**Thermoresponsive cell culture surfaces for promoting cell adhesion**

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**Statement of Purpose:** Our group have developed poly(*N*-isopropylacrylamide) (PIPAAm)-grafted 1st-generation thermoresponsive culture dish for recovery of cultured cells (Yamada N. Makromol Chem Rapid Commun. 1990;11:571-576.) and cell sheets for regenerative medicine (Kobayashi J. Sci Technol Adv Mater. 2010;11:014111) only by reducing temperature. In this study, we have developed next-generation thermoresponsive cell culture dishes for promoting cell adhesion. One approach is the introduction of ionic groups onto the thermoresponsive cell culture surfaces. In general, positively charged surfaces enhance the binding with negatively charged molecules such as sialic acid and chondroitin sulfate proteoglycan on the cellular membrane via electrostatic interaction. Here, positively charged poly(*N*-isopropylacrylamide-*co*-*n*-butyl methacrylate-*co*-3-acrylamidopropyl trimethylammonium chloride) (poly(IPAAm-*co*-BMA-*co*-APTAC)-grafted cell culture dishes were prepared by electron beam irradiation. Another approach is the introduction of specific ligands toward receptors on the surface of cellular membrane. Anti-CD90 antibody-immobilized thermoresponsive cell culture surfaces were also prepared as a model. CD90 is expressed many type of cells, including T cells, thymocytes, neurons, endothelial cells, fibroblasts and some types of stem cells such as mesenchymal stem cell.
**Methods:** Positively charged poly(IPAAm-*co*-BMA-*co*-APTAC)-grafted tissue culture polystyrene dish (TCPS) was prepared by electron beam irradiation with changing molar ratio of IPAAm/BMA/ APTAC at a total concentration 55% (w/w). Next, anti CD90 antibody-immobilized thermoresponsive surface was prepared as described previously (Nishi M. Biomaterials. 2007;28: 5471-5476.). Briefly, poly(*N*-isopropylacrylamide-*co*-2-isopropylacrylamide) (poly(IPAAm-*co*-CIPAAm)-grafted surfaces was prepared by electron beam irradiation. Then, the surfaces were biotinylated by condensing reaction between 5-(biotinamido)pentylamine and carboxyl groups on the surfaces. The surfaces were incubated with PBS containing streptavidin (50 μg/mL) for 1.5 h. After rinsing with PBS, dishes were finally incubated with PBS containing biotinylated anti-human CD90 antibody (5 μg/mL) for 1.5 h. On both type of thermoresponsive surfaces, cells were cultured at 37 °C in a humidified atmosphere with 5% $CO_2$. Cell detachment from the surfaces was performed by lowering temperature to 20 °C. Morphology and number of cells were monitored under a phase contact microscope at various time points.
**Results:** Firstly, initial adhesion of Chinese hamster ovary (CHO) cells and low temperature-induced detachment of endothelial cells (ECs) on positively charged poly(IPAAm-*co*-BMA-*co*-APTAC)-grafted surfaces was investigated. The maximum adhesion of CHO cells after 1 hour was observed on the surface with 6 mol% APTAC and 5 mol% BMA in feed. By lowering temperature to 20 °C, behavior of EC detachment was observed after seeding of ECs for 24 hours. The detachment time of ECs were delayed by the increase in the introduction of APTAC, because the strong electrostatic interaction was induced via quaternary amine groups of APTAC. Consequently, the enhancement of the cell adhesion was achieved on the surfaces with the optimum surface charge densities.

Secondly, anti CD90 antibody-immobilized thermoresponsive surfaces were utilized for enhancing adhesion of floating cells. Floating cells (Ty-82) didn't adhere on isotype antibody-immobilized surfaces, and the cells aggregated (Figure 1A). By contrast, Ty-82 cells selectively adhere on anti CD90 antibody-immobilized thermoresponsive surfaces (Figure 1B). This result indicated that Ty-82 adhered onto the CD90 antibody-immobilized surfaces through affinity interaction, not through nonspecific interactions. Moreover, the cells were detached from the surfaces by lowering temperature to 20 °C with pipetting (Figure 2). These indicate that hydrated PIPAAm chains diminished affinity interaction of anti CD90 antibody from cell surface CD90 by lowering temperature.

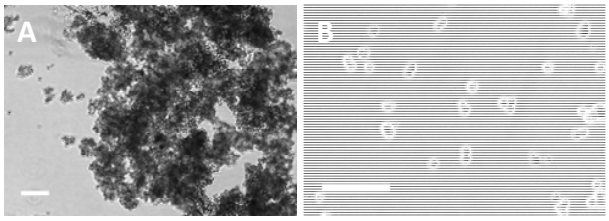


Figure 1. Adhesion of Ty-82 cells in serum-free medium after 3 hours at 37 degrees on (A) isotype antibody and (B) anti CD90 antibody-immobilized thermoresponsive cell culture surfaces. Scale bars: 100 μm

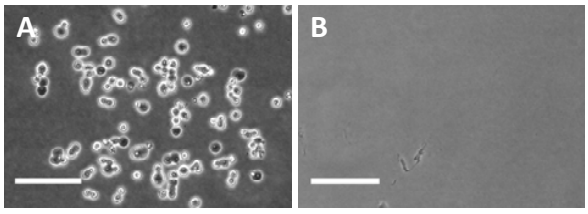


Figure 2. Detachment of Ty-82 cells from anti CD90 antibody-immobilized thermoresponsive cell culture surfaces by lowering temperature to 20 degrees for 2 hours (A) with pipetting (B). Scale bars: 100 μm

**Conclusions:** Two types of thermoresponsive cell culture surfaces for enhancing selective cell adhesion were successfully prepared. These surface technologies would be useful for primary cell culture and selective cell attachment/detachment system such as cell separation.