Ultrathin Surface Coating of Thermoresponsive Block Copolymers for Fabricating Cell Sheets

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Statement of Purpose: Our research group has developed an unique sheet-like cell manipulation technology, "cell sheet engineering", for reconstructing damaged tissues and organs, and some clinical trials are now in progress [1, 2]. Confluently cultured cells can be harvested as cellular monolayers, "cell sheets", from thermoresponsive polymer-grafted culture substrates prepared by electron beam induced graft-polymerization of *N*-isopropylacrylamide (IPAAm) via incubation at a temperature below PIPAAm's lower critical solution temperature (LCST). Herein, we proposed the facile fabrication method of thermoresponsive culture surfaces by the ultrathin coating of PIPAAm-based block copolymers on commercial culture substrates for thermally regulated surface adhesion of cells. In addition, the effect of chain length of thermoresponsive polymers was also investigated for optimizing cell sheet harvest.

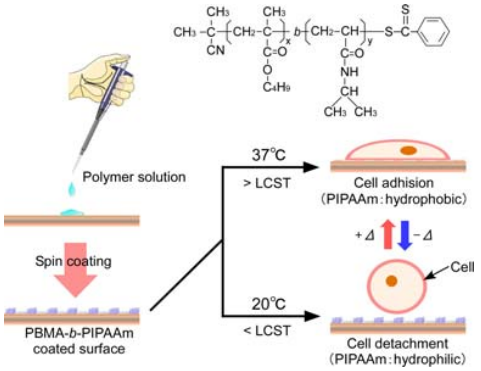

Figure 1. Schematic illustration of thermoresponsive block copolymer coating for thermally induced cell adhesion/detachment.

Methods: Well-defined poly(n-butyl methacrylate)-*b*-poly(*N*-isopropylacrylamide) (PBMA-*b*-PIPAAm) with various PIPAAm molecular weights [M_w of PBMA; 11500 and M_w of PIPAAm; 2800 (coded as B/IP-1), 13600 (B/IP-2), and 35700 (B/IP-3)] were prepared by RAFT polymerization. Each polymer was dissolved in mixed solvent of acetonitrile/DMF (5/1) (0.3 w/v%), and deposited on commercial tissue culture polystyrene (TCPS) substrate or cell culture insert (PET membrane with 1.0 μm pores) using a spin coater. Grafted PIPAAm amount was determined by ATR/FT-IR. Polymer layer thickness were also estimated by ellipsometric measurements in dry state. To investigate temperature-dependent cellular behavior, bovine carotid artery endothelial cells (BAECs) were seeded at 5 x 10^3 cells/cm^2 on various polymer-coated surfaces and cultured at 37°C. After the 3-days culture, the cell adhering culture surfaces were incubated at 20°C, and then the cells were observed microscopically for various time periods.

Results: PBMA blocks strongly interacted with hydrophobic culture surfaces and inhibited the release of block polymers from the surfaces, unlike IPAAm homopolymer. Coated amounts of PIPAAm chains ranged from 0.72 to 1.60 μg/cm^2, and the values increased with increasing in the molecular weight of PIPAAm chains. Thickness of polymer layers in dry state were 15-19 nm. Chain length of PIPAAm chains significantly affected temperature-dependent cellular behavior. Cell adhesion became lower with increasing PIPAAm chain length. Cell proliferation profiles for B/IP-1 and B/IP-2 coated surfaces were comparable to bare TCPS, while the surface coated with B/IP-3 possessing the largest molecular weight (M_w: 35700) showed a low cell adhesive property and growth rate and difficulty for reaching to confluent cell culture (Figure 2, 37°C). On the other hand, longer PIPAAm chain promoted cell detachment via a low temperature to 20°C (below PIPAAm's LCST) (Figure 2, 20°C). In addition, block polymer coated surfaces allowed the confluently cultured cells to be harvested as the cell sheets with intact deposited extracellular matrix. We also applied polymer coating method to porous PET membranes for obtaining stratified cellular constructions without using feeder layer cells [3]. Cell sheets could be recovered from porous membrane, while detachment period became longer to 2 hours (B/IP-2 coated TCPF: 20 min) due to the difference of hydrophobicity between polystyrene and PET and the subsequent aggregate state of coated polymers.

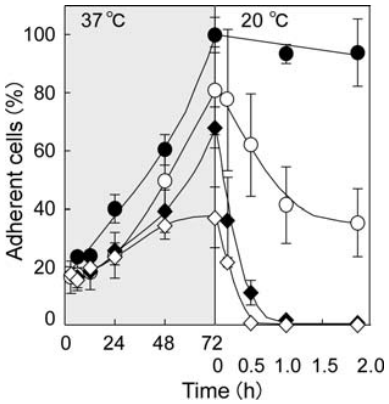

Figure 2. Cell adhesion/growth on and detachment from TCPS (closed circle) and polymer coated surfaces (B/IP-1: open circle; B/IP-2: closed diamond; B/IP-3: open diamond). mean ± SD, n=3.

Conclusions: Nano-scaled thin coating of PIPAAm-based polymers on commercial culture surfaces allowed cells to detach from the surfaces by reducing temperature. Chain length and mobility of grafted PIPAAm greatly affected both cell adhesion/proliferation and detachment. In addition, cell sheets with intact deposited extracellular matrix were able to be harvested from the smart surfaces.

References:

1) Yamato M., Okano T. Materials Today, 2004;7:42-47.
2) Matsuda N., et al. Adv Mater, 2007;19:3089-3099.
3) Murakami D., et al. J Artif Organs, 2006;9:185-191.