Cell Separating Surface using Hydrophobized Thermoresponsive Copolymer Brush

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Introduction: A strong demand for cell separation methods has been increased for various applications. Especially, in the fields of regenerative medicine, an effective cell separation method 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*-isopropylacrylamide-*co*-butylmethacrylate) (P(IPAAm-*co*-BMA), thermo-responsive copolymer, brush grafted surfaces with various BMA contents 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: Silane layer comprising of 2-(*m*/*p*-chloromethylphenyl)ethyltrimethoxysilane, an ATRP initiator, was formed on an glass coverslipsas shown in the first step in Figure 1, and dense P(IPAAm-*co*-BMA) brush were modified on the initiator modified surface through surface-initiated ATRP as shown in the second step in Figure 1. BMA feed composition was varied from 0 to 5mol%. Human umbilical vein endothelial cells (HUVEC), neonatal human dermal fibroblasts (NHDF), were used as model cells for observation of adhesion at 37 °C and detachment at 10 and 20 °C on the surfaces. GFP expressing HUVEC (GFP-HUVEC) and NHDF were used for observation of cell separating behavior.

Results: Characterization of the prepared P(IPAAm-*co*-BMA) brush grafted surfaces was performed by ATR/FT-IR, ^{1}H-NMR and GPC measurements. BMA composition of copolymer increased with increasing feed molar ratio of the monomer. Contact angle measurement and fibronectin adsorption measurement revealed that surface hydrophobicity increased with BMA composition of grafted copolymer. On observation of cells adhesion and detachment on the prepared copolymer brush, cells adhesion was enhanced with increasing BMA composition of copolymer, probably due to the enhanced fibronectin adsorption. For investigating the effective temperature for recovering these cells, cells detachment properties was observed at 10 and 20 °C (Figure 2). HUVECs and NHDFs exhibited excellent cell detachment properties at 20 and 10 °C, respectively. Using these cells' intrinsic thermo-sensitive properties for detachment, HUVEC and NHDF were separated. A mixture of GFP-HUVEC and NHDF was allowed to adhere on the surface at 37 °C, and the external temperature was reduced to 10 °C for recovering NHDFs. After 30 min incubation at 10 °C, the temperature was elevated to 20 °C for recovering HUVECs. In incubation period at 10 °C, NHDFs promptly detached while HUVECs adhered on copolymer brush surfaces. Then, incubation period at 20 °C, adhered HUVECs were recovered from the copolymer brush surface. Thus, the higher ratio of NHDFs and HUVECs was recovered in medium during the 10 °C and 20 °C incubation periods, respectively.

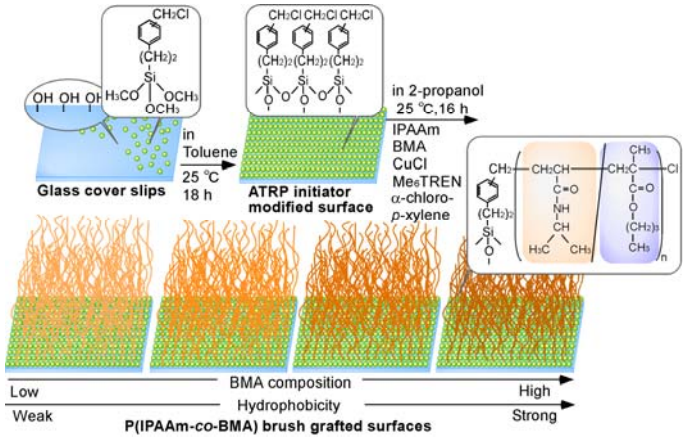

Figure 1. Scheme for preparation of hydrophobized thermoresponsive copolymer brush on glass with various BMA composition.

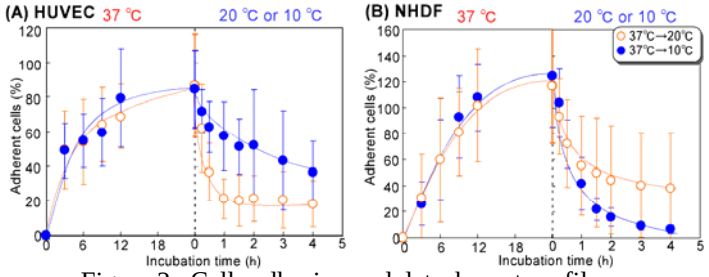

Figure 2. Cells adhesion and detachment profiles on Hydrophobized thermoresponsive copolymer brush surfaces (BMA 5mol%)

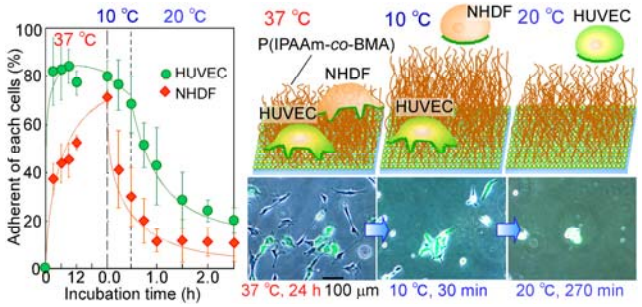

Figure 3. Cell separation using hydrophobized thermoresponsive copolymer brush by multistep temperature change

Conclusions: Hydrophobized thermoresponsive copolymer brush can separate cells by using cells intrinsic thermo-sensitive properties for their detachment. The prepared surfaces would be useful as cell separation materials for microfluidics devices or cell separation chromatography matrices.