Cell Sheet Engineering: Intelligent Polymer Patterned Surfaces for Tissue Engineered Liver

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Summary: We describe a new culture system utilizing the temperature-responsive polymer grafted surface for designing of cell position and layered tissue reconstruction. Organizing of the hepatic tissue structure by controlling the culture system, that is patterned co-culture and layered cell sheet co-culture achieved by moving the cultured cells from the culture surface, resulted in regulation of the hepatocyte function. The technique for cell sheet manipulation would promote the liver tissue engineering in quality.

Keywords: cell sheet; co-culture; hepatocyte; poly (*N*-isopropylacrylamide); temperature-responsive culture surface

Introduction

Development of tissue engineering has been accelerated with new technologies. Previously, various cell-based constructs such as cartilage, bone, blood vessel, and urinary bladder has achieved by using biodegradable polymer as culture scaffold. These constructs are randomly formed of cell-material combination. However in liver tissue engineering, it is difficult to reconstruct of functional tissue by only this relatively simple technique. Because liver has many kind of cells, more complex but systematic three-dimensional tissue structure, vascular-connection and highly metabolic cellular function. Further advanced technique is required for the basic cell culture system to achieve the controllable cell positioning, 'patterning' culture and

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reconstruction of *in vivo* like 'layered tissue' composed of cell sheet units. We here report the novel cell manipulation technique utilizing intelligent temperature-responsive culture surface.

Temperature-Responsive Cell Culture Surface

The cell culture surface we have developed responds reversibly and dynamically to temperature changes.[1] The temperature-responsive polymer, poly (*N*-isopropylacrylamide) (PIPAAm),[2] was covalently grafted to tissue culture polystyrene (TCPS) dishes by electron beam irradiation. The PIPAAm-grafted surfaces are relatively hydrophobic at 37°C compared to the TCPS dishes, because the PIPAAm chains are aggregated due to dehydration and lie compactly on the surface. At reducing temperature below the lower critical solution temperature of 32°C, the grafted PIPAAm rapidly hydrates and becomes hydrophilic. On the PIPAAm surface, cells adhere, spread and proliferate at 37°C as well as TCPS dish, and detach at the lower temperature 20°C without enzymatic digestion or divalent cation chelators. Loose interaction between the cell layer and the culture surface caused by structural change of hydrated PIPAAm resulted in cell detachment via their tractional forces[3] without cell injury. Therefore, the PIPAAm surface induced both proliferating single cell detachment together with their producing extracellular matrix (ECM), and confluent cell layer 'cell sheet' detachment with underlying ECM and remaining cell-cell junction. The detaching units including the cell and the ECM were gradually floated in culture medium as temperature decrease.

Temperature-Responsive Patterned Surface

Since electron beam is easily shielded by thin masks, patterned graft of PIPAAm is achieved. (Fig.1). In addition, the phase transition temperature can be controlled by copolymerization with other monomers. Therefore, PIPAAm-patterned surfaces have two advancements comparing with other patterning surfaces. The cells are movable from PIPAAm-patterned surfaces keeping the cell sheet shapes as well as micropatterning.[4] And various cell types co-cultured can be combined limitlessly while the cells show resemblance in adherent abilities. When electron beam was radiated through a mask having many circular holes onto tissue culture polystyrene (TCPS) dishes with the monomer solution, PIPAAm was grafted as circular domains, which is temperature-responsive, and outside was naked TCPS, which is cell adhesive temperature-independently. On

these culture dishes, parenchymal hepatocytes and nonparenchymal cells could be co-cultured as following. First, parenchymal hepatocytes were seeded and adhered on the whole surfaces at 37°C, then hepatocyte were detached only from the PIPAAm domains by reducing temperature below 32°C. Finally, nonparenchymal cells were seeded on the same surfaces at 37°C. The endothelial cells and fibroblasts adhered not on the hepatoytes but on PIPAAm domains.

In order to prepare cell non-adhesive domains, highly hydrophilic polymers such as poly(*N,N'*-dimethylacrylamide) and polyacrylamide are grafted by electron beam irradiation. As shown in Figure 1b, square patterned PIPAAm surfaces were achieved by using square glass coverslips for the graft of highly hydrophilic polymers onto PIPAAm-grafted surfaces. The glass slips shielded electron beam so that highly hydrophilic polymers were grafted only outside the slip. With the patterned surfaces, square cell sheets are prepared. The cultured cell positioning including the area shape and size could be regulated by combination of the temperature-responsive polymer and mask patterns.

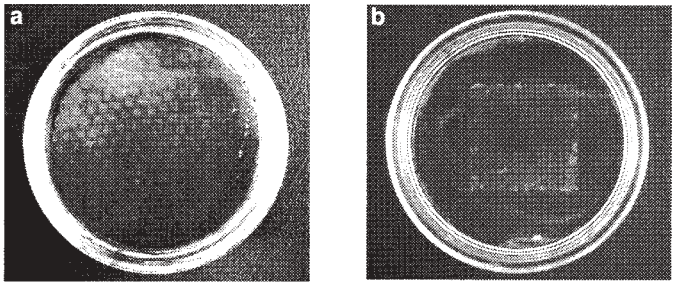

Figure 1. Macroscopoic views of patterned cell culture. Cells were cultured on PIPAAm grafted culture dish surface with porous (a) and square (b) patterns.

