PDMS (Sylgard 184, Dow Corning) using replicate molding and soft lithography. The channel-structured PMDS substrate and a flat PDMS film were then irreversibly bonded immediately after air plasma treatment, and two 3-mm thick square PDMS sheets with a 2-mm i.d through hole were irreversibly sealed to the two accessing holes of the channel serving as the culture medium reservoirs. The picture of the fabricated PDMS chip, together with the channel layout, is shown in Fig 1.

After sealing, the inner surface of the PDMS channel was grafted with PNIPAAm via benzophenone-initiated photopolymerization [4]. Briefly, the channel was first flushed by a acetone solution of benzophenone for 10 min, and washed afterward with water. After the microchannel was filled with the mixed monomer solution containing NIPAAm, NaIO4 and benzyl alcohol, the PDMS chip that was put on a heat sink on ice was irradiated by UV lights emitted from a high pressure mercury lamp for 25 min. Finally, the channel was flushed with acetone and deionized water, respectively. After grafting, the PNIPAAm modified channels were coated by gelatin.

Cells were cultivated in the PNIPAAm grafted microchannel under static condition with changing culture medium every 12h. Firstly, cell suspension with a density of approx. 4×10⁶ cells/ml was infused into a micro reservoir and suctioned into the channel by the synergetic action of capillary and hydrostatic forces. After the channel was full of cell suspension and the reservoirs were sealed with films, the chip was put in an incubator where temperature was maintained at 37°C. When the cells reached 80-90% confluence, the chip was moved out of the incubator and left at room temperature. After 10-15 min, the cultivated cells were detached from the bottom of the channel, and could be in-line transferred to a downstream chip, which was connected to the upstream chip via a fine tubing, with a gentle flow induced by a minor negative pressure.
Figure 1: The PDMS microfluidic chip used for cell-culture/harvest. (a) The photopicture of the chip. (b) The layout of the micro channel. (c) The cross section view of the chip.

RESULTS AND DISCUSSION

Without using trypsin-digestion, harvesting of cells from the PNIPAAm grafted surfaces via simply lowering temperature was gentle and harmless to cells. As shown in Figure 2a, the mean survival rate of the COS7 harvested from the PNIPAAm-g-PDMS surfaces by the present method was 89.1%, and that of COS7 harvested from the native PDMS surfaces by trypsin digestion was 80.6%. The viability of COS7 from the PNIPAAm-g-PDMS surfaces was significantly higher than that from the PDMS surfaces ($P=0.016$). This superiority was more outstanding when the developed method was used to harvest highly sensitive cells such as human mesenchymal stem cells (hMSCs) (see Figure 2b).

Figure 2: The survival rates of COS7 and hMSCs. (a) The COS7 cells cultivated on the native PDMS surfaces were harvested by 0.25% trypsin/EDTA treatment for 30 s, and that cultivated on PNIPAAm-g-PDMS surfaces were harvested by lowering of temperature. (b) The hMSCs cultured on the TCPS surfaces were harvested by 0.25% trypsin/EDTA treatment for 5 min, and that cultured on PNIPAAm-g-PDMS surfaces were harvested by lowering of temperature in combination of 0.1% trypsin/EDTA treatment for 1 min.

Figure 3: Morphology of the cells cultured in both PNIPAAm-g-PDMS microchannels (a)-(f) and native PDMS channels (a’)-(e’) during a cell passage operation. (a) and (a’): three days after the cells had been cultivated on the upstream channel; (b) and (b’): 15 min after (a) and (a’) were left at the room temperature; (c) and (c’): after the channel was flushed with a gentle flow; (d) and (d’): the cells transferred to the downstream channel; (e) and (e’): 2 days after the cells had been sub-cultivated on the downstream channel.

A twin chip connected with fine tubing was used for the on-chip cell harvest and passage operation. Figure 3 (a-e) shows the morphology of the cells cultivated with the PNIPAAm-g-PDMS twin chip during a cell passage operation. As a comparative, Figure 3 (a’-e’) shows the morphology of the cells cultivated with a native PDMS twin chip of the same
size and under the same conditions. After culture for 3 days, the cells in both types of channels reached confluence (see Figure 3a and Figure 3a'). Then, both chips were moved out of the incubator and left at room temperature. After 15 min, the cells in the PNIPAAm-g-PDMS channel detached from the channel bottom and broke into small clusters of different sizes (Figure 3b). The detached cells could be transferred to the downstream chip by a gentle flow (Figure 3c and Figure 3d). Moreover, the transferred cells were able to spread again and proliferated in the channel of the downstream chip (Figure 3e). In contrast, the cells cultured in the native PDMS channel still spread perfectly on the surface of the upstream channel after leaving at room temperature for 15 min (Figure 3b'), consequently, they could not be transferred to downstream channel for sub-cultivation (see Figure 3c', Figure 3d' and Figure 3e').

CONCLUSION
COS7 cells cultivated in the PNIPAAm-g-PDMS channel could be harvested by lowering temperature to about 20°C without need of trypsin digestion. The harvested cells have a relatively high viability and are capable of sub-cultivation. Because trypsin was not involved in the cell harvesting process, the integration of a series of such operations as thermomodulated-cell culture, harvest and passage on the upstream of microfluidic channel network would not make troubles for downstream cell assay operations.

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REFERENCES

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