Biofunctional Thermo-Responsive Polymeric Surface with Micropatterns for Controlling Cell Attachment and Detachment

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Statement of Purpose: Thermo-responsive polymeric surfaces can allow adhered cells and cell sheet to be spontaneously recovered by lowering temperature without proteolytic enzymes. At 37 ºC, thermo-responsive poly(*N*-isopropylacrylamide) (PIPAAm)-grafted surface is slightly hydrophobic, allowing cells to proliferate under normal conditions. A decrease in temperature below 32 ºC, however, results in the rapid hydration of the polymer surface, leading to the spontaneous detachment of the cells as a single cell and/or a uniform tissue sheets. Using cell sheet engineering we can altogether avoid to use scaffolds, and to fix and suture them for conventional tissue engineering approaches using isolated cell injections and scaffold based technologies whose applicability is often limited. The direct transplantations have been applied to corneal epithelia, periodontal ligament cells, myoblast cells, and oesophageal epithelia. In this study, hydrophilic micro-patterns were immobilized on a thermo-responsive surface for controlling cell adhesion and proliferation as a next generation of cell sheet engineering.

Methods: Patterned thermo-responsive surface was fabricated as follows: *g*-line positive photoresist was spin-coated on thermo-responsive culture dishes (UpCell®) and photoirradiation was performed by a visible light through a quartz mask (pattern size: 5 μm black/white repeated pattern), followed by the development using a developer solvent. Twenty-five wt% acrylamide aqueous solution was added on the photoresist-patterned culture dish, followed by radical polymerization on the developed position for 2 h. The following two studies using the patterned surface will be presented: (1) an acceleration of cell sheet recovery, and (2) cell separation. In the first study, 3T3s with 1.0×10^5 cells/cm^2 densities were seeded on the patterned surface in DMEM supplemented with 10% FBS at 37 ºC for 24 h. Then, the confluent 3T3 cell sheets were recovered by lowering temperature at 20 ºC. In the second study, mixture of 3T3, human umbilical vein endothelial cells (HUVEC), and HeLa cells were harvested on the patterned surface in the DMEM at 37 ºC. Then the three kinds of cells were recovered by the exchange media or lowering temperature (Figure 1).

Results: In the first research, 3T3s adhered and proliferated at 37 ºC until confluent cell sheet on the patterned surface as well as a conventional thermo-responsive surface. The rate of 3T3 cell sheet detachment on the patterned surface was approximately five times accelerated than that on the conventional thermo-responsive surface, indicating that hydrophilic patterns supported the spontaneous detachment of cell sheet. In the second research, 3T3, HUVEC, and HeLa cells were used for controlling the cell attachment and detachment. Adhered 3T3 cells spontaneously detached themselves on the patterned surface after reducing temperature as mentioned above. On the other hand, HUVECs adhered on the patterned surface until 12 h incubation at 37 ºC, but the adhered HUVECs detached themselves through the next 12 h incubation at 37 ºC. In addition, the attachment of HeLa cells was hardly observed on the patterned surface. In Figure 2, HeLa cells were mainly detected in the cell suspension (cell suspension, Figure 1A) and HUVEC were detected in the cell suspension (cell suspension, Figure 1B). In addition, 3T3 were recovered by lowering temperature and the ratio of 3T3 was found to be approximately 90% (cell suspension, Figure 1C), meaning that three kinds of cells were able to be separated without any labels.

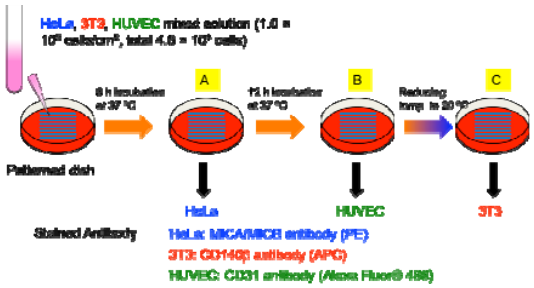

Figure 1 Illustration of cell separation on the patterned thermoresponsive surface. The mixture of 3T3, HUVEC, and HeLa cells were harvested on the patterned surface at 37 ºC. The media was exchanged at 12 (A) and 24 h (B), and then the cells were recovered by lowering temperature at 20 ºC (C).

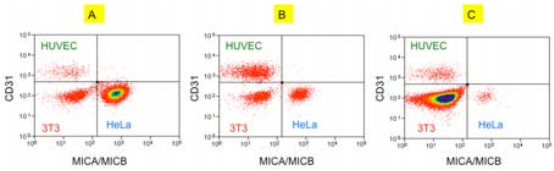

Figure 2. Density plots of separated cells by flow cytometry. (A), (B), and (C) indicate the cell suspensions obtained in the process of Figure 1A, B and C.

Conclusions: Patterned thermo-responsive surface was produced for rapid cell sheet recovery and cell separation. In conclusion, the patterned thermo-responsive surface has a potential for breakthrough in the next generation of biomaterials.

References:

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