Co-culturing of parenchymal hepatocyte with nonparenchymal cells had positive effect on continuous hepatic albumin expression. Interestingly the different culture conditions of hepatocytes co-cultured with endothelial cells, and with fibroblasts, and without co-cultured cells,

resulted in different hepatic albumin expression (Figure 2). Higher albumin expression was observed when hepatocytes were co-cultured with endothelial cells. This culture condition would reflect the environment of hepatocytes along sinusoids in liver lobules.

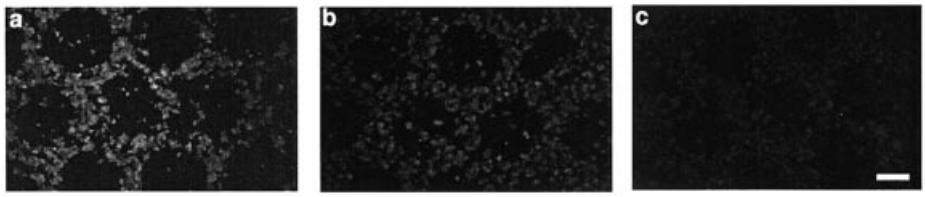

Figure 2. Immunostaining of albumin in hepatocytes. Hepatocytes were co-cultured with endothelial cells (a) or fibroblasts (b) on day 7. These co-cultured cells are present in the circular domains having 1-mm diameter. Homotypic culture shows the basal albumin expression (c). Bar, 100 μm.

Layered Cell Sheets Co-Culture

Volume increase of tissue engineered constructs is needed for clinical use. However, little is developed about systematic technique for three-dimensional hepatic tissue reconstruction. For example, it is well known that hepatic spheroids show lower cell viability in the inner mass when the size is increased. Therefore, the novel technique for reconstructing the three-dimensional tissue structures have been required. Beyond the plane limitation of the co-culture by extending the PIPAAm-grafted surfaces, we established a contiguous layered co-culture system formed by cell sheets of hepatocytes and endothelial cells to achieve the functional units like liver lobules. The vascular endothelial cells were cultured on the PIPAAm-grafted dishes to obtained the movable endothelial cell sheets. The endothelial cell sheets recovered from the PIPAAm-grafted surfaces are moved onto the cultured hepatocytes directly (Figure 3). In this co-culture system, the double layered structures and the hepatic albumin expression were maintained during prolonged culture more than one months.[5] The endothelial cells overlaid on the hepatocytes also showed

higher uptake of acetylated-LDL than the homotypically cultured control. The cell sheet manipulation and layered tissue reconstruction could not be limited to the combination of these cell types. These techniques to modulate spatial cell positioning and cell-cell interactions, and cell functions would open the novel way in tissue engineering toward the ideal tissue reconstruction.

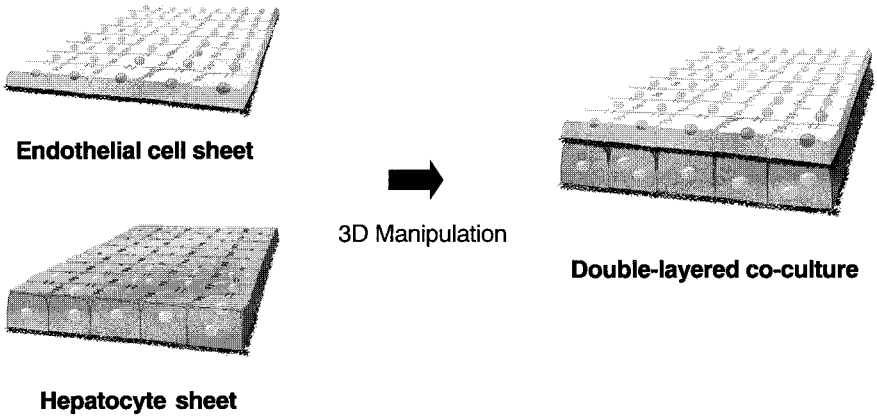

Figure 3. Schematic drawing of the double layered cell sheets co-culture by three-dimensional manipulation of the endothelial cell sheet onto the hepatocyte monolayer.

[1] N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, Y. Sakurai, *Macromol. Chem. Rapid Commun.* **1990**, *11*, 571.

[2] YH. Bae, T. Okano, SW. kim, *J. Polym. Sci. Polym. Phys.* **1990**, *28*, 923.

[3] M. Yamato, M. Okuhara, F. Karikusa, A. Kikuchi, Y. Sakurai, T. Okano, *J. Biomed. Mater. Res.* **1999**, *44*, 44

[4] M. Hirose, OH. Kwon, M. Yamato, A. Kikuchi, T. Okano, *Biomaceomolecules* **2000**, *1*, 377.

[5] M. Harimoto, M. Yamato, M. Hirose, C. Takahashi, Y. Isoi, A. Kikuchi, T. Okano, *J. Biomed. Mater. Res.* **2002** (*in press*).