Control of cell function on carbohydrate-conjugated phosphorylcholine polymer surfaces

Yasuhiko IWASAKI, Utae TAKAMI, Yurika SHINOHARA, Kazunari AKIYOSHI
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University

Introduction: In natural environment, carbohydrates on the cell surface contribute to most communications between the cell and its environments. We have been studying 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers synthesized as biomimetics to cell membrane structures. The MPC polymers exhibit a surface property that resists nonspecific protein adsorption and cell adhesion, i.e., "biofouling" [1]. It was hypothesized that carbohydrate-immobilization on a MPC polymer surface might be promising way to control protein/material or cell/material interactions. In this study, MPC polymers having carbohydrate units (lactose residues) were newly synthesized and control of cell adhesion and function on the polymer surface was demonstrated.

Methods: Poly[MPC-co-*n*-butyl methacrylate (BMA)] (PMB) and poly(MPC-co-BMA-co-LAMA) (PMBL) were synthesized by radical polymerization using 2,2'-azobisisobutyronitrile as an initiator. The structure of PMBL is shown in Figure 1.

The synthetic polymers were coated on disk-shaped PBMA or PET plates (ϕ=14 mm) by solvent evaporation technique. Surface analyses of polymer films were performed by contact angle measurement and X-ray photoelectron spectroscopy (XPS).

Human hepatoma cell line (HepG2) cells were seeded onto each polymer plate at a density of $2x10^4$ cells/mL of DMEM medium containing 10% FBS. The cell density on a polymer surface at varied culture periods was determined LDH-cytotoxic test. The morphological evaluation of cells cultured on polymer surfaces was carried out using the confocal microscope system. The amount of albumin secreted from HepG2 cultured on polymer surfaces was determined by ELISA.

Results/Discussion: The time dependence of density of HepG2 cells on polymer surfaces was shown in Figure 2. On PBMA surface, a lot of cells were adhered and the density was increased with an increase in the culture time. In contrast, cell adhesion was reduced on PMB surface because adsorption of cell adhesive protein could be reduced on the surface. According to HepG2 cells have asialoglycoproteinreceptor (ASGPR), which is galactose-recognizing receptor, the cell adhesion was induced on the phosphorylcholine polymer surface having LAMA units. The cell density increased with an increase in the composition of LAMA unit in the copolymers and was almost similar to that on PBMA when the LAMA composition was 3 %. Mouse fibroblast (NIH-3T3) cells were also cultured on the polymer surfaces. NIH-3T3 cells do not have ASGPR. On PBMA surface, NIH-3T3

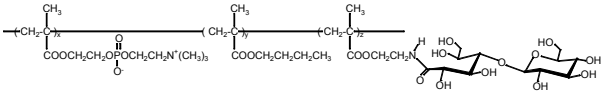

Figure 1. Structure of PMBL

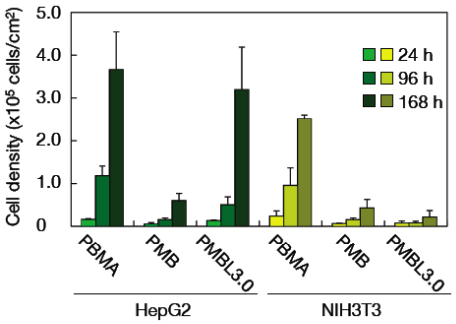

Figure 2. Cell density on polymer surfaces

cells adhered and proliferated as well as HepG2 cells. On the other hand, the adhesion of NIH-3T3 cells was reduced on the polymer surfaces having MPC units. This result indicates that the ligand/receptor interaction at the polymer/cell interface was preferably worked on the MPC polymers.

Figure 3 shows confocal micrographs of HepG2 cells cultured on PBMA and PMBL3.0 (MPC/BMA/LAMA =20/77/3 in mol%) for 96 h. On PBMA surface, the monolayer adhesion of cells was observed and each cell was well spread. On the other hand, HepG2 cells cultured on PMBL3.0 formed spheroids with multilayer adhesion. The uniform-sized spheroids were studded on PMBL3.0 surface.

The amount of albumin secreted from HepG2 cells cultured on PBMA and PMBL3.0 was compared. When the amount of albumin secreted from HepG2 cells for 24 h after 2 weeks-cultivation on polymer surfaces was determined, the amount on PMBL3.0 was approximately ten times compared with that on PBMA.

Conclusions: The biomembrane like surface phosphorylcholine polymer conjugated with carbohydrates was prepared. On this polymer surface ligand/receptor interaction was preferably performed and the surface structure strongly influenced cell functions.

References: [1] Iwata R et al., *Biomacromolecules* 2004;5:2308.

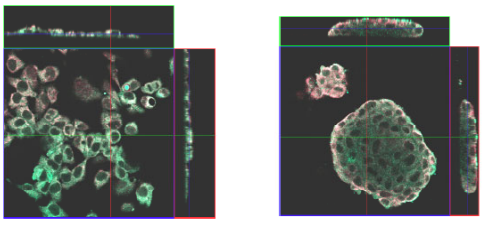

Figure 3. Confocal micrographs of HepG2 cells on polymer surface