Temperature-Controlled Capture and Release of Heparin-Binding Proteins and Cells on Heparin-Immobilized Thermoresponsive Cell Culture Substrates

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Statement of Purpose: Supplements including serum proteins and growth factors in culture medium are typically essential for maintaining cellular survival. As receptors and their ligands tend to be down-regulated through endocytosis, frequent dosing of the supplements is required for sustained stimulation of the receptors. Covalently immobilized growth factors on cell culture surfaces facilitates the long-term stimulation of the receptors without the down-regulation (Ito Y. Soft Matter. 2008;4:46-56.). However, isolation and passage processes using enzymatic treatments such as trypsinization may induce irreversible damage of the receptors, leading to the reduction of cellular functions (Okano T. J Biomed Mater Res. 1993;27:1243-1251.). In order to harvest cultured cells nonenzymatically with maintaining cellular functions, heparin-immobilized thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm)-grafted cell culture surface was designed. Immobilized heparin molecules on shrunken PIPAAm were able to capture heparin-binding proteins such as basic FGF (Arisaka Y. Biomaterials. 2013;34:4214-4222.) and heparin-binding EGF-like growth factor (HB-EGF) (Arisaka Y. Regen Ther. 2016;3:97-106.), which stimulated cultured cells (Figure 1, left). In addition, cultured cells were recovered as a sheet when lowering temperature to 20 °C (Figure 1, right). In this paper, the stimulation and activation of cultured cells on HB-EGF bound heparin-immobilized thermoresponsive cell culture surfaces were evaluated using molecular biological approaches. Detachment of cultured cell sheets by lowering temperature was also examined.

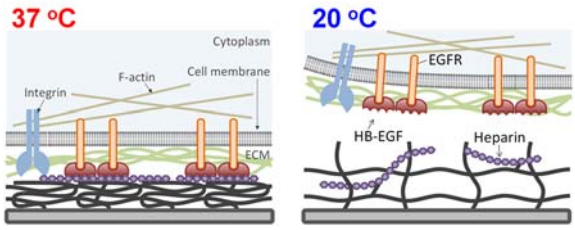

Figure 1. Schematic of temperature-controlled capture and release of heparin-binding proteins and cells.

Methods: Heparin-immobilized thermoresponsive surfaces were prepared as described previously (Arisaka Y. Biomaterials. 2013;34:4214-4222.). HB-EGF was bound onto the heparin-immobilized thermoresponsive surfaces by though affinity interaction in PBS at 37 °C for 24 h (Arisaka Y. Regen Ther. 2016;3:97-106.). Amounts of bound HB-EGF on the heparinized surface were quantified using [^{125}I]-labeled HB-EGF. A431 cells, which had an excess of EGF receptors, and rat primary hepatocytes were cultured on thermoresponsive cell culture surfaces with stimulation by soluble or immobilized HB-EGF in medium containing 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2.

Results: In order to confirm the activation and intracellular signaling of the EGF receptor, phosphorylated EGF receptor was semi-quantitatively determined by Western blotting (Figure 2). Time-course of HB-EGF stimulation revealed that phosphorylated EGF receptor was maximum in the early stage of stimulation, and gradually decreased with increasing incubation time. There are no obvious difference between soluble and immobilized HB-EGF. However, residual EGF receptors on immobilized HB-EGF were maintained compared with soluble HB-EGF stimulation. Therefore, internalization of EGF receptor on heparin-immobilized surface was suppressed by affinity interaction during a few days incubation. We also confirmed that 80-90% of bound HB-EGF was remained on the surface during 4-day incubation in the medium containing 10% FBS, indicating stable binding of HB EGF on the surfaces. Hepatic functions such as albumin secretion were maintained and higher compared to those on PIPAAm-grafted surfaces with soluble HB-EGF stimulation (Arisaka Y. Regen Ther. 2016;3:97-106.).

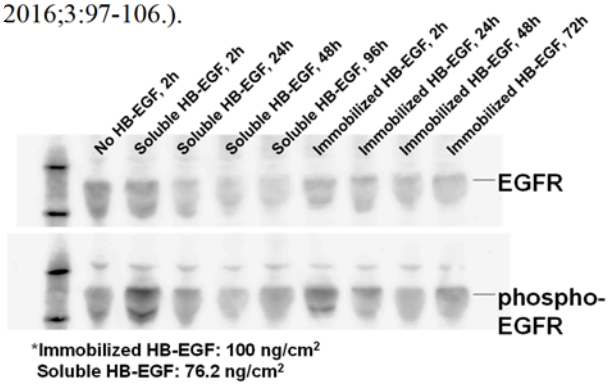

Figure 2. Western botting of EGFR and phosphorylated EFGR on hepatocytes

Finally, when lowering temperature to 20 °C, the cultured hepatocyte sheets were detached from the surface. This detachment was considered to be through the reduction of affinity binding between HB-EGF and immobilized heparin with increasing the mobility of heparin and steric hindrance of swollen PIPAAm chains (Figure 1, right).

Conclusions: Heparin-immobilized thermoresponsive cell culture surfaces facilitated temperature-controlled capture and release of heparin-binding proteins and cells. A cultivation technology using heparin-immobilized thermoresponsive cell culture surfaces is considered to have a potential to provide transplantable liver tissues with maintaining hepatic functions.

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