Polymer thickness-dependency of cell adhesion on poly(*N*-isopropylacrylamide)-grafted glass surfaces

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Introduction: We have developed temperature-responsive culture surfaces by grafting poly(*N*-isopropylacrylamide) (PIPAAm) on tissue culture polystyrene (TCPS) with electron beam irradiation.[1,2] Various types of cells adhere and spread on the surfaces at 37°C, but these cells spontaneously detach from the surfaces upon reducing temperature below 32°C without need for proteolytic enzymes. Here, we grafted PIPAAm onto glass cover slips (PIPAAm-G) surfaces by electron beam irradiation to examine the PIPAAm thickness-dependency of cell adhesion on the surfaces with comparison to that on PIPAAm-grafted TCPS.

Methods: Glass cover slip surfaces were cleaned by oxygen plasma treatment, and placed in a 500 mL separable flask with 3 mL 3-methacryloxypropyltrimethoxysilane (MPTMS). Cover slips were rinsed with toluene, methanol, and distilled water, and dried for 3 h at 160°C. Specific procedures for the preparation of PIPAAm grafted surfaces are described previously.[1,2] Briefly, *N*-isopropylacrylamide in 2-propanol solution (concentration of 5-50 wt%) was spread onto silanized glass surfaces, then electron beam was irradiated. Thickness of PIPAAm-grafted layer was obtained by atomic force microscopy (AFM) after polymer specific ablation with UV excimer laser and photomasks as described previous report.[4] Bovine aortic endothelial cells were seeded onto PIPAAm-G and cultured with DMEM in the presence of 10% FBS at 37°C. Adherent cell number on each surface was counted periodically on phase contrast photographs. For cell detachment, PIPAAm-Gs were transferred to a CO_2 incubator set at 20°C after non-adherent cells were removed by changing culture medium. Remaining adherent cell numbers on each surface were counted.

Results and Discussion: At the optimized laser fluence, only the grafted PIPAAm layer was selectively ablated in the limited area through photomasks without damaging basal glass cover slips.[3] AFM revealed that thickness of the grafted PIPAAm layer was increased along the initial monomer concentration. Figure 1 shows AFM images and their cross section profiles of the ablated domains for 5 wt% and 40 wt% PIPAAm-G. The depth of ablated domains was approximately 3.6 and 8.9 nm for 5 wt% PIPAAm-G and 40 wt% PIPAAm-G, respectively.

Cell adhesion and detachment were examined on PIPAAm-Gs with different grafted PIPAAm thickness (Figure 2). Only when the thickness was below 5 nm, cell adhesion was observed on PIPAAm-Gs. Interestingly, similar polymer thickness dependency of cell adhesion was observed on PIPAAm-grafted TCPS, but cells adhered on the surfaces having 15 nm thickness of grafted PIPAAm. All the cells adhered on the temperature-responsive surfaces were detached upon reducing temperature below 32°C.

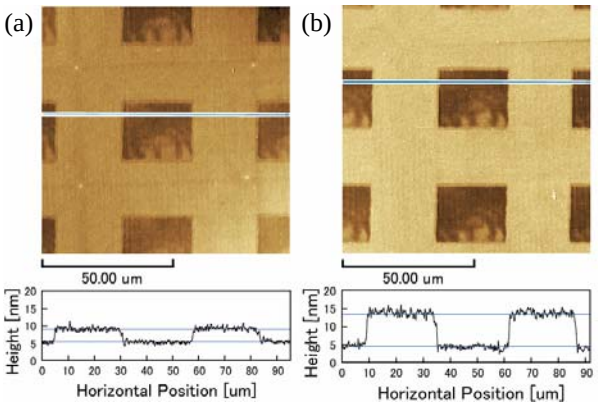

Figure 1. AFM images of the ablated surface of 5 wt% PIPAAm-G(a) and 40 wt% PIPAAm-G(b).

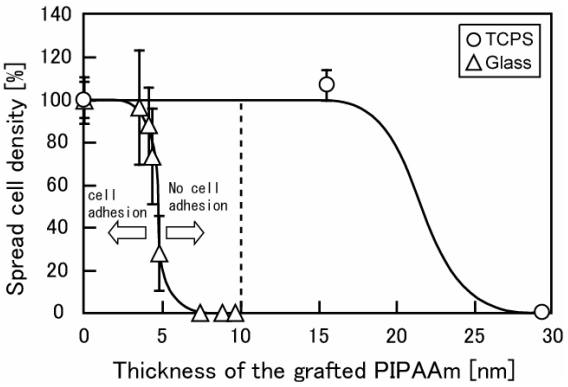

Figure 2. Polymer thickenss dependent cell attachment for PIPAAm grafted surfaces.

Conclusions: Cell adhesion and detachment on PIPAAm-grafted surfaces depends on the thickness of immobilized PIPAAm as well as the basal substrate.

References:

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