Precisely Designed Thermo-responsive Polymer Brush Surface for Cell Separation

Kenichi Nagase,[1] Ayaka Kimura,[2] Yuri Hatakeyama,[2] Tatsuya Shimizu,[1] Katsuhisa Matsuura,[1] Masayuki Yamato,[1] Naoya Takeda,[2] Teruo Okano[1]

1. Tokyo Women's Medical University, Tokyo, Japan, 2. Waseda University, Tokyo, Japan

Introduction: With progress in biomedical technologies, a strong demand for cell separation methods has been increased for various applications. Especially, in the fields of regenerative medicine, an effective cell separation technology that can provides adequate purity, yield, and function after separation have been needed for fabricating transplantable tissues without modification of cell surfaces. In the present study, poly(*N*-isopropyl acrylamide) (PIPAAm), thermo-responsive polymer, brush grafted surfaces with various brush length were prepared by surface-initiated atom transfer radical polymerization (ATRP). Temperature-dependent adhesion and detachment properties of human cells were observed for investigating the possibility of the surface as a cell separating materials.

Methods: Glass coverslips with a silane layer comprising of 2-(*m/p*-chloromethylphenyl)ethyltri methoxysilane, an ATRP initiator, was prepared as shown in the first step in Figure 1, and dense PIPAAm brush were modified on the initiator modified surface through surface-initiated ATRP as shown in the second step in Figure 1. Four types of human cells, human umbilical vein endothelial cells (HUVEC), neonatal human dermal fibroblasts (NHDF), human aortic smooth muscle cells (SMC), and human skeletal muscle myoblast cells (HSMM) were used as model cells for observation of adhesion at 37 °C and detachment at 20 °C on the surfaces. GFP expressing HUVEC (GFP-HUVEC) and HSMM were used for observation of cell separating behavior.

Results: Characterization of the prepared PIPAAm brush grafted surfaces was performed by ATR/FT-IR and GPC measurements. Amount of grafted PIPAAm, indicating PIPAAm brush length, were increased with feed IPAAm monomer concentration. Estimated graft density exhibited a relatively higher value (>0.1 chains/nm^2), indicating that ATRP reaction formed densely packed PIPAAm brush on glass substrates. Figure 2 show the cell adhesion and detachment profiles on the prepared surfaces. On short PIPAAm brush grafted surface, four types of human cells adhered with comparable adhesion rates. However, the recovery rate of adheres cells was relatively low, because the hydration of grafted short PIPAAm brush was insufficient for cell detachment. On the contrary, long PIPAAm brush grafted surface, almost all cells were unable to adhere, because the relatively higher hydrophilic PIPAAm brush suppressed the adhesion of these cells. PIPAAm brush with a moderate brush length, four types of cells was able to adhere and detach after incubation at 20 °C. Using the moderate length of PIPAAm brush, a mixture of GFP-HUVEC and HSMM was allowed to adhere on the surface at 37 °C, and then to be recovered at 20 °C (Figure 3). GFP-HUVEC detached from the surfaces promptly at initial incubation at 20 °C, and then HSMM gradually detached, indicating that the high ratios of HUVEC and HSMM

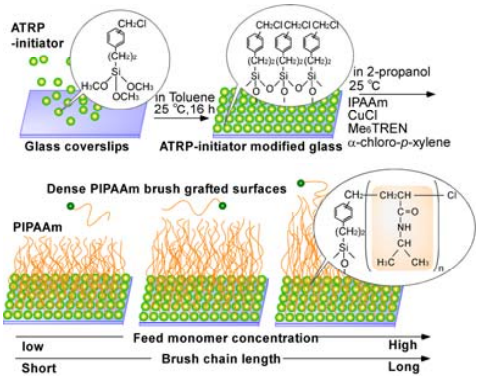

Figure 1. Scheme for preparation of thermo-responsive polymer brush on glass with various chain lengths

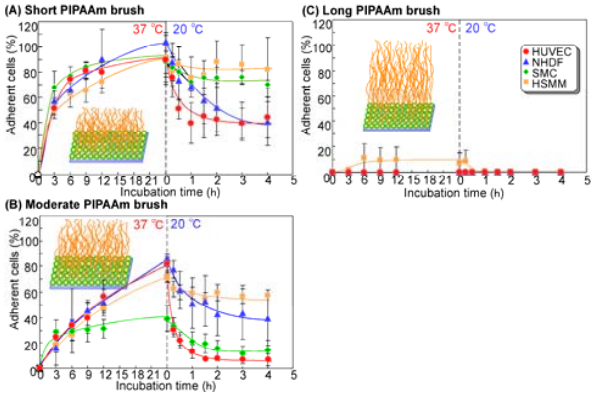

Figure 2. Cells adhesion and detachment profiles on PIPAAm brush surfaces

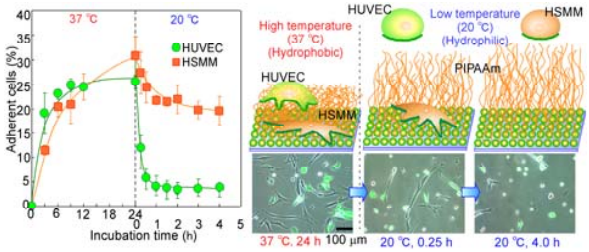

Figure 3. Cell separation using difference in cells' detachment properties

were obtained in the initial and subsequent periods of 20 °C incubation, respectively.

Conclusions: Precisely designed PIPAAm brush was able to separate cells by the utilization of different detachment properties of cells from the surfaces. The prepared surfaces would be useful as cell separation materials for microfluidics devices or cell separation chromatography matrices.