Selective Biorecognition and Preservation of Cell Function on Carbohydrate-Immobilized Phosphorylcholine Polymers

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To obtain synthetic materials capable of selectively recognizing proteins and cells, and preserving their functions, biomembrane mimetic polymers having a phospholipid polar group and carbohydrate side chains were designed. Poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-*n*-butyl methacrylate (BMA)-*co*-2-lactobionamidoethyl methacrylate (LAMA)] (PMBL) was synthesized and coated on substrates by solvent evaporation. Selective binding of galactose-recognized lectin, RCA_{120}, to a PMBL surface was investigated by measurement of surface plasmon resonance. The binding of RCA_{120} to the PMBL surface was confirmed by a remarkable change in resonance angle. The apparent affinity constant of RCA_{120} to PMBL3.0 (3.0 mol % LAMA unit in the feed) per LAMA unit was 2.77×10^5 M^{-1}. When a glucose-recognized lectin, concanavalin A, was in contact with PMBL, no change in the resonance angle was observed, and any nonspecific fouling of protein on PMBL was effectively reduced. Cells of the human hepatocellular liver carcinoma cell line (HepG2) having asialoglycoprotein receptors (ASGPRs) were seeded on polymer surfaces. On poly(BMA) (PBMA), many adherent cells were observed and were well-spread with monolayer adhesion, but cell adhesion was reduced on poly(MPC-*co*-BMA) (PMB). HepG2 adhesion was observed on PMBL because the cell has ASGPRs; the number of cells adhering to the PMBL polymer surfaces increased with an increase in the density of galactose residues on the surface. In contrast, adhesion of NIH-3T3 cells to PMBL was reduced in a manner similar to that on PMB because the NIH-3T3 cells did not have ASGPRs. Cell adhesion to the PMBL surface was well-regulated by ligand−receptor interactions. Furthermore, some of the cells adhering to the PMBL surface had a spheroid form, and similarly shaped spheroids were scattered on the surface. Although poly(BMA-*co*-LAMA) (PBL) has galactose residues, the adherent cells were spread in a manner similar to those on PBMA. The MPC units in PMBL contribute to make a spheroid formation of HepG2 cells. The amount of albumin secreted from a cell was compared with the chemical structure of the substrate. The spheroid shaped cells cultured on the PMBL surface secreted much more albumin than did the spreading cells that adhered to the PBMA. In conclusion, the biomembrane mimetic carbohydrate-immobilized phosphorylcholine polymers produced a suitable surface for biorecognition and preservation of cell function.

Introduction

Control of cell adhesion and the function of synthetic polymers are important in molecular separation, biosensors, and development of biomedical materials.[1,2] In this article, we explore the ability of biomembrane mimetic polymer surfaces to recognize specific biosubstances from their physiological conditions.

Using physicochemical and biological approaches, various surface conditions, such as a wettability gradient,[3] topological patterns,[4,5] peptide immobilization,[6−13] and carbohydrates[14−20] have been applied to control cell attachment. The physicochemical or topological systems enable control of the surface density of adherent cells, but these surfaces cannot recognize the specific type of cell. By ligand immobilization on a synthetic surface, recognition selectivity could be improved. Arg−Gly−Asp (RGD) immobilized surfaces have been studied extensively to induce cell adhesion based on ligand−receptor interactions.[6,11−13] While these approaches improved cell adhesion, the selectivity of RGD is limited because the RGD sequence is not unique for a specific cell. The immobilization was generally performed on conventional polymers, and nonspecific interactions occurred between the cell and the synthetic surface.

Carbohydrates are also one of the most reliable candidates as ligands immobilized on a surface because carbohydrates on a cell surface contribute to most forms of communication between living cells and their environment.[16] In particular, galactose residues are preferably conjugated polymers to interact with hepatocytes, which are asialoglycoprotein receptors (ASGPRs). The ASGPR is a lectin for receptor-mediated endocytosis found at the hepatocyte cell surface, which is bound to galactose/*N*-acetylgalactosamine (GalNAc)-terminated ligands

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Table 1. Synthetic Results and Surface Characterization of PMBL

	molar fraction (MPC/BMA/LAMA)				XPS elemental data (%)[a]			
abbreviation	in feed[b]	in copolymer[c]	M_w (10^5)	M_w/M_n	C	O	N	P
PMBL0.5	0.200/0.795/0.005	0.192/0.801/0.006	4.7	2.1	71.4	25.1	1.3	2.2
					(72.8)	(23.9)	(1.7)	(1.6)
PMBL1.0	0.200/0.790/0.010	0.172/0.817/0.011	3.7	1.7	70.5	25.3	1.8	2.4
					(73.2)	(23.8)	(1.6)	(1.5)
PMBL3.0	0.200/0.770/0.030	0.180/0.801/0.019	3.4	1.8	70.2	26.5	1.6	1.7
					(72.5)	(24.3)	(1.7)	(1.5)
PMB	0.300/0.700/−	0.314/0.686/−	7.4	1.8	71.0	25.2	1.3	2.5
					(69.7)	(25.4)	(2.4)	(2.4)
PBL1.0	−/0.990/0.010	−/0.992/0.008	2.4	1.5	−	−	−	−
					(79.4)	(20.5)	(0.1)	(−)

