Surface Design of Heparin-Functionalized Thermoresponsive Cell Culture Substrates for Maintaining Hepatic Functions and Harvesting Cultured Hepatocyte Sheets

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Statement of Purpose: Our laboratory have developed a unique technique for harvesting cultured cell sheets (Kushida A. J Biomed Mater Res. 1999;45:355-362.) only by lowering temperature from thermoresponsive poly(*N*-isopropylacrylamide) (PIPAAm)-grafted cell culture surface, which exhibits temperature-dependent hydrophilic/hydrophobic changes (Yamada N. Makromol Chem Rapid Commun. 1990;11:571-576.). These cultured cell sheets have been utilized to create and transplant functional tissues for treating a wide range of diseases. Hepatocyte sheet-based tissue engineering is also an attractive method for the treatment of liver diseases (Ohashi K. Nat Med. 2007;13:880-885.). Hepatocyte sheets were effectively engrafted and exhibited liver-specific functionalities. This indicated that the transplanted hepatocyte sheets were connected to host blood vessels thanks to the environment in living body. By contrast, cultured hepatocytes rapidly lose their phenotypic functions on isolation from the native *in vivo* microenvironment of the liver. As cell-sheet-based therapy has evolved, our attention has been focused on some challenging issues such as regenerating functional liver tissues. In this paper, heparin-functionalized PIPAAm-grafted cell culture surface (Arisaka Y. Biomaterials. 2013;34:4214-4222.), which interacts with heparin-binding proteins such as heparin-binding EGF-like growth factor (HB-EGF), was been designed for maintaining hepatic functions during the cultivation (Figure 1).

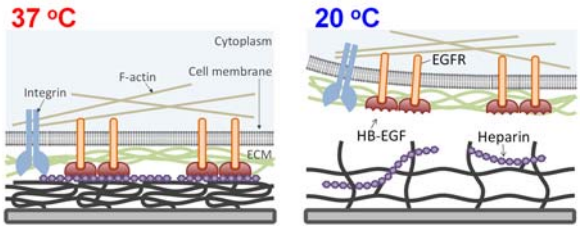

Figure 1. Affinity regulation between heparin-binding protein and immobilized heparin by conformational change of PIPAAm.

Methods: Heparin-functionalized thermoresponsive surfaces were prepared as described previously (Arisaka Y. Biomaterials. 2013;34:4214-4222.). Briefly, poly(IPAAm-*co*-CIPAAm)-grafted surfaces on tissue culture polystyrene (TCPS) dishes was prepared by electron beam irradiation. Heparin was covalently immobilized on the poly(IPAAm-*co*-CIPAAm)-grafted dishes by condensing reaction. Then, affinity binding of HB-EGF was performed on the heparinized thermoresponsive surfaces by incubation of HB-EGF solution at 37 °C for 24 h. Amounts of bound HB-EGF on the heparinized surface were quantified using [^{125}I]-labeled HB-EGF. Primary rat hepatocytes were seeded on the dishes in DMEM with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2.

Results: The addition of soluble HB-EGF in the cell culture media was essential for the survival of hepatocytes. When the medium contained less than 10 ng/cm^2 of soluble HB-EGF, the hepatocytes were not able to adhere and form their cell sheets. By contrast, hepatocytes adhered and formed their sheets on heparin-functionalized thermoresponsive surface with 10 ng/cm^2 of bound HB-EGF. In addition, the secretion of albumin on bound HB-EGF was maintained and higher than that on PIPAAm-grafted surfaces with soluble HB-EGF (Figure 2). Therefore, bound HB-EGF gave a high activity of maintenance of hepatocyte adhesion and function compared with soluble HB-EGF. Finally, by lowering temperature to 20 °C, the cultured cell sheets were detached from the surface through the reduction of affinity binding between HE-EGF and immobilized heparin with increasing the mobility of heparin and the swollen PIPAAm.

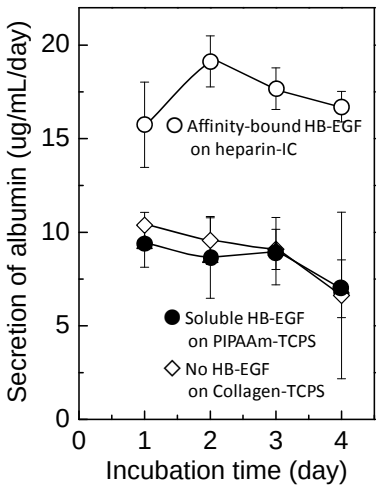

Figure 2. Albumin secretion of cultured hepatocyte sheets was determined by ELISA.

Conclusions: The function of hepatic cell sheet was maintained by using heparin-functionalized thermoresponsive cell culture surfaces. Creation of functional liver tissues is considered to have a potential to treat liver disease.

Acknowledgements: This work was financially supported by the Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems "Cell Sheet Tissue Engineering Center (CSTEC)", and by Grant-in-Aid for Scientific Research on Innovative Areas "Bio Assemble" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.