Studies of Shear Stress-dependent Cell Detachment from Temperature-responsive Cell Culture Surfaces by Using Microfluidic Devices

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Statement of Purpose: We have proposed cell sheet engineering as a novel approach of tissue engineering field, using poly(*N*-isopropylacrylamide) (PIPAAm) gel modified tissue culture polystyrene (PIPAAm-TCPS).[1] At 37°C, cells were well adhered and spread on PIPAAm-TCPS, and grow confluently. By lowering temperature to 20°C, cells detached from hydrophilic PIPAAm surface spontaneously and formed a single contiguous sheet. In our previous studies, the surface properties were evaluated by contact angle, FT-IR/ATR, XPS, AFM, cell attachment and detachment assay, and fluorescent labeling. However, these techniques focused on either the surface properties or cell attachment and detachment behavior. Cell-material interactions were not quantitatively estimated by these techniques. In order to quantitatively evaluate cell detachment process from PIPAAm-TCPS, microfluidic chamber made of PDMS was constructed on the temperautre-responsive cell culture surfaces and the laminar flow within the chamber generated shear forces applied to the cells. Shear stress-dependent cell detachment from the surface was studied with different shear stress varying by resistance of each channel. This approach provided the basis for the theoretical analysis of interaction between cells and PIPAAm layer.

Methods: Microfluidic device consisted of PDMS chip sealed on PIPAAm-TCPS was constructed using the modified liquid-crystal-display projector (LCDP) apparatus.[2] The microfluidic device had one inlet and five outlets with five parallel test channels (400 μm in width, 50 μm in depth) (Figure 1). By changing the length of test channel, the flow rate of each channel was designed as 3.0 : 2.5 : 2.0 : 1.5 : 1.0 from Outlet A to Outlet E. Bovine aortic endothelial cells (BAECs) were seeded in each microchannel from Outlet C. After 24h incubation at 37°C, the microfluidic device was moved on a cold plate (20°C). The cold medium (20°C) was introduced into the microfluidic device, while the flow fate at Inlet was 2.0 ml/h. Cell morphology in detachment process was monitored by CCD camera for 1h. Cell numbers at various time points in each observation area were counted on printed capturer frames from video file. The critical shear force of cell detachment was obtained by comparing the shear stress calculated from flow rate and the average cell detachment time in each microchannel.

Results: Using the modified LCDP apparatus, the width and depth of obtained test microchannel were 410-412 μm and 51.5-55.0 μm, respectively. The resultant microfluidic device from Outlet A to Outlet E worked as designed, while the ratio of measured flow rates was 3.1 : 2.3 : 2.1 : 1.4 : 1.1. After incubation for 24h at 37°C, BAECs adhered and spread on the substrate of microchannels. Moreover, the proliferation of cells was observed in microchannels by further cell culture period, indicating that the fabrication of microchannel did not weaken the physiological properties of BAECs. By reducing the temperature to 20°C, BAECs shrunk, and changed their shape from flat to round without flow. These results was almost the same as that in general PIPAAm-TCPS, strongly suggesting the fabrication of microfluidic device on PIPAAm-TCPS did not deteriorate temperature-responsive properties of PIPAAm layer. After 24h culture, by flowing the cold medium through the microchannels, BAECs were peeled off from the substrates. The time-dependents of cell detachment from each channel were shown in Figure 1. Cells in microchannel A detached from the substrate most quickly, since the shear stress in microchannel A was stronger than those in other microchannels. These results strongly suggested the rate of cell detachment depend on the shear stress in microchannel. The higher flow rate caused a quick cell detachment. The average detachment time from microchannel A to E were 11.7 min, 14.3 min, 15.3 min, 20.3 min, and 21.8 min, respectively.

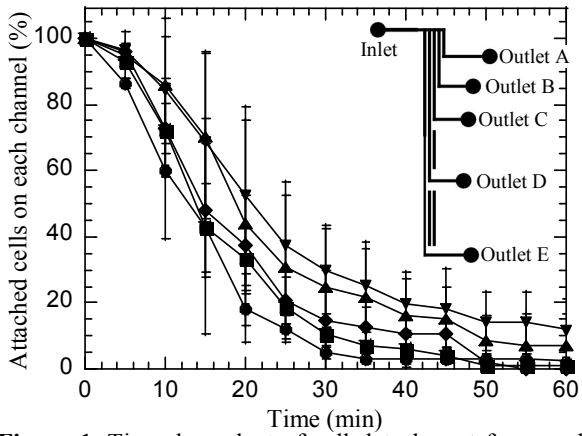

Figure 1. Time-dependent of cell detachment from each channel at 20°C. (●: Channel A, ■: Channel B, ◆: Channel C, ▲: Channel D, ▼: Channel E.) The schematic diagram of microfluidic device is shown in the upper right corner.

Conclusions: The microfluidic device was fabricated on temperature-responsive cell culture surface successfully. Cells adhesion behavior in microchannels was equivalent to those on general PIPAAm-TCPS at 37°C. When reducing the temperature to 20°C and starting the flowing, BAECs detached from the substrates of microchannels. The cells detachment behaviors were quantified by shear stress. The unique microfluidic device could be estimated various cell-PIPAAm layer interaction.

References:

1. Okano T. J Biomed Mater Res. 1993; 27: 1243-51.
2. Itoga K. Biomaterials. 2004; 25: 2047-2053.