[a] Takeoff angle = 90°. Theoretical elemental data calculated from bulk concentration are in parentheses. [b] [Monomer] = 1.0 M, [AIBN] = 5 mM, temperature = 60 °C, and solvent is EtOH/DMSO. [c] Determined by phosphorus analysis and anthrone sulfuric acid method.

in a calcium-dependent manner.[17,18] Although the ASGPR does not function physiologically as an adhesion receptor, galactose-containing polymers have been used to induce adhesion in primary hepatocytes.[19−21] Various polymers bearing galactose residues as ligands were prepared for drug delivery[22] and cellular matrices.[23] While these approaches have been quite successful, limitations remain in terms of selective recognition.[24] Indeed, most earlier reports did not focus on the reduction of nonspecific interactions.

We have been studying 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers synthesized as biomimetics of phospholipids in a biomembrane.[25−28] MPC polymers have a surface that resists nonspecific protein adsorption and cell adhesion (i.e., biofouling).[29,30] Biofouling reduces the functionality of a material and can induce an unexpected bioreaction. Further, it has been shown that cells in contact with MPC polymers do not exhibit activation or an inflammatory response.[31,32] Although nonfouling phenomena of MPC polymer surfaces have been extensively studied, carbohydrate-immobilized MPC polymers have not been synthesized for creating biorecognition surfaces.

We hypothesized that MPC polymer surfaces bearing carbohydrates might perform in the same way as biomembrane mimetic surfaces, which can interact with a specific cell and control its function. In this study, an MPC copolymer with galactose residues has been synthesized for the first time, and we present here the effectiveness of the surface in controlling cell−material interactions and preserving cell function.

Materials and Methods

Polymer Synthesis. *n*-Butyl methacrylate (BMA) was purified by conventional distillation. MPC was synthesized as previously reported.[33] 2-Lactobionamidoethyl methacrylate (LAMA) was synthesized by reacting lactobionolactone and 2-aminoethyl methacrylate hydrochloride.[34] Other reagents and solvents were obtained commercially in extra-pure grade and used without further purification.

Poly(MPC-*co*-BMA) (PMB), poly(BMA-*co*-LAMA) (PBL), and poly(MPC-*co*-BMA-*co*-LAMA) (PMBL) were synthesized by conventional radical polymerization using 2,2′-azobisisobutyronitrile (AIBN) as an initiator. BMA was used as a comonomer because it enables the processing of a polymer membrane. The mole fraction of the MPC and LAMA units in the polymer was determined by phosphorus analysis and the anthrone sulfate method, respectively. The number-averaged and weight-averaged molecular weights of the polymers were determined with a Tosoh gel permeation chromatography (GPC) system with a refractive index detector and size exclusion columns (Polymer Laboratories Ltd.) and MIXED-C with a poly(methyl methacrylate) standard (PMMA, Tosoh standard sample) in $CHCl_3$ or $CHCl_3$/methanol (6:4, vol/vol). The results of polymerization are summarized in Table 1.

Preparation of Sample Plates for Cell Culture Experiments. Poly(ethylene terephthalate) (PET) plates (14 mm in diameter; Wako Pure Chemical Industries, Ltd.) were immersed in an ethanol or toluene solution of the polymer (0.5 wt %) and dried under a vapor atmosphere of the solvent at room temperature for 30 min. This procedure was repeated twice, after which the plates were dried in vacuo. The surface properties of the polymer-coated PET plates were analyzed by surface contact angle measurement (G-1, Erma) and X-ray photoelectron spectroscopy (XPS; Kratos-Shimazu) with a magnesium anode (takeoff angle of 90°). Survey scan spectra of C_{1s}, O_{1s}, N_{1s}, and P_{2p} were taken.

