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Hybrid immobilization of galactosyl lactose and cellobiose on a gold substrate to modulate biological responses

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ABSTRACT

Bioactive *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose (4′-galactosyl lactose) was site-selectively modified at a reducing end with thiosemicarbazide (TSC). As-synthesized 4′-galactosyl lactose-TSC was immobilized on a gold substrate with cellobiose-TSC as a spacer through spontaneous self-assembly chemisorption *via* S—Au bonding. Quartz crystal microbalance analysis suggested the successful formation of self-assembled monolayers (SAMs) of 4′-galactosyl lactose-TSC and/or cellobiose-TSC. Galactose-binding lectin exhibited the highest affinity for hybrid SAMs with an equimolar ratio of the two oligosaccharide-TSCs, while glucose-binding lectin showed decreasing adsorption with a decrease in cellobiose-TSC ratios. Human hepatocellular carcinoma cells, which recognize galactose residues, efficiently adhered to the hybrid SAMs. Higher enzymatic deethoxylation of ethoxyresorufin *via* cytochrome P450 appeared on hybrid SAMs. These results suggested that clustering of the bioactive sugars was involved in the cellular responses, possibly *via* biological carbohydrate–protein interactions. This approach to designing carbohydrate-based scaffolds should provide a basis for the functional development of glyco-decorated biointerfaces for cell culture applications.

1. Introduction

Carbohydrates are abundantly expressed on the outer surfaces of cytoplasmic membranes of mammals as well as viral, bacterial, protozoan and fungal pathogens (Hayashi et al., 2004). Biological regulation of cell attachment and growth is mostly governed by carbohydrate-mediated communications at cellular interfaces *via* protein–carbohydrate (nonreducing ends) interactions (Hu et al., 2010; Wang, Yin, Wang, & Wang, 2012; Wu, Yao, Bai, Du, & Ma, 2010). For tissue engineering applications, innovative cell culture scaffolds that possess various biological functions for cellular activation and organization are required (Kumar et al., 2011; Peter et al., 2010; Tsai, Chen, Li, Lai, & Liu, 2012). Thus, carbohydrate-based scaffolds for cell culture applications may be useful for advancing cell engineering technology.

Lactose, *O*-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, is a major disaccharide found notably in mammalian milk, and can mediate various biological phenomena *in vivo* (Bajdik et al., 2009; Miyagawa, Carmelita, Kasuya, & Hatanaka, 2007; Potes, Kerry, & Roos, 2012). Recently, the galactose residues of lactose and its oligomers have attracted attention as functional bioregulators for *in vitro* cell culture applications. Especially, it is well known that various carbohydrate-binding proteins, called a lectin, on cell membranes have a broad range of interfacial biofunctions including cell–cell adhesion and proliferation (Vasta & Ahmed, 2008). On these grounds, Akaike et al. reported that poly-*N*-*p*-vinylbenzyl-D-lactoneamide (PVLA) was useful as a hepatocyte culture matrix by noting that asialoglycoprotein receptors, one of the calcium-dependent (C-type) lectins, on rat liver cells can interact with the galactose branch of the PVLA backbone (Uchida, Serizawa, Ise, Akaike, & Akashi, 2001). Gotoh, Niimi, Hayakawa, and Miyashita (2004) reported the hepatocyte culture behavior of lactose-immobilized silk fibroins and their resulting galactose-mediated biological functions. Thus, continued research in glycomaterial engineering should help in the development of practical cell culture scaffolds.

