Immobilization of bFGF on Heparinized Thermoresponsive Cell Culture Surface for Enhancing Cell Growth

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Statement of Purpose: Our laboratory have developed poly(*N*-isopropylacrylamide) (PIPAAm)-grafted thermoresponsive cell culture dish for recovery of cultured cells only by reducing temperature (Yamada N. Makromol Chem Rapid Commun. 1990;11:571-576.). For the introduction of bioactive molecules onto the surface, poly(IPAAm-*co*-2-carboxylisopropylacrylamide) (poly(IPAAm-*co*-CIPAAm))-grafted culture dishes having carboxyl groups were developed (Ebara M. Biomacromol. 2003;4:344-349.). Utilizing this surface, RGD peptide-immobilized thermoresponsive culture dish was prepared for serum-free cell culture (Ebara M. Biomacromol. 2004;5:505-510.).

In this study, we developed heparinized thermoresponsive culture dish for enhancing cell growth. Heparin is a sulfated glycosaminoglycan (GAG), consisting predominantly of a repeating disaccharide motif comprised of β-D-glucuronic acid and *N*-acetyl-*α*-*D*-glucosamine residues connected through 1→4 glycosidic linkages. Heparin has affinity interactions with various growth factors and their receptors such as fibroblast growth factor (FGF), vessel endothelial cell growth factor (VEGF) and platelet-derived growth factor (PDGF). Thus, various growth factors would be immobilized on the heparinized thermoresponsive culture dish without deactivation. Here, we investigated the adhesion of mouse fibroblast cells (NIH3T3) on heparinzed poly (IPAAm-*co*-CIPAAm)-grafted surfaces in serum-containing media, and on basic FGF (bFGF)-immobilized surfaces under serum-free conditions.

Methods: Poly(IPAAm-*co*-CIPAAm)-grafted TCPS with IPAAm/CIPAAm molar ratio of 99/1 (C1), 98/2 (C2), and 97/3 (C3) was prepared as described previously (Ebara M. Biomacromol. 2003;4:344-349.). PIPAAm-grafted TCPS was used as a control surface. Heparin was immobilized on the poly(IPAAm-*co*-CIPAAm)-grafted dishes by condensing reaction (Hep-C1, Hep-C2, Hep-C3 and Hep-PIPAAm, respectively). Then, 2 mL of bFGF solution (1 μg/cm^2) was added on the dishes, and the dishes were incubated at 37 °C for 24 h (bFGF/Hep-C1, bFGF/Hep-C2, bGF/Hep-C3and bFGF/Hep-PIPAAm, respectively). These dishes were sterilized with ethylene oxide gas. The amount of grafted polymer was determined by attenuated total reflectance Fourier transform infrared (ATR-FTIR) measurement. NIH3T3 cells were cultured on the dishes in DMEM with 10% fetal bovine serum (FBS) or an insulin-transferrin-sodium selenite supplement at 37 °C in a humidified atmosphere with 5% CO_2. NIH3T3 cells were seeded at an initial cell density of 5×10^3 cells/cm^2 onto the dishes. Cell morphology was monitored under a phase contact microscope at various time points.

Results: Amounts of grafted poly (IPAAm-*co*-CIPAAm) on the surfaces were determined to be 5 μg/cm^2 by ATR/FT-IR measurement. Successful introduction of heparin on poly (IPAAm-*co*-CIPAAm)-grafted surfaces was confirmed by the reduction of zeta potential on heparinized poly (IPAAm-*co*-CIPAAm) surfaces compared with un-heparinized surfaces.

In the DMEM containing 10% FBS, adhesion and growth of NIH3T3 cells were observed on both poly(IPAAm-*co*-CIPAAm) and heparinized poly(IPAAm-*co*-CIPAAm) surfaces at 37 °C. NIH3T3 cells did not adhere on the heparinized and un-heparinized surfaces with over 2 mol% CIPAAm contents. This was due to the enhanced surface hydration by introduction of carboxyl and sulfate groups. Pronounced cell adhesion was observed on PIPAAm, Hep-PIPAAm, C1 and Hep-C1 surfaces (Figure 1). Moreover, adhered cells detached from these surfaces by decreasing temperature to 20 °C. Growth rate of NIH3T3 cells on heparinized Hep-C1 was faster than that on C1.

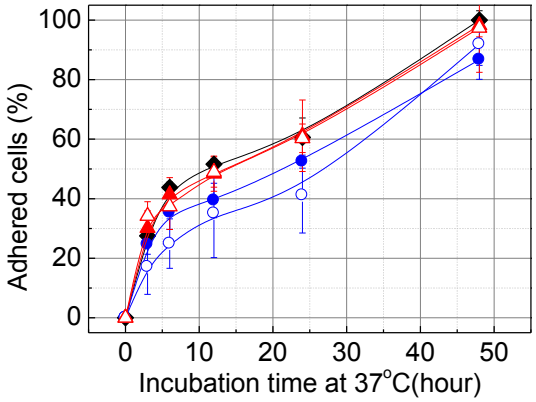

Figure 1. Adhesion of NIH3T3 cells on TCPS (diamonds), PIPAAm (closed circles), C1 (open circles), Hep-PIPAAm (closed triangles) and Hep-C1 (open triangles) at 37 °C in 10% FBS-containing medium.

To confirm the effects of serum, serum-free medium containing an insulin-transferrin-sodium selenite supplement was used. Under this condition, cell adhesion was greatly reduced compared with 10% FBS-containing media. On bFGF-immobilized heparinized poly(IPAAm-*co*-CIPAAm) surfaces (bFGF/Hep-C1, bFGF/Hep-C2, bFGF/Hep-C3), most cells adhered within 3 hours. These results indicated that growth factors such as bFGF in the FBS-containing medium were condensed on the surface through affinity interactions with heparin.

Conclusions: Heparinized thermoresponsive polymer-grafted surfaces were successfully prepared by electron beam irradiation and condensing reaction. Cell growth was by heparinize due to the condensation of growth factors in FBS. This cell culture surface would be useful for rapid fabrication of cell sheets.