Selective Binding of Lectins on Polymer Surfaces. The binding of lectins on the polymer surfaces was determined by asurface plasmon resonance (SPR; Moritex) sensor. Gold-spattered sensor chips were purchased from Moritex, and the polymers were further coated using a spin coater (ACT-220A, ACTIVE Co., Ltd.) at 4000 rpm from a 0.25 wt % solution. The running buffer for the SPR was a phosphate buffered solution (PBS; 150 mM sodium chloride, pH 7.4). Galactose-recognizing lectin (RCA_{120}; Vector Laboratories, Inc.) or glucose binding lectin, concanavalin A (Con A; Vector Laboratories, Inc.), was in contact with the polymer-coated sensor chips. After the lectin solution was in contact with the polymer surfaces for 10 min, the surfaces of the sensor chips were rinsed with running buffer. The change in resonance angle due to the adsorption/desorption of lectin was monitored. Apparent affinity constants per LAMA unit (K_a) and changes in maximum angle ($\Delta\theta_{max}$) were calculated from the slope and intercepts according to Langmuir (eq 1)[35]

$$[\text{lectin}]/\Delta\theta = [\text{lectin}]/\Delta\theta_{max} + 1/\Delta\theta_{max}K_a \qquad (1)$$

Cell Culture Experiments. Before the cell culture experiments were performed, protein adsorption on the polymer surfaces from the culture medium was determined because cell adhesion is strongly dependent on protein adsorption. PBMA-, PMBL-, and PBL-coated PET plates were placed in contact with PBS to equilibrate their surfaces. The plates were then soaked in the culture medium (Eagle's MEM; Nissui Pharmaceutical) containing 10% fetal bovine serum (FBS) at 37 °C. The amount of adsorbed proteins on the polymer surfaces from 10% FBS was determined by the micro-BCA method.[36]

Human hepatocellular liver carcinoma cell line (HepG2) cells and mouse fibroblasts (NIH-3T3) were purchased from RIKEN Cell Bank. The cells were maintained in a culture medium containing 10% FBS at 37 °C in a humidified atmosphere of air containing 5% CO_2. For cell maintenance, the contents of the flasks were detached by trypsin treatment. The concentration of the cells was adjusted to 2.0×10^4 cells/mL. The cells (1 mL suspension) were seeded on the polymer surfaces and continuously cultured for specific periods in the CO_2

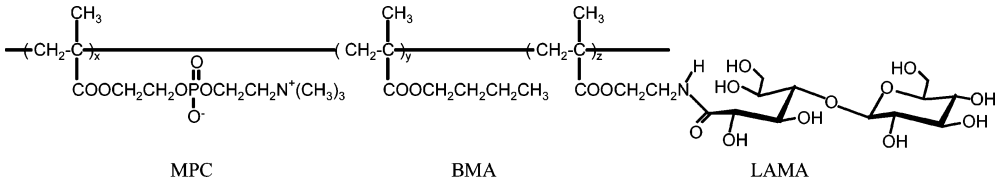

Figure 1. Structure of PMBL.

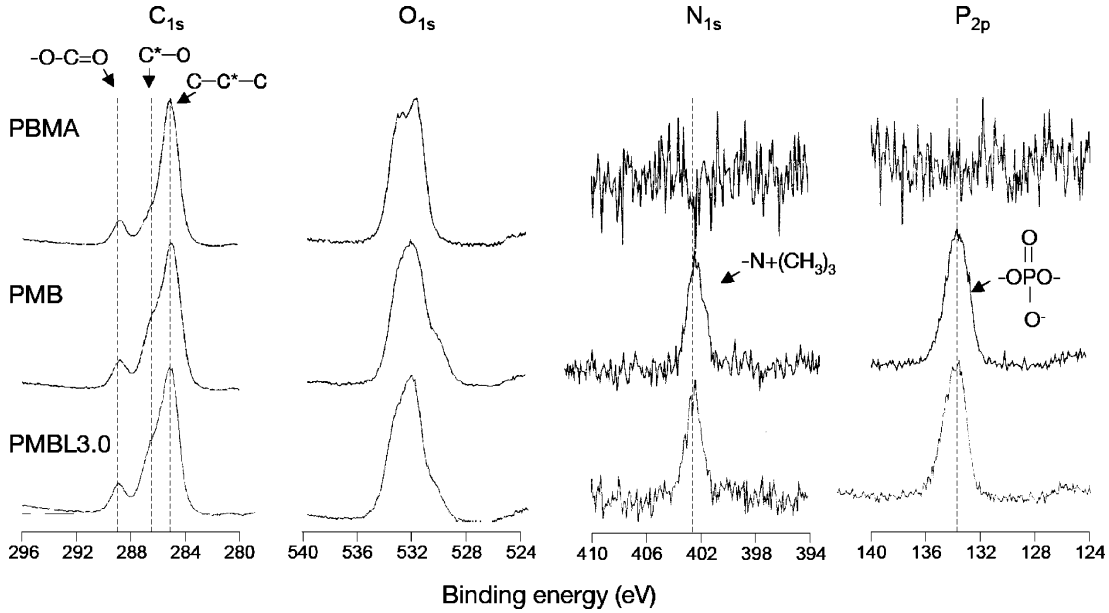

Figure 2. XPS spectra of polymer surfaces.