In our previous studies, various types of oligo-/polysaccharides such as cellulose and its derivatives (Kitaoka, Yokota, Opietnik, & Rosenau, 2011; Yokota, Kitaoka, Opietnik, Rosenau, & Wariishi, 2008; Yokota, Kitaoka, Sugiyama, & Wariishi, 2007; Yokota, Kitaoka, & Wariishi, 2008), chitin (Yoshiike & Kitaoka, 2011; Yoshiike, Yokota, Tanaka, Kitaoka, & Wariishi, 2010), chitosan (Yoshiike et al., 2010) and hyaluronan (Tanaka, Yoshiike, Yoshiyama, & Kitaoka, 2010) were immobilized on a gold substrate through a

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self-assembly chemisorption technique, and some were tested in cell culture applications (Tanaka et al., 2010; Yokota, Kitaoka, & Wariishi, 2008; Yoshiike & Kitaoka, 2011; Yoshiike et al., 2010). Only reducing end groups of carbohydrates were selectively modified with thiosemicarbazide (TSC). The resultant carbohydrate-TSC derivatives were fixed on the gold (Au) surfaces *via* a covalent S—Au bond, called vectorial chain immobilization. This approach has provided unique biointerfaces containing aligned carbohydrates whose bioactive nonreducing ends are in direct contact with the surfaces of cultured cells. Rat liver cells (IAR-20) (Yokota, Kitaoka, & Wariishi, 2008), human hepatocellular carcinoma cells (HepG2) (Yoshiike & Kitaoka, 2011) and mouse fibroblasts (NIH-3T3) (Tanaka et al., 2010; Yoshiike et al., 2010) were preferentially adhered to these biointerfaces. In particular, the combination of chitin and cellulose having high affinity for each other at the molecular level (Takegawa, Murakami, Kaneko, & Kadokawa, 2010) demonstrated the unique biological response of HepG2 cells on the hybrid nanolayers prepared using respective chito-/cello-hexaose (Yoshiike & Kitaoka, 2011). Further expansion of this technique is expected to be applicable for the functional design of bioactive glyco-scaffolds.

In this study, bioactive *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose (4′-galactosyl lactose; GL) and cellobiose (CL) as a spacer were site-selectively modified at each reducing end group with TSC. The hybrid glyco-nanolayers were fabricated in various molar ratios of both oligosaccharides according to our previous study (Yoshiike & Kitaoka, 2011). Herein, the combination of longer-chain bioactive GL (trisaccharide) and shorter-chain spacer CL (disaccharide) was applied to expect higher bioactivity due to the presented galactose residues on the scaffold surfaces. The self-assembly behavior of carbohydrate-TSCs was monitored by quartz crystal microbalance (QCM) analysis, and the as-formed self-assembled monolayers (SAMs) were characterized by a lectin-binding assay on a QCM apparatus to assess the essential interaction between as-designed glyco-SAMs and carbohydrate-binding proteins at the molecular level. We evaluated the hydrophilic properties of carbohydrate-SAMs by measuring the sessile-droplet contact angle of water. HepG2 cells, which contain galactose receptors, were used to investigate the biofunctional properties of the GL/CL hybrid nanolayers by examining their initial adhesion and their cytochrome P450 activity.

2. Experimental

2.1. Materials

Pure *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose (GL), resorufin, resorufinethylether and 3-methylcholanthrene were purchased from Wako Pure Chemical Ind. Ltd., Japan. Cellobiose (CL), thiosemicarbazide (TSC), sodium cyanoborohydride ($NaCNBH_3$) and dicumarol were purchased from Sigma–Aldrich Corp., USA. Microcover glass (diameter: 15 mm, Matsunami Glass Ind. Ltd., Japan) was used as a transparent substrate for thin-layer gold sputtering. Water was purified with a Milli-Q system (Millipore Co. Ltd., Japan). Human liver carcinoma cells (HepG2) were provided by the Japanese Collection of Research Bioresources, Japan. Dulbecco's Modified Eagle's Medium (DMEM), glutamine, penicillin–streptomycin, trypsin–EDTA and MEM non-essential amino acids solution (NEAA) were purchased from Life Technologies Corp., Japan. Fetal bovine serum (FBS) was obtained from Biowest Co. Ltd., France. Tissue culture polystyrene (TCPS) dishes and TCPS plates (24 well) were obtained from Sumitomo Bakelite Co. Ltd., Japan. Concanavalin A (ConA from *Canavalia ensiformis*, Wako Pure Chemical Ind. Ltd., Japan) and *Ricinus communis* agglutinin (RCA_{120}, Cosmo Bio Co. Ltd., Japan) were used for the lectin-binding assay to assess the biological recognition of glucose and galactose residues, respectively, on the SAM surfaces. Other chemicals were reagent grade and used without further purification.

