Smart Coating of Poly(*N*-isopropylacrylamide)-Based Block Copolymers for Cell Sheet Harvest

Masamichi Nakayama [a], Naoko Yamada [b], Hideko Kanazawa [b], Teruo Okano [a]
[a] Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University (TWIns),
[b] Graduate School of Pharmaceutical Sciences, Keio University

Statement of Purpose: Polymeric biomaterials for tissue engineering have been investigated to apply in aspects of regenerative medicine. As our unique approach of cell manipulation technology, "cell sheet engineering" as a novel concept for reconstruction of various tissues has been developed using thermoresponsive polymer-grafted culture substrates prepared by electron beam induced graft-polymerization of *N*-isopropylacrylamide (IPAAm). By using the smart culture surfaces, confluently cultured cells are harvested as sheet-like cellular structures, "cell sheet" with reducing temperature below the PIPAAm's lower critical solution temperature (LCST). Herein, we proposed the convenient fabrication method of thermoresponsive cell culture surfaces by the deposition of PIPAAm-based block copolymers for thermally controlled cell adhesion/detachment. The smart polymer-coated surfaces with various grafting polymer amounts and film thicknesses were obtained by various coating conditions and were investigated for optimizing cell sheet harvest.

Methods: Well-defined block copolymers of poly(*n*-butyl methacrylate)-*b*-poly(*N*-isopropylacrylamide) (PBMA-*b*-PIPAAm) (M_n; 25000, monomer units; BMA/IPAAm; 79/120) were prepared by RAFT polymerization. The obtained polymers were dissolved in mixed solvent of acetonitrile/*N*,*N*-dimethylformamide (5/1 in v/v) (polymer concentrations; 0.1, 0.3, and 0.5 w/v%), and deposited on commercial tissue culture polystyrene (TCPS) substrates (cut into the size of 24 x 24 mm) using a spin coater (3000 rpm, 30 sec). Polymer-coated surfaces were dried at room temperature for overnight in vacuo. The substrates were rinsed with pure water thoroughly, followed by drying under vacuum. Amounts of grafted PIPAAm segments were determined by ATR/FT-IR. Thicknesses of coated polymer layer were also estimated by ellipsometric measurements in dry state. For the investigation of temperature-dependent cellular behavior, bovine carotid artery endothelial cells (BAECs) were seeded at 1 x 10^5 cells/cm^2 on the various polymer-coated surfaces, followed by incubation at 37°C. After the 3-days culture of BAECs, the cell adhering culture surfaces were incubated at 20°C, and then the cells were observed microscopically and/or visually for various time periods.

Results: Thermoresponsive block copolymers of PBMA-*b*-PIPAAm were deposited on the TCPS surfaces via spin coating method. Coated amounts of PIPAAm chains ranged from 0.87 to 1.81 μg/cm^2 and increased with increasing in polymer concentration for spin coating. PBMA blocks significantly inhibited the release of block polymers from the surfaces, unlike IPAAm homopolymer as the control. Thicknesses of PBMA-*b*-PIPAAm layers in dry state were controllable by varying the concentration for polymer deposition and determined to be 7.0, 15.4 and 23.3 nm for the concentration of 0.1, 0.3, and 0.5w/v%, respectively. Polymer layer thicknesses extremely affected cellular behavior. BAECs adhered and proliferated on the PBMA-*b*-PIPAAm coated surfaces with layer thickness less than 15.4 nm, as similar to commercial culture dishes under conventional cell culture condition at 37°C. However, cells were difficult to adhere on the surfaces with 23 nm-layers and to achieve confluent condition (Figure 1 left, 37°C). On the other hand, adherant cells spontaneously peeled from polymer-coated surfaces with thickness more than 15.4 nm by reducing the culture tempeature below the PIPAAm's LCST (Figure 1 right, 20°C). Consequently, block polymer coated surfaces with 15 nm-thickness successfully allowed the confluently cultured cells to be harvested as the cell sheets with intact deposited extracellular matrix by low-temperature treatment.

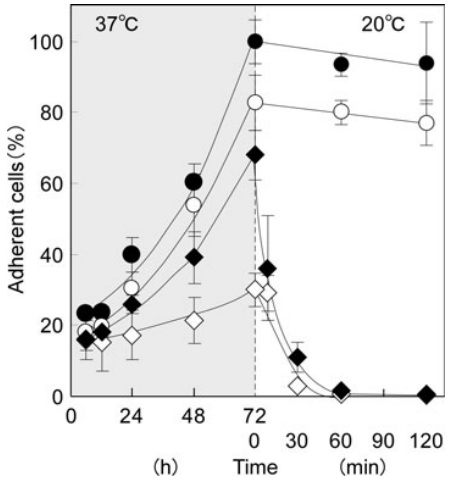

Figure 1. Cell adhesion/proliferation (37°C, left) on and detachment (20°C, right) from the PBMA-*b*-PIPAAm coated surfaces; closed circle: TCPS, open circle: 7.0 nm-layer, closed diamond: 15.4 nm-layer, and open diamond: 23.3 nm-layer. mean ± SD, n=3.

Conclusions: In summary, thermoresponsive cell culture surfaces for cell sheet fabrication were prepared via PIPAAm-based block copolymer deposition on commercial TCPS in nano-scaled thickness. Deposited amounts of thermoresponsive polymer segments and thickness of coated polymer layers were achieved to control by changing polymer concentration. Thickness of coated polymer layers significantly affected cellular behavior including cell adhesion/growth and low-temperature treated cell detachment. In addition, cell sheets were successfully harvested from the block copolymer coated surface by simply reducing temperature below the PIPAAm's LCST for confluently cultured cells.

References:

1) Kikuchi A, Okano T. J Control Release 2005; 101: 69-84.

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