Micropatterned Thermoresponsive Polymer Brush Surfaces for Preparation of Cell Sheets with Well-defined Alignment

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Statement of Purpose: A tissue-like cellular monolayer "cell sheet" has been developed using thermoresponsive cell culture substrates. Cell sheets can be harvested using thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm) grafted substrates with intact deposited extracellular matrix (ECM) simply by reducing temperature below the PIPAAm's lower critical solution temperature (LCST) of 32°C. Recently, our group has demonstrated that thermoresponsive polymer brush surfaces can be prepared by PIPAAm grafting to solid surfaces through surface-initiated reversible addition-fragmentation chain transfer radical (RAFT) polymerization process using dithiobenzoate (DTB) compounds as chain tranfer agents (CTA). While this grafting technique allows cell sheets to be harvested effectively by adjusting the chain lengths and graft densities of PIPAAm brushes, it also gives chain transfer active DTB groups to the grafted PIPAAm termini. Therefore, this RAFT-mediated grafting can be used to obtain block copolymer brush surfaces. In this study, as a new type of functionalized thermoresponsive surface, the stripe-like micropatterned surface comprising the block copolymer brush domains and PIPAAm brush domains was fabricated for controlling cell orientation and harvesting the cell sheets with maintaining their orientational structures.

Methods: PIPAAm was grafted on azoinitiator, 4,4'-azobis (4-cyanovaleric acid) (V-501) immobilized glass surfaces through a surface-initiated RAFT polymerization process as reported previously (IPAAm: 1.0 mol/L, 4-cyanopentanoic acid dithiobenzoate (as the CTA): 0.5 mmol/L, 70°C, 20 h) [1]. Positive photoresist was spin-coated onto the PIPAAm brush surfaces. After prebaking for 1 h at 80°C, the photoresist-coated substrates was irradiated with visible-light to make stripe patterns (50 μm in width). To convert the exposed DTB groups of PIPAAm brushes with maleimide (Mal) groups, the patterned surfaces were immersed in Dulbecco's phosphate buffered saline (PBS) solution (pH 7.4) containing 10 mmol/L 2-aminoethanol, 30 mmol/L Mal, and 1 mmol/L sodium hydrosulfite for 20 h at 20°C in a nitrogen atmosphere. After removing residual photoresist, the patterned surfaces (Mal/DTB-PIPAAm) were immersed in 1,4-dioxane containing the distilled *N*-acryloylmorpholine (AcMo) (1 mol/L) and V-501 (1 mmol/L), and then the reaction solution was heated at 70°C for 20 h. Since AcMo was polymerized from the DTB-terminated PIPAAm chains as the macro-CTAs to form second blocks on the PIPAAm brush surfaces, block copolymer PIPAAm-*b*-PAcMo was grafted selectively on the DTB-PIPAAm regions. As a result, PIPAAm brushes and PIPAAm-*b*-PAcMo brushes were patterned with 50 μm stripes on the glass substrate.

Normal human dermal fibroblasts (NHDFs) were seeded onto the patterned surfaces, and then the media was changed every 2 days for 3-5 days. After being confluent, NHDFs adhering on the patterned surfaces were incubated at 20°C, 5% CO_2, and then the harvested cell sheet was observed microscopically and visually.

Results: NHDFs adhered site-specifically on the patterned polymer brush surfaces even in serum-containing culture media. Subsequent further incubation at 37°C allowed the patterned cells to migrate and proliferate on all regions of the patterned surfaces, since cell-repellent properties of PIPAAm-*b*-PAcMo were insufficient for inhibiting cell invasion from the neighboring cell-adhensive PIPAAm regions. The aligned cells finally became confluent with maintaining the same orientation on all regions of patterned surfaces. After immunostaining for the aligned cell layers, fluorescence images also showed the orientation of actin fibers and fibronectin in the cell layers with deposited ECM on the surfaces. These indicated that the alignment of cytoskeleton and ECM proteins were also regulated by the micropatterning of the polymer brushes. The aligned cells were harvested as the cell sheet only by reducing the culture temperature below the PIPAAm's LCST to 20°C. The cell sheet harvested from the patterned surface possessed a different shrunken rate between vertical and parallel sides of the cell alignment (approx. 3: 1 of aspect ratio (width: height)). This indicates that the harvested cell sheet maintains the alignment of cells and related proteins.

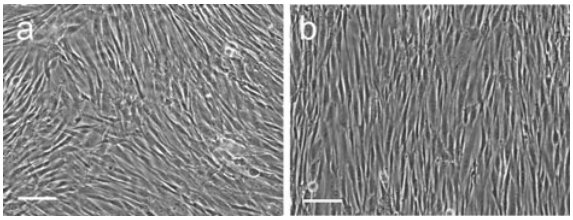

Figure 1. Microscopic images of adherent fibroblasts on (a) PIPAAm brush surface and (b) PIPAAm/PIPAAm-*b*-PAcMo patterned brush surface. Scale bar: 100 μm.

Conclusions: RAFT-mediated block copolymerization achieved the stripe-like micropatterning of PIPAAm brush domains and PIPAAm-*b*-PAcMo domains. NHDFs were aligned on the patterned polymer brush surfaces simply by one-pot cell seeding. The alignment of cells and ECM proteins is promising for showing the mechanical and biological aspects of cell sheets harvested from the patterned surfaces. Engineered cell sheets such as achieved in this work may promise for creating tissue-mimicking structures with specific biological functions in cell sheet engineering.

References: Takahashi H. et al. Biomacromolecules. 2010; 11: 1991-1999.