

## End-functional Poly(*N*-isopropylacrylamide) Brushes for Efficiently Promoting Cell Adhesion and Detachment

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**Statement of Purpose:** A unique sheet-like cell manipulation method, cell sheet engineering, has been developed for recovering the lost functions of tissues and organs using poly(*N*-isopropylacrylamide) (PIPAAM) grafted cell culture surfaces [1]. Recently, we reported newly designed thermoresponsive surfaces with densely packed linear polymer structures, PIPAAm brushes, which were prepared by a surface-initiated reversible addition-fragmentation chain transfer (SI-RAFT) polymerization [2]. This grafting method enables to construct well-defined PIPAAm brush structures and introduce various functional groups to polymer termini. Herein, the effects of terminal electrostatic property and polymer chain length of PIPAAm brushes were investigated for promoting cell adhesion and detachment in response to environment temperature.

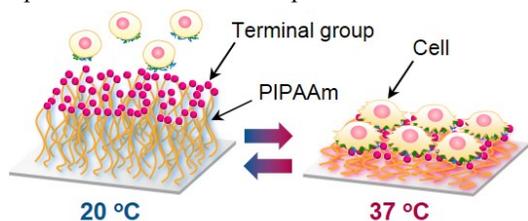


Figure 1. Schematic illustration of end-functional thermoresponsive PIPAAm brush surfaces for thermally induced cell adhesion/detachment.

**Methods:** PIPAAm grafting on glass coverslip was performed by SI-RAFT polymerization, and various PIPAAm chain lengths were synthesized by changing initial monomer feeds. Polymer terminal trithiocarbonate group was reduced to thiol group and then reacted with cationic 3-acrylamidopropyl trimethylammonium or nonionic *N*-isopropylacrylamide. PIPAAm brushes were characterized by ATR/FT-IR spectroscopy, gel permeation chromatography, static contact angle measurement, and zeta-potential measurement. Bovine carotid artery endothelial cells (BAECs) were seeded and cultured on individual surfaces at 37°C. In addition, the cells were incubated at 20°C and were observed microscopically for various time periods.

**Results:** Molecular weight of grafted PIPAAm on the surfaces was controllable by changing initial monomer concentration [ $M_n$  of PIPAAm: 43000 (coded as IP-1); 48000 (IP-2); and 59000 (IP-3)]. Grafted PIPAAm amounts were ranged from 1.6–3.2  $\mu\text{g}/\text{cm}^2$ , and the density of grafted PIPAAm chains was calculated to be 0.2 chain/ $\text{nm}^2$ . Surface wettability of PIPAAm grafted surfaces increased hydrophilicity with increasing PIPAAm chain lengths, regardless of terminal groups. Moreover, surface zeta potentials of PIPAAm brush surfaces possessing terminal positive charges were higher than those of nonionic-terminated ones, independent of temperature changes across the lower critical solution

temperature (LCST) of PIPAAm. Amount of adhering BAECs on terminally positive-charged PIPAAm brush surfaces at 37 °C was larger than those on non-charged ones, and terminal electrostatic effect was gradually reduced with increasing PIPAAm chain length (Figure 2). Especially, the PIPAAm brush with a PIPAAm's molecular weight of 59000 (IP-3) showed a low cell adhesive property even above the LCST at 37 °C, and confluent cell culture was unable to be achieved. On the other hand, cell detachment profiles at 20 °C were promoted with increasing PIPAAm chain length with the negligible effect of terminal groups. Consequently, cell sheet fabrication was successfully improved by optimizing PIPAAm chain lengths with the terminal introduction of positive charges.

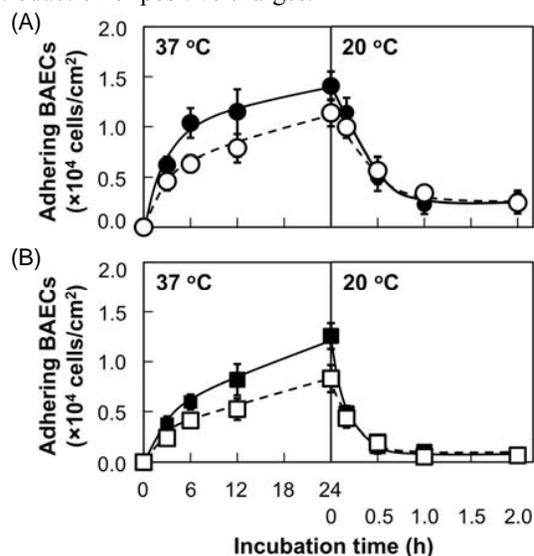


Figure 2. Cell adhesion and detachment on/from (A) IP-1 ( $M_n$ : 43000) and (B) IP-2 ( $M_n$ : 48000) surfaces. Closed and open marks indicated terminally cationic- and nonionic-PIPAAM brushes, respectively.

**Conclusions:** This study presented the effect of terminal electrostatic charge and chain length of PIPAAm brush surfaces on temperature-dependent cellular behavior. Terminal cationic moieties enhanced cell adhesion at 37°C due to electrostatic interaction with cells, and these electrostatic effect was gradually reduced with increasing PIPAAm chain length. In contrast, the low-temperature-induced cell detachment was promoted with increasing PIPAAm chain length without any effect of terminal functionality. Introduction of terminal electrostatic function to PIPAAm brushes with controlled chain length would contribute to cell sheet-based tissue engineering.

### References:

- 1) Yamato M., Okano T. *Materials Today*, 2004; 7:42-47.
- 2) Matsuzaka N., et al. *Biomacromolecules*, 2013;19: 3089-3099.