2.2. Preparation of GL/CL hybrid nanolayers

An outline of the preparation of hybrid glyco-nanolayers composed of GL and CL is illustrated in Fig. 1. Terminal TSC-labeling of GL and CL molecules at each reducing end was successfully carried out by aqueous reductive amination with $NaCNBH_3$ under mild conditions as previously reported (Tanaka et al., 2010; Yoshiike & Kitaoka, 2011; Yoshiike et al., 2010). Either powdery GL (20.0 mM final concentration) or CL (29.2 mM) was dissolved in Milli-Q water, and then the TSC reagent (110 mM) was added to the solutions, followed by stirring at 70 °C for 72 h in the presence of $NaCNBH_3$ (2 M). As-synthesized GL-TSC and CL-TSC as shown in Fig. 1 were precipitated in ethanol, and rinsed using sufficient ethanol by repeated (at least five times) centrifugation (3000 rpm, room temperature) to remove excess $NaCNBH_3$ and residual TSC.

Piranha-washed clean glass plates were coated with Au by ion sputtering (VPS-020, ULVAC Inc., Japan) at 4 mA current for 3 min below 1.5 mPa of vacuum level. The Au layer thickness was *ca.* 23 nm (Yoshiike & Kitaoka, 2011). The Au-coated plates were soaked at room temperature for 24 h in the designated concentration of carbohydrate-TSC aqueous solutions containing GL-TSC and CL-TSC at molar ratios of 1:0, 3:1, 1:1, 1:3 and 0:1. The treated plates were then washed with sufficient Milli-Q water at least three times to give GL/CL hybrid nanolayers (pure GL-SAM; nominally 75% GL-SAM, 50% GL-SAM and 25% GL-SAM; and pure CL-SAM).

2.3. Analytical characterization

A QCM apparatus (AFFINIXQ, Initium Inc., Japan) equipped with a 27 MHz AT-cut, Au-coated quartz crystal was used to quantify the amounts of carbohydrate-TSC molecules chemisorbed on the Au surface of the QCM sensor chip. An aqueous solution of carbohydrate-TSCs (1.0 mM, 15 μL, GL-TSC:CL-TSC = 1:0, 3:1, 1:1, 1:3 or 0:1 molar ratio) was injected into a sample chamber containing 8.0 mL of Milli-Q water at pH 7 for 24 h. The frequency changes of the sensor chip were monitored on line at 25 °C with stirring at 1000 rpm. The approximate amounts of chemisorbed oligosaccharides were calculated according to our previous study (Yoshiike & Kitaoka, 2011).

Subsequently, the adsorption behavior of the lectins onto the carbohydrate-SAMs was monitored by QCM analysis. Each carbohydrate-SAM was set into a sample chamber containing 8.0 mL of phosphate buffered saline (PBS) solution with calcium chloride dehydrate and magnesium chloride hexahydrate at pH 7 and 25 °C with stirring at 1000 rpm. After adding 30 μL of a blocking agent (4× Blocking Reagent for AFFINIXQ, Initium Inc., Japan), the excess amount of the blocking agent was sufficiently removed by rinsing with PBS solution. After stabilization of the frequency variation, either ConA or RCA_{120} (1.0 μM, 8.0 μL) was added four times at an interval of 30 min during the 2 h monitoring of continuous frequency changes.

2.4. Cell culture assay

Hybrid nanolayers on Au-coated glass plates were placed at the bottom of each well of 24-well TCPS plates, and suspended HepG2 cells (1.0 mL) were seeded on each substrate (1.0×10^5 cells mL^{-1}, *i.e.* 1.0×10^5 cells per well). After incubation for 24 and 96 h with DMEM supplemented with 10% (v/v) FBS, 1% (v/v) penicillin–streptomycin and 1% (v/v) NEAA in a 5% CO_2 atmosphere at 37 °C, unattached cells were completely removed