incubator with 95% humidity. After the cells were incubated on the polymer samples for the specific periods, the polymer plates were rinsed 3 times with PBS. The plates were then transferred to a new 24-well tissue culture plate. Triton X-100 (0.5 wt %, 1 mL) was introduced into each well, and the cells were incubated for 30 min. The Triton X-100 solution (250 μL) was collected, and the concentration of LDH from the adherent cells was measured with an LDH-Cytotoxic Test kit (Wako Pure Chemical Industries, Ltd.).[37,38]

Morphological observation of the HepG2 cells cultured on the polymer surfaces was performed using a confocal laser scanning microscope (LSM510, Zeiss). Cultured cells were fixed at 37 °C with 4% para-formaldehyde in Dulbecco's PBS for 20 min. The fixed surfaces were washed with PBS and permeabilized with 0.5% Triton X-100 in PBS for 2 min. The surfaces were then blocked with 0.1% BSA in PBS for 90 min. To enable observation of the cell membrane, a DMSO solution (10 μL) of 10 μM Texas Red-DHPE (Molecular Probes Inc.) and 0.1% BSA/PBS (1 mL) was poured into each well and stored for 30 min. After being rinsed with 0.1% BSA/PBS (1 mL) 3 times, F-actin synthesized in the cells was stained for assay by immunocytochemistry. The cells were in contact with primary antibodies [anti-human actin goat polyclonal antiserum (Sigma Chemical)], rinsed with PBS, and then incubated with FITC conjugated secondary antibody [FITC conjugated anti-goat IgG rabbit IgG (Sigma Chemical)]. The surfaces were then sufficiently rinsed with 0.1% BSA/PBS after which they were mounted for observation.

Albumin secreted from the HepG2 cells cultured on the polymer surfaces was determined by an enzyme linked immunosorbent assay (ELISA). After the HepG2 cells on the polymer surfaces were cultured for 96, 168, or 360 h, the culture medium was entirely changed, and additional culturing was continued for 24 h. The supernatant of the medium was collected, and the concentration of albumin was determined.

Statistical Analysis. The data are represented as the mean + the standard deviation (SD). Statistical comparisons ($n = 4$) were performed with the Student's t test.

Results and Discussion

Polymer Synthesis. To control biorecognition, polymer surfaces containing phospholipid polar groups and carbohydrates were designed as biomimetics of the outer surface of a biomembrane. Carbohydrate residues were added to the phosphorylcholine polymer surfaces as recognition sites. Figure 1 shows the structures of the polymers synthesized in this study. The polymerization of MPC, BMA, and LAMA was carried out in a DMSO mixture. As indicated in Table 1, the feed could be used to control the composition of each monomer unit. Copolymers containing various amounts of carbohydrate units were thus obtained. PET plates coated with the synthesized polymers from a 0.5 wt % solution were prepared by solvent evaporation. Elemental analysis of polymer-coated PET surfaces was performed by XPS. In the case of the PET plates coated with MPC polymers, nitrogen and phosphorus peaks were observed at 402.5 and 133.0 eV, respectively (Figure 2). O_{1s} shows a low binding energy component around 530 eV, which is most probably due to the PO_4 environment in phosphorylcholine. The XPS elemental concentration was summarized in Table 1. The concentration of oxygen was slightly increased by the addition of the LAMA unit to the copolymer. This increase is due to the hydroxyl groups of the carbohydrates and the LAMA units located on the surface of the sample plates.

Selective Binding of Lectin on Polymer Surfaces. To identify the selective interaction of lectin on the PMBL surface, the binding behavior was monitored by SPR. Figure 3 shows time courses of the change in the resonance angle of SPR responding to the addition of RCA_{120} to a PMBL3.0-coated gold surface in PBS (pH 7.4). The change in the resonance angle ($\Delta\theta$) due to RCA_{120} binding to PMBL3.0 was remarkable and well-correlated with the concentration of RCA_{120}. The apparent binding constant of RCA_{120} to PMBL3.0 per LAMA unit was

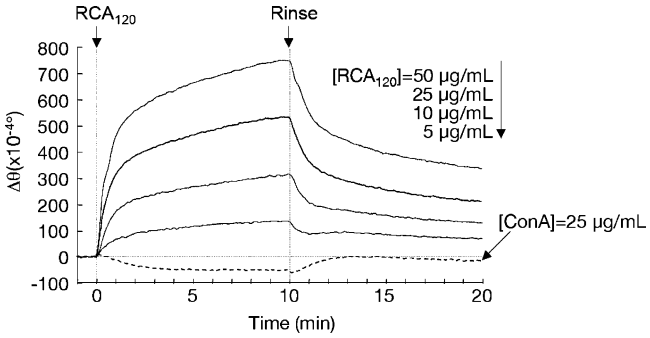

Figure 3. SPR curve on PMBL3.0 after contact with RCA120.

