Controlled End-functionality of Thermoresponsive Polymer Brushes for Regulating Thermally Induced Surface Cell Adhesion Behavior

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Statement of Purpose: Thermally regulated cell adhesion behavior has been achieved on thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm) grafted surfaces. By using the modified surfaces, sheet-like cellular architectures, "cell sheets", are harvested noninvasively from the surfaces by sole temperature changes across PIPAAm's lower critical solution temperature (LCST) at 32°C [1)]. Herein, end-functional PIPAAm brush surfaces as novel smart cell culture substrates were prepared by a surface-initiated reversible addition-fragmentation chain transfer radical polymerization (SI-RAFT) [2)]. In addition, we investigated the effects of PIPAAm's end-functionalities on thermoresponsive surface properties and cell adhesion/detachment behavior.

Methods: PIPAAm grafting was performed by SI-RAFT (sIP-D) on glass coverslips with immobilizing dodecyl trithiocarbonate derivative as a chain transfer agent (CTA) (sCTA). Terminal dodecyl groups of grafted PIPAAm chains were substituted to hydrophilic maleimide groups (sIP-M) through a terminal reduction reaction and subsequent coupling reaction under deoxidized condition. PIPAAm-grafted surfaces were characterized by ATR/FT-IR spectroscopy and static contact angle measurement using captive bubble method in water. Temperature-dependent cell adhesion of bovine carotid artery endothelial cells on sCTA, sIP-D and sIP-M, respectively, were investigated at temperatures from 20 to 37°C. In addition, adhered cells were incubated at 20°C for detachment kinetics of cell sheets.

Results: Surface PIPAAm grafting and substitution of PIPAAm's termini were confirmed by XPS analysis. In addition, the grafted PIPAAm amounts were determined by ATR/FT-IR spectroscopy. The peaks of grafted polymers were detected around 1650 cm^{-1} derived from the amide carbonyl group of PIPAAm. The grafted amounts of PIPAAm on both sIP-D and sIP-M were approximately 1.0 $\mu g/cm^2$. Subsequently, wettability changes of thermoresponsive polymer-grafted surfaces were also characterized by contact angle measurements. The results showed that PIPAAm grafting provided the increase in surface hydrophilicity compared with sCTA, confirming PIPAAm grafting on glass surfaces through our grafting method. Furthermore, the PIPAAm-grafted surfaces for both sIP-D and sIP-M exhibited temperature-dependent wettability changes across PIPAAm's LCST around 32°C. In addition, wettability of sIP-D (contact angle: 37.7 ± 2.2°) was larger than that of sIP-M (contact angle: 33.3 ± 1.7°) at 31°C. Subsequently, temperature-dependency of cell adhesion for the individual end-functional PIPAAm brush surfaces were investigated (Figure 1). Incubated at 37°C, cells were adhered on both sIP-D and sIP-M, while cell adhesion was significantly suppressed at 27°C, unlike non-PIPAAm grafted (sCTA). These results suggested that temperature-induced cell adhesion behavior was influenced by the surface property changes of hydrophilic/hydrophobic states across the LCST of grafted PIPAAm chains. Of great interest, cell adhesion-initiating temperatures were affected by the hydrophobicity of polymer termini. This was due to that terminal chemistry altered the hydration state of grafted PIPAAm chains and brought about the shifts of original PIPAAm's LCST (30°C)[3)]. Furthermore, cell sheets were harvested from sIP-D and sIP-M by reducing temperature to 20°C, regardless of end-functionalities (sIP-D: 29±2 min and sIP-M: 23±2 min incubation).

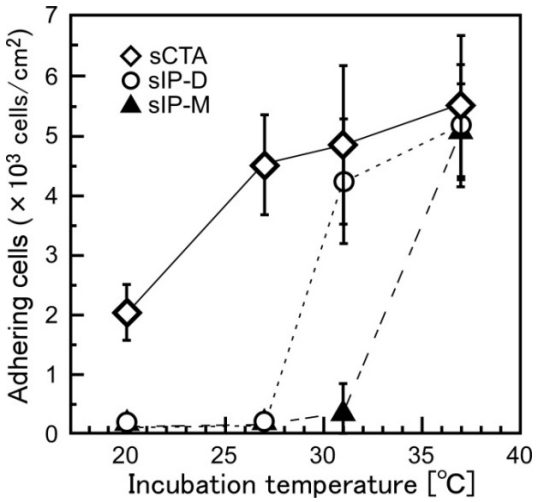

Figure 1. Cell adhesion behavior on individual surfaces at various temperatures.

Conclusions: End-functional PIPAAm brush surfaces have been successfully prepared through SI-RAFT polymerization. In addition, PIPAAm brush surfaces with various end-functional groups were able to be controlled about cell adhesion temperature. These results expected that construction of patterned co-culture cell sheet by changing surface hydrophilic/hydrophobic characteristics using culture temperature.

References: 1) M. Yamato, T. Okano, *Mater. Today*, 7, 42-47 (2004). 2) H. Takahashi *et al.*, *Biomacromolecules*, *11*, 1991-1999 (2010). 3) J. E. Chung *et al.*, *Colloids Surfaces B: Biointerfaces*, 9, 37-48 (1997).