

Control of cell function on carbohydrate-immobilized phosphorylcholine polymer surfaces

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INTRODUCTION: We hypothesized that 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer surfaces bearing carbohydrates might perform as biomembrane mimetic surfaces, which can interact with a specific cell. In this study, MPC copolymers with galactose residues have been synthesized and we present here the effectiveness of the surface in controlling cell/material interaction and preserving cell function.

METHODS: Poly[MPC-co-n-butyl methacrylate (BMA)] (PMB), poly[BMA-co-2-lactobionamidoethyl methacrylate (LAMA)] (PBL), and poly(MPC-co-BMA-co-LAMA) (PMBL) were synthesized by conventional radical polymerization¹.

Human hepatocellular liver carcinoma cell line (HepG2) cells and mouse fibroblasts (NIH-3T3) were purchased from RIKEN Cell Bank. The concentration of the cells was adjusted to 2.0×10^4 cells/ml. The cells were seeded on the polymer surfaces and continuously cultured for specific periods. The polymer plates were then rinsed with PBS. The plates were soaked into Triton X-100 aqueous solution. The Triton X-100 solution was collected and the concentration of LDH from the adherent cells was measured.

Morphological observation of the HepG2 cells cultured on the polymer surfaces was performed by a confocal laser scanning microscope.

RESULTS: Figure 1 shows the time-dependent surface density of HepG2 and NIH-3T3 cells on a polymer surface after culture for given periods. On a PBMA surface, many HepG2 cells adhered and the density increased with an increase in culture time. In contrast, the cell adhesion was reduced on the PMB surface because adsorption of

the cell-adhesive protein on the surface could be reduced (data not shown). When the LAMA composition was 3% in PMBL, the density of adherent HepG2 cells was similar to that on PBMA for every culture period. NIH-3T3 cells adhered and proliferated as well as the HepG2 cells on the PBMA surface. On the other hand, the adhesion of NIH-3T3 cells was reduced on the polymer surfaces having MPC units.

Figure 2 shows confocal micrographs of HepG2 cells cultured on PBMA, PBL1.0, and PMBL1.0 for 168 h. On PBMA and PBL1.0 surfaces, monolayer cell adhesion was observed and each cell was spread. At the outline of the pseudopod formation of the adherent cells, actin was easily observed. In contrast, HepG2 cells cultured on PMBL1.0 formed spheroids with multilayer adhesion.

DISCUSSION & CONCLUSIONS:

Carbohydrate-immobilized phosphorylcholine polymers (PMBL) were newly synthesized to produce biomembrane mimetic surfaces, which perform selective recognition of proteins and cells. Surfaces coated with PMBL effectively reduced nonspecific interaction, and specific ligand/receptor interaction was clearly demonstrated. Changing the types of carbohydrates enables changes in the types of biorecognition. The polymers have great potential for bioreaction, molecular separation, targeting, sensing, etc.

REFERENCES: ¹ Y. Iwasaki, U.Takami, Y.Shinohara, et al (2007) *Biomacromolecules*, in press.

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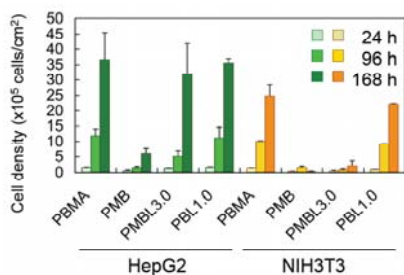


Fig. 1: Cell density on polymer surfaces.

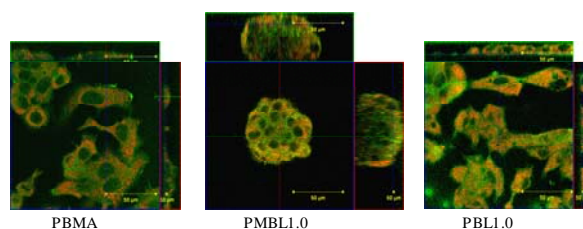


Fig. 2: Fluorescence micrographs of adherent cells.