DYNAMICALLY CELL SEPARATING THERMO-RESPONSIVE BIOINTERFACES HAVING DENSE POLYMER BRUSHES
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ABSTRACT
Poly(N-isopropylacrylamide) (PIPAAm) brush with various brush lengths grafted glass surfaces were prepared as a cell separating intelligent interface through a surface-initiated atom transfer radical polymerization (ATRP) with a CuCl/Me₆TREN catalytic system. Prepared PIPAAm brush surfaces as a cell separating intelligent interfaces were characterized by observing the adhesion and detachment behavior of four types of human cells. PIPAAm brush with a moderate brush length exhibited the different cell detachment rates among individual cell types. These results indicated that precisely designed PIPAAm brush functioned as an intelligent cell separating interface by utilizing the intrinsic cell detachment properties of individual cells.

KEYWORDS
Cell separation, Intelligent materials, Temperature-responsive polymer, Polymer brush, Regenerative medicine.

INTRODUCTION
With progress in biomedical technologies, regenerative medicine that reproduces the lost functions of the tissue and organs has been becoming one of the promising therapies for patients. Especially, cells based regenerative medicine and tissue engineering has been progressing rapidly, and a number of clinical trials have already started [1-4]. In this approach, an effective cell separation and purification technology that can provides an adequate purity, yield, and function after separation have been needed for preparing and constructing tissues, because the purity of cells or individual cell contents in co-cultured cells is important for fabricating functional tissues. To date, various types of cell separation methods have been developed such as field-flow fractionation (FFF), affinity adsorption, and flow sortings [5,6]. Especially, fluorescence-activated cell sorting (FACS) and magnetic cell sorting (MACS) are widely used as precise cell separation methods. However, these cell separation methods require the modification of cell surfaces with fluorescent antibody or magnetic particles, leading to a serious problem upon the transplantation of separated cells to human body. Thus, a cell separation method that requires no modification on the surface of cell is preferable for utilizing separated cells for transplantation. With investigating new cell separating tools, our laboratory has paid attention to PIPAAm modified surfaces as a cell separating material [7]. In this study, PIPAAm brush grafted surfaces with various brush lengths were prepared by a surface-initiated ATRP. Temperature-dependent adhesion and detachment properties of human cells were observed for investigating the possibility of the surface as a cell separating material.

EXPERIMENT
Glass coverslips with a silane layer comprising of 2-(m/p-chloromethylphenyl)ethyltrimethoxysilane, 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. Prepared PIPAAm brush surface was characterized by attenuated total reflection fourier transform infrared spectroscope (ATR/FT-IR) and gel permeation chromatography (GPC) measurement of PIPAAm in ATRP reaction solution for estimating brush length. Four types of human cells, human umbilical vein endothelial cells (HUVEC), normal 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.

Figure 1. Scheme of the preparation of PIPAAm brush grafted glass surface as an intelligent biointerface for cell separation.
RESULTS AND DISCUSSION
Characterization of the prepared PIPAAm brush grafted surfaces was summarized in Table 1. 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/nm²), indicating that ATRP reaction formed densely packed PIPAAm brush on glass substrates.

Table 1. Characterization of PIPAAm brush

<table>
<thead>
<tr>
<th>IPAAm monomer in ATRP (mmol/L)</th>
<th>Amount of PIPAAm (µg/cm²)</th>
<th>Molecular weight (Mn)</th>
<th>Grafted density (chains/nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>0.39</td>
<td>8200</td>
<td>0.29</td>
</tr>
<tr>
<td>875</td>
<td>0.76</td>
<td>12800</td>
<td>0.36</td>
</tr>
<tr>
<td>1000</td>
<td>1.29</td>
<td>15600</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Figure 2 show the cells morphology on prepared-PIPAAm-brush-grafted surfaces and Figure 3 shows 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.

Figure 2. Cells morphologies on PIPAAm brush grafted surfaces at 37 °C (hydrophobic) and 20 °C (hydrophilic).

Figure 3. Cell adhesion and detachment profiles on the PIPAAm brush surfaces.

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Additionally, PIPAAm brush with a moderate brush length exhibited the different cell detachment rates among individual cell types. The difference in the detachment ratios among cells was speculated to be applicable to a good cell separation.

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

Thus, 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 microfluidics or cell separation chromatography matrices.

CONCLUSION

Dense PIPAAm brushes having various brush lengths were grafted onto glass surfaces, and these prepared surfaces were utilized for cell separation. On moderate PIPAAm brush surfaces, four types of cells were able to adhere and detach themselves after incubation at 20 °C. Using the surface, a mixture of GFP-HUVEC and HSMM was allowed to be separated by changing temperature. Thus, precisely designed PIPAAm brush was able to separate cells by the utilization of different detachment properties of cells from the surfaces.

ACKNOWLEDGEMENT

Part of the present research was financially supported by the “Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program),” initiated by the Council for Science and Technology Policy from the Japan Society for the Promotion of Science (JSPS).

REFERENCES


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