Ex Vivo Gene Delivery for Fabrication of Hepatocyte Sheet Tissues Secreting Angiogenic Factors

Jun Kobayashi,[1] Hyukjin Lee,[2] Masayuki Yamato,[1] Teruo Okano[1] ([1]Tokyo Women's Medical University, [2]Ewha Womans University)

Introduction

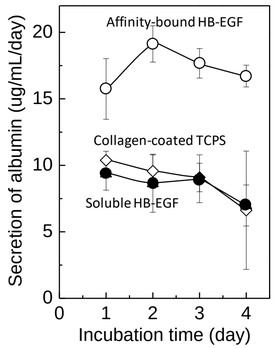
Liver tissue engineering is an attractive method for the treatment of liver diseases, as well as an *in vitro* tissue model for drug screening. Creation of liver tissues using hepatocytes is one of promising methods for the treatment of liver diseases such as enzyme deficiencies and hemophilia. Our laboratory developed temperature-responsive cell culture dish bound with growth factor, heparin-binding EGF-like growth factor for the creation of transplantable hepatocyte sheets with maintaining hepatic functions.[1] Transplantation of hepatocyte sheets was able to be engrafted in a pre-vascularized subcutaneous site, resulting in long-term secretion of hepatocyte-specific enzyme.[2] For effective engraftment of transplanted tissues, we focused on the fabrication of hepatocyte sheet tissues secreting angiogenic factors using *ex vivo* non-viral transfection of pDNA or mRNA encoding VEGF.

Methods

Heparin-modified poly(*N*-isopropylacrylamide) (PIPAAm)-grafted surface was prepared as described previously.[1] HB-EGF was bound onto the heparin-modified temperature-responsive surfaces by though affinity in PBS for 24 h at 37 ˚ C. Rat primary hepatocytes were cultured on the temperature-responsive 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. In addition, the expression of transgenes including enhanced green fluorescent protein (EGFP) and human VEGF genes on cultured rat primary hepatocytes was analyzed after the transfection of pDNA or mRNA using Lipofectamine 2000.

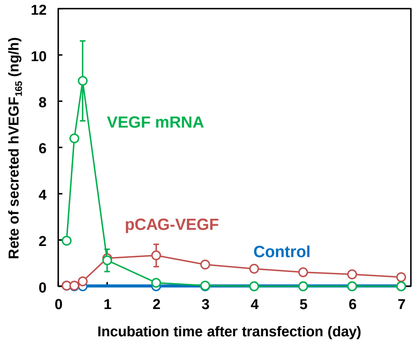
Results and Discussion 1

The secretion of albumin from cultured hepatocytes on HB-EGF-bound heparin-modified temperature-responsive surface was significantly higher compared to that on PIPAAm-grafted surfaces with soluble HB-EGF. The cultured cell sheets were detached from the surface through the reduction of affinity binding between HB-EGF and immobilized heparin with increasing the mobility of heparin and the swollen PIPAAm chains by lowering temperature to 20 ˚ C. Therefore, functional hepatocyte sheets were fabricated using the heparin-modified temperature-responsive surfaces.



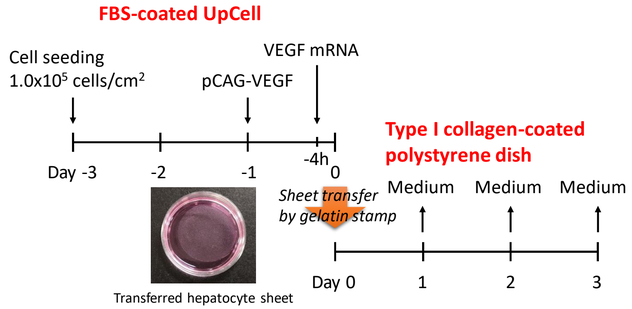
Results and Discussion 2

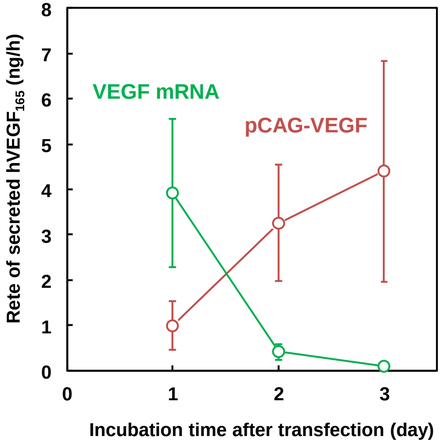
VEGF transgene was transfected into cultured hepatocytes on collagen-coated surface. Transfected hepatocytes with VEGF mRNA rapidly secreted VEGF within 24 hours, resulting in the cumulative VEGF secretion of ca. 140 ng. By contrast, the transfection of VEFG pDNA exhibited much lower secretion of VEGF than that of mRNA. However, the hepatocytes transfected with VEGF pDNA continuously secreted during 7 days of culture and the cumulative secretion of VEGF was reached to ca. 3 mg. Thus, rapid expression of angiogenic factors from non-proliferative hepatocytes can be achieved by using mRNA transfection.



Results and Discussion 3

VEGF gene-transfected hepatocyte sheet was able to be transferred onto another culture dish using a gelatin stamp. It was confirmed that the transferred hepatocytes sheet secreted human VGEF.





Summary

A heparin-modified temperature-responsive cell culture surface facilitated temperature-controlled capture and release of HB-EGF and hepatocytes. A combination of the heparin-modified temperature-responsive cell culture surface and mRNA delivery technology is considered to have a potential to provide the engraftment of transplanted liver tissues with maintaining hepatic functions.

Acknowledgement: This work was supported by JSPS KAKENHI grant numbers JP18H03537 and JP18KK0415

References: 1) Y. Arisaka, J. Kobayashi, et al., Regen Ther 2016; 3, 97.; 2) K. Ohashi, et al., Nat Med. 2007; 13, 880.