Thermoresponsive Polymer Brush Interfaces; Surface Characterization for Controlled Cell Adhesion

Aya Mizutani,[1] Akihiko Kikuchi,[2] Jun Kobayashi,[3] Yoshikatsu Akiyama,[3]
Masayuki Yamato,[3] Hideko Kanazawa,[1] and Teruo Okano[3]
[1]Kyoritsu Univ. of Pharm., [2]Dept. Mater. Sci. & Tech., Tokyo Univ. of Sci., [3]Tokyo Women's Med. Univ., 21stCOE

Introduction: We have been investigating cell sheet engineering for regenerative medicine using thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm) grafted cell culture surfaces. Grafted PIPAAm is introduced by electron beam irradiation method to form cross-linked thin hydrogel structure.[1, 2] In the present research, we focused on the structure of PIPAAm thin layer, and designed densely grafted PIPAAm brush surfaces using surface-initiated atom transfer radical polymerization (ATRP).[3] We investigated surface characteristics of PIPAAm brushes and discussed influence of PIPAAm brushes on adhesion and detachment of bovine carotid artery endothelial cells (ECs).

Methods: Poly(*p*-chloromethylstyrene)-coated polystyrene substrates were soaked in Milli-Q water containing 0.3M IPAAm, 3.0mM copper chloride (I), 0.3mM copper chloride (II), and 3.0mM tris(2-(dimethylamino)ethyl)amine (Me_6TREN) under nitrogen atmosphere. Polymerization proceeded at 25°C. Surfaces having different amount of grafted PIPAAm was prepared by changing reaction times. The amount of grafted PIPAAm was determined by ATR/FTIR. Prepared surfaces were abbreviated to ATX, where X is the amount of surface-grafted PIPAAm in μg/cm^2. PIPAAm layer thickness in a dry state was determined by ellipsometry. Water contact angles of prepared interfaces were determined by captive bubble method in Milli-Q water at 37°C and 20°C. Adhesion and detachment behavior of ECs at predetermined temperature were then observed. ECs were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) on PIPAAm grafted surfaces at 37°C for 24 hours, and then transferred to the incubator set at 20°C and again incubated for 2 hours to determine cell adhesion and detachment. Adsorption of bovine plasma fibronectin (FN) to the surface was also examined. The PIPAAm-grafted surfaces were incubated with 10μg/mL FN in phosphate buffered saline (PBS) solution at 37°C for 18h. After that supernatant FN solution was collected to estimate concentration of FN by micro BCA assay.

Results and Discussion: Figure 1 shows contact angle changes and cell adhesion on PIPAAm brush surfaces at 37°C and 20°C. PIPAAm layer thickness for AT6.3 and AT8.9 was 30.4 and 64.7nm, respectively, as determined by ellipsometry. Contact angles gradually decreased as the amount of grafted PIPAAm increases regardless of temperature. As prepared surfaces have large amount of grafted PIPAAm with relatively high density, these chains exist in expanded conformation.[4, 5] This affects cell adhesion behavior. EC adhesion on the PIPAAm brush surfaces at 37°C decreased with increasing amount of grafted PIPAAm. On the surfaces of AT7.9 and AT8.9, cell adhesion was almost negligible. Adhered cells on AT6.3 and AT6.7 detached completely from the surfaces by decreasing temperature to 20°C. In general, FN adsorbed in greater amounts to hydrophobic than hydrophilic surfaces as found in the literature.[6-8] Similar trend was also found to AT6.3 and AT8.9. FN adsorption on relatively hydrophobic AT6.3 was larger than that on AT8.9 in the present research. In the polymer brush surfaces, grafted PIPAAm exists in extended conformation, which influences polymer chain hydration and protein adsorption. Protein adsorption should influence the cell adhesion. Thus, polymer conformations defined by polymerization methods should be influential factor on cell adhesion behavior.

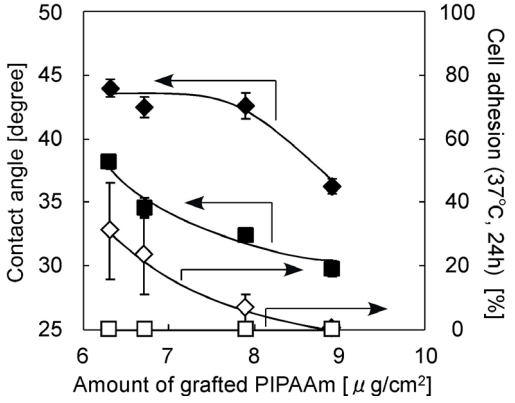

Figure 1 Surface characterization of thermoresponsive surfaces prepared by ATRP. Diamond: 37°C, square: 20°C, respectively.

Conclusions: Grafted chains of the PIPAAm brush surfaces prepared by ATRP exist in more extended conformations which promote polymer chain hydration. Hydration of polymer chains leads to the depression of cell adhesion through minimized FN adsorption.

References

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