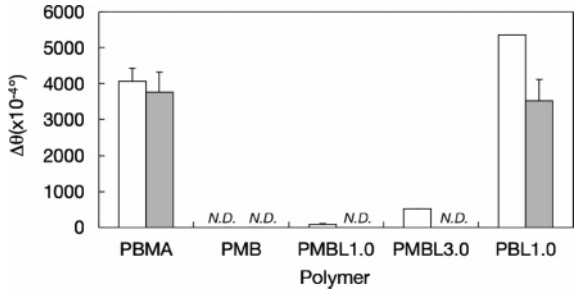

Figure 4. Change in the resonance angle of polymer-coated SPR sensors after contact with lectin for 10 min: (white) RCA_{120} and (gray) Con A.

$2.77 \times 10^5\ M^{-1}$. Because these values are comparable to the affinity constant between the lactose-carrying polymer and RCA_{120}, it can be said that the conjugate is strongly bound to the lectin by the glycocluster effect.[39,40] When Con A was in contact with the PMBL3.0-coated gold surface, the resonance angle was minimally changed, as shown in Figure 3. The $\Delta\theta$ value of various SPR sensor-coated polymers due to lectin binding is summarized in Figure 4. On PBMA and PBL1.0, $\Delta\theta$ was remarkable after contact both with RCA_{120} and with Con A. This result indicates that nonspecific adsorption of lectin occurred on these surfaces. In contrast, $\Delta\theta$ was only detected on PMBL when RCA_{120} was in contact with the surface; it increased with an increase in the fraction of the LAMA unit in PMBL. Although the absolute amount of RCA_{120} adsorbed on the PMBL1.0 surface was much lower than that adsorbed on the PBL1.0 surface, nonspecific adsorption of Con A was completely reduced on the PMBL1.0 surface.

On the PMB surface, the resonance angle was minimally changed. The behavior of protein adsorption resistance on MPC polymer surfaces has been reported in past literature.[29] The phosphorylcholine group of MPC polymers is very hydrophilic, which means that hydrophobic interaction with proteins is unlikely. Moreover, phosphorylcholine polymers minimally interact with the water structure around the polymer.[41] This is a unique property of phosphorylcholine polymers in comparison with the properties of conventional hydrophilic polymers. In addition, electrostatic interactions between a phosphorylcholine polymer surface and proteins are weak because the charge potential (ζ-potential) of phosphorylcholine is neutral.[42] Therefore, a phosphorylcholine polymer has several features that reduce nonspecific protein adsorption. Consequently, this polymer is one of the best polymer materials for forming nonbiofouling surfaces.

The XPS concentrations of oxygen and phosphorus of PMBL-coated surfaces were higher than the theoretical amount calculated from the bulk concentration, as is shown in Table 1. Thus, both functions of MPC and LAMA units represent preferable surface properties. A few mole percent of LAMA units incorporated into the copolymer did not influence the nonfouling properties of the MPC polymers and worked well as recognition sites for specific proteins.

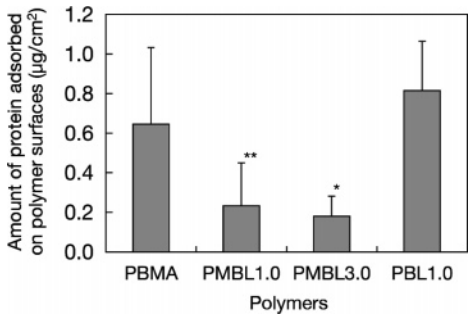

Figure 5. Amount of serum protein adsorbed on polymer surfaces after contact with cell culture medium for 60 min. *$p < 0.01$ PMBL3.0 vs PBMA and PMBL1.0 and **$p < 0.05$ PMBL1.0 vs PBL1.0.

