Heparin functionalized thermoresponsive cell culture surfaces for regulating affinity interaction with basic fibroblast growth factor and enhancing cell sheet formation

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Statement of Purpose: Our group have developed thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm)-grafted cell culture surfaces for recovery of cultured cells (Yamada N. Makromol Chem Rapid Commun. 1990;11:571-576.) and cultured cell sheets for regenerative medicine (Kobayashi J. Sci Technol Adv Mater. 2010;11:014111.) only by reducing temperature. Currently, cell sheet-based therapies have been applied in clinical settings. In order to achieve the promotion of cell sheet technology, we have investigated next-generation thermoresponsive cell culture surfaces. Here, heparin-functionalized thermoresponsive cell culture surfaces were developed for achieving rapid cell sheet formation by enhancing the growth of cultured cells. For immobilizing bFGF in active form, poly(*N*-isopropyl-acrylamide-*co*-2-carboxyisopropylacrylamide) (poly(IPAAm-*co*-CIPAAm))-grafted surfaces were modified with heparin, which has an affinity interaction with bFGF. Furthermore, when the temperature was lowered to 20 °C, immobilized bFGF would be released with detaching cells and cell sheets, because hydration of PIPAAm chains on the surfaces reduced affinity interaction with basic fibroblast growth factor through steric hindrance of hydrated PIPAAm chains. In this study, the growth of NIH/3T3 cells on bFGF-immobilized heparinized-thermoresponsive surfaces was investigated and compared with those on PIPAAm surfaces in the presence of soluble and physically adsorbed bFGF. Eventually, the confluently cultured cells on these surfaces were harvested by low temperature treatment.

Methods: Poly(IPAAm-*co*-CIPAAm)-grafted surfaces was prepared on tissue culture polystyrene (TCPS) dishes as described previously (Ebara M. Biomacromolecules 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. Then, immobilization of bFGF was performed on the heparinized thermoresponsive surfaces by incubation of bFGF solution at 37 °C for 24 h. Amounts of immobilized bFGF on heparin-poly(IPAAm-*co*-CIPAAm) grafted surface were quantified using [^{125}I]-labeled bFGF. NIH3T3 cells were cultured on the dishes in DMEM with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2. Cell morphology was monitored under a phase contact microscope at various time points.

Results: NIH/3T3 cells on both TCPS and PIPAAm-grafted surfaces grew to confluence for 5 days. However, the minimum incubation period of NIH/3T3 on bFGF-immobilized heparin-modified thermoresponsive surfaces was reduced to 3 days. In addition, two- to threefold number of cells were proliferated on bFGF-immobilized heparin-modified thermoresponsive surfaces compared with those on both bFGF-physisorbed surface and PIPAAm surface with soluble bFGF after 3-day cultivation (Figure 1). These result indicated that heparin-mediated immobilization of bFGF on the surfaces would reinforce the formation of bFGF-FGF receptor complex. By contrast, the activity of physisorbed bFGFs on PIPAAm-grafted surfaces decreased by non-specific and randomly oriented adsorption (Figure 1). Eventually, cultured cell sheets on bFGF-immobilized heparin-modified thermoresponsive surfaces were harvested by lowering temperature to 20 °C for 45 min. Fluorescence immunostaining revealed that bFGF and extracellular matrix such as fibronectin were released and recovered with the cell sheets from the heparinized thermoresponsive surfaces. Thus, affinity interaction between bFGF and immobilized heparin was reduced by lowering temperature.

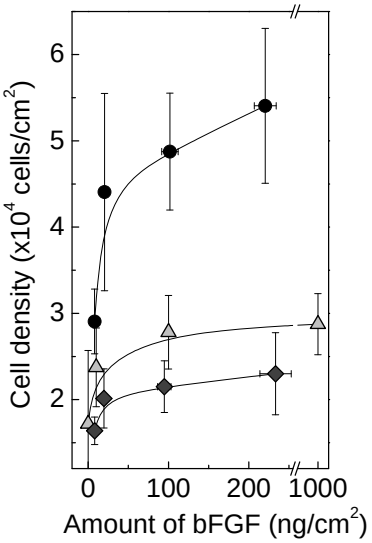

Figure 1. Relationship between the amount of bFGF and the cell density on thermoresponsive cell culture surfaces after 3-day incubation at 37 °C. Symbols represent bFGF-immobilized heparin-IC1 (circle), PIPAAm surfaces with soluble bFGF (triangle) and bFGF-physisorbed PIPAAm surfaces (diamond). The prepared poly(IPAAm-*co*-CIPAAm) grafted TCPS were coded as "IC*X*", where *X* indicates the molar percentages (mol%) of CIPAAm to the total monomers in feed.

Conclusions: bFGF-immobilization via surface-immobilized heparin on thermoresponsive surface allowed cells to grow effectively and to be harvested as a contiguous cell-sheet only by regulating affinity interaction between bFGF and immobilized heparin with temperature changes. The cultivation technology using bFGF-immobilized heparinized thermoresponsive cell culture surfaces will be useful in reducing the culture time and the cost of cell sheet fabrication.