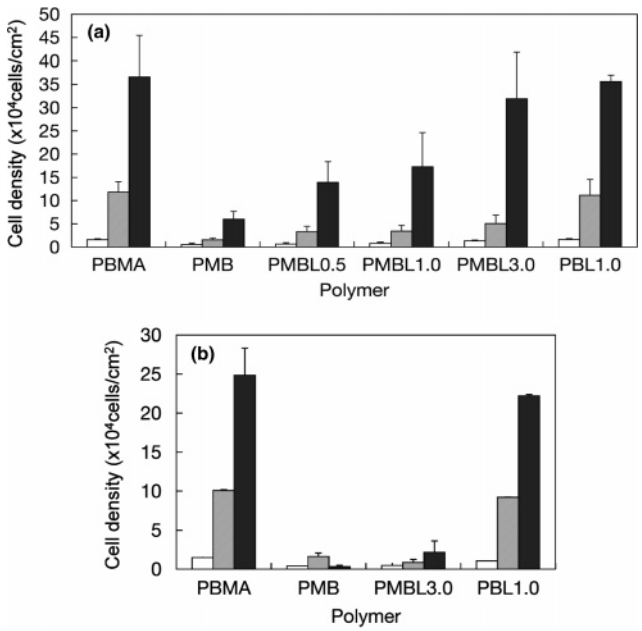

Figure 6. (a) Surface density on polymer surfaces after contact with HepG2 cells: (white) 24 h; (gray) 96 h; and (black) 168 h. (b) Surface density on polymer surfaces after contact with NIH/3T3 cells: (white) 24 h; (gray) 96 h; and (black) 168 h.

Cell Culture Experiment. The amount of adsorbed protein on the polymer surfaces is shown in Figure 5. The PMBL1.0 and -3.0 surfaces significantly reduced protein adsorption as compared to that on PBL1.0. The amount of protein adsorption on PMBL is similar to that on PMB, as previously described.[29] The amount of protein adsorption on PMBL is a level for the reduction of nonspecific cell adhesion.[27]

Figure 6a,b shows the time-dependent surface density of HepG2 and NIH-3T3 cells on a polymer surface after culturing for given periods, respectively. On a PBMA surface, many cells adhered, and the density increased with an increase in culture time. In contrast, cell adhesion was reduced on the PMB surface because adsorption of the cell adhesive protein on the surface could be reduced. Because the HepG2 cells have ASGPRs, which are galactose-recognizing receptors, cell adhesion was induced on the phosphorylcholine polymer surface having LAMA units. The cell density increased with an increase in the composition of LAMA units in the copolymers. When the LAMA composition was 3%, the density was similar to that on PBMA for every culture period (Figure 6a, PMBL3.0: *$p >$ 0.05 vs PBMA and PBL1.0 and **$p < 0.01$ vs PMB).

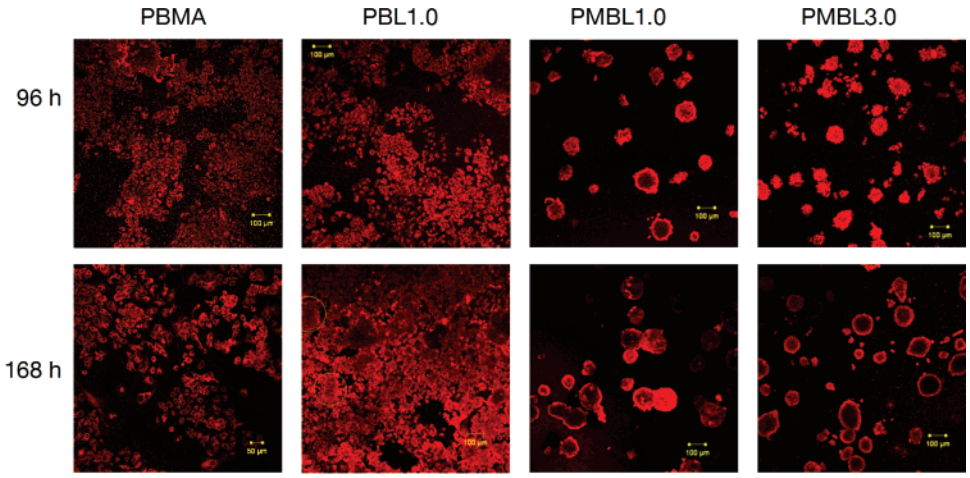

Figure 7. Fluorescence micrographs of polymer surfaces in contact with HepG2 cells for 96 and 168 h.

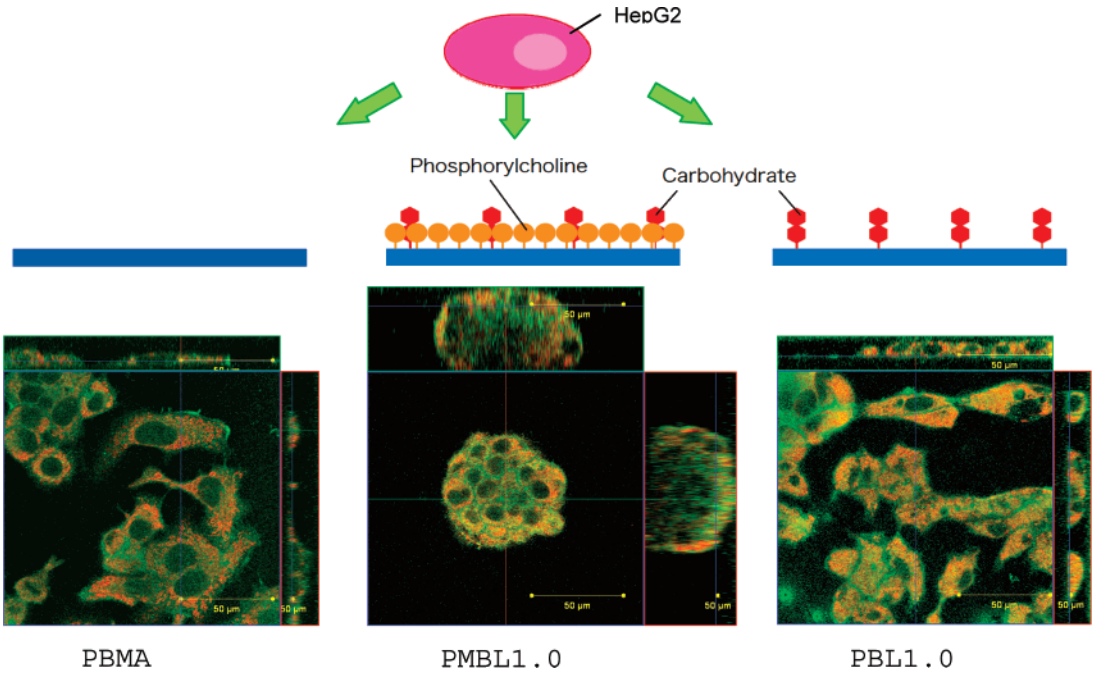

Figure 8. Confocal micrographs of adherent cells: (Green) F-actin and (red) phospholipid double layer.

NIH-3T3 cells do not have ASGPRs. On the PBMA surface, NIH-3T3 cells adhered and proliferated as well as the HepG2 cells did because much cell adhesive protein was adsorbed on the PBMA.[27] The LAMA unit in PBL did not affect NIH-3T3 cell adhesion, and a large number of NIH-3T3 cells adhered to the PBL1.0 surface. Because the PBL1.0 surface has a large amount of adsorbed protein containing cell adhesive protein such as fibronectin, integrin-mediated adhesion of NIH-3T3 cells on the PBL1.0 surface must have occurred. In contrast, the adhesion of NIH-3T3 cells was reduced on the polymer surfaces having MPC units (Figure 6b, PMBL3.0: $*p < 0.01$ vs PBMA and PBL1.0 and $**p > 0.05$ vs PMB). This result indicates that the ligand−receptor interaction at the polymer−cell interface worked preferably on the MPC polymers.

Spheroid Formation of HepG2 Cells. Figure 7 shows fluorescence micrographs of HepG2 cells cultured on polymer surfaces for 96 and 168 h. The membrane of the adherent cells was stained by Texas Red-DHPE. The adherent HepG2 cells on the PMBA surface were spread, and the morphology of each cell was easily observed. The HepG2 cells were also spread on the PBL1.0 surface, but some aggregation was observed. In contrast, the HepG2 cells observed on the PMBL1.0 and PMBL3.0 surfaces formed spheroids. The number of spheroids on PMBL increased with an increase in the number of LAMA units in the copolymer. The average diameter of the spheroids under each condition was calculated by measuring 50 random samples. Each amount was found to be as follows: 86.1 ± 26.7 μm (PMBL1.0, 96 h), 92.0 ± 28.9 μm (PMBL3.0, 96 h), 115.7 ± 28.6 μm (PMBL1.0, 168 h), and 125.8 ± 29.1 μm (PMBL3.0, 168 h). The number of cells forming a spheroid was calculated from the data shown in Figures 6a and 7 and was around 25 cells for the 96 h culture and 125 cells (PMBL1.0) and 160 cells (PMBL3.0) for the 168 h culture. The size of the spheroids increased with an increase in the number of LAMA units, indicating that the units play a role in holding the spheroids to the polymer surface.

For spheroid formation, a round-bottomed plate with a nonbinding surface was generally used. Although the process was very successful, only one large spheroid per well was obtained, and nutrition did not reach inside the cells of a spheroid, resulting in necrosis. In addition to the process of using round-bottomed wells with a nonbinding surface, spheroid formation of hepatocytes has been studied on polymer surfaces controlled chemically and/or physically. Akaike and co-workers

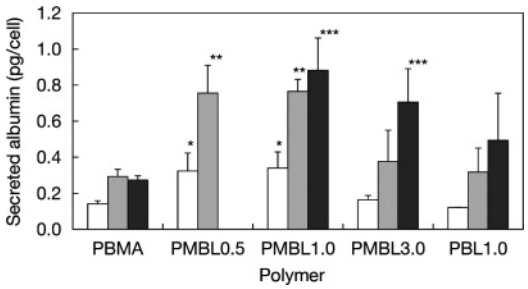

Figure 9. Amount of albumin secreted from adherent cells for 24 h after culturing of HepG2 cells on polymer surfaces for 96 (white), 168 (gray), and 360 h (black). *p < 0.05 vs PBMA in 96 h culture; **p < 0.01 vs PBMA in 168 h culture; and ***p < 0.01 vs PBMA in 360 h culture.

are leading this field with the use of poly(*p*-*N*-vinylbenzyl-D-lactonamido) (PVLA). They reported that spheroid formation of hepatocytes on a PVLA surface could be observed with the addition of an epidermal growth factor (EGF).[43] The spheroid formation was also influenced by the morphologies of polymer surfaces such as honeycomb[44] and nanofibril[45] structures. On a PMBL surface, spheroid formation was induced on a flat surface and without the need for any growth factor or cytokine.

Figure 8 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 pseudo-pod formation of the adherent cells, actin was easily observed. In contrast, HepG2 cells cultured on PMBL1.0 formed spheroids with multilayer adhesion.

The mechanism of spheroid formation induced on PMBL surfaces is considered a nonintegrin-mediated adhesion phenomenon. The PMBL surfaces effectively reduce protein adsorption; the number of integrin binding sites on the surface is fewer than that on PBMA or PBL1.0 surfaces.

Albumin Secretion from Adherent HepG2. The functioning of HepG2 cells cultured on polymer surfaces was compared by measuring the amount of albumin secreted by the cells. Figure 9 shows the amount of albumin secreted by HepG2 cells for 24 h after 96 h (4 days), 168 h (7 days), and 360 h (15 days) of culturing on the polymer surfaces as determined by ELISA. At up to 168 h of culturing, the amount of albumin secreted per cell that adhered onto PMBL0.5 and -1.0 was significantly higher (*p < 0.05, 96 h and **p < 0.01, 168 h) than that on PBMA and PBL1.0. After culturing for 360 h, high amounts of albumin secreted from cells that adhered onto PMBL1.0 and -3.0 were observed (***p < 0.01). On PMBL0.5, reproducible data for 360 h of culturing were not obtained because the spheroids were easily detached from the surface. The density of the LAMA unit on the PMBL1.0 surface appeared to be optimal for preserving the ability to secrete albumin. Lu et al. recently reported that the functional maintenance of hepatocytes was enhanced with NIH/3T3 fibroblasts.[46] Although the mechanism has not been explained in detail, cell contact with NIH/3T3 cells constitutes a better condition for hepatocytes. PMBL has phosphorylcholine groups similar to the cell surface, and the surface may present a more suitable situation for hepatocyte growth as compared to that of PBMA or PBL.

There has been great interest in the potential utility of a bioartificial liver (BAL) device as a bridge support for patients who have suffered massive liver injury during the period until the regeneration of their own hepatocytes or until liver transplantation. Although preservation of cell function in the material surface is very important for this application, conventional polymer materials such as polysulfone, polyolefine, and polyurethane have generally been used.[47,48] Surface modification is important in further improving the function of BAL devices. We have reported that MPC polymers could be applied in the surface modification of hollow fibers that have the possibility of producing hemodialyzer and liver assist bioreactor devices.[49,50] We have applied PMBL as a coating material for polypropylene hollow fiber mini-modules, which are the subject of a long-term cell culture study. PMBL can reduce nonspecific biofouling, thus efficiently preserving the mass transport ability of hollow fiber membranes. In addition, the polymer can provide a more suitable environment for cell cultivation than that of conventional hollow fiber materials.

Conclusion

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 interactions, and a specific ligand−receptor interaction was clearly demonstrated. In this study, we used HepG2 cells to recognize galactose. The morphology of the cells and their functions on a PMBL surface were significantly different from those on a PBMA surface. LAMA units and MPC units of PMBL are important for supporting hepatocytes and for reducing nonspecific interactions at the cell−material interface, respectively. Yin et al. compared the morphology of cancer cells and primary cells on galactose-immobilized substrates.[51] They stated that no significant difference in spheroid formation based on these cells was observed. Therefore, the primary hepatic cell might form spheroids on the PMBL surface. The primary cell function on the PMBL surface will be reported in the near future.

We clarified that carbohydrates immobilized on the phosphorylcholine polymer surface were easily distinguished from the specific protein and cells. Changing the types of carbohydrates enables changes in the types of biorecognition. The polymers have great potential for bioreaction, molecular separation, targeting, sensing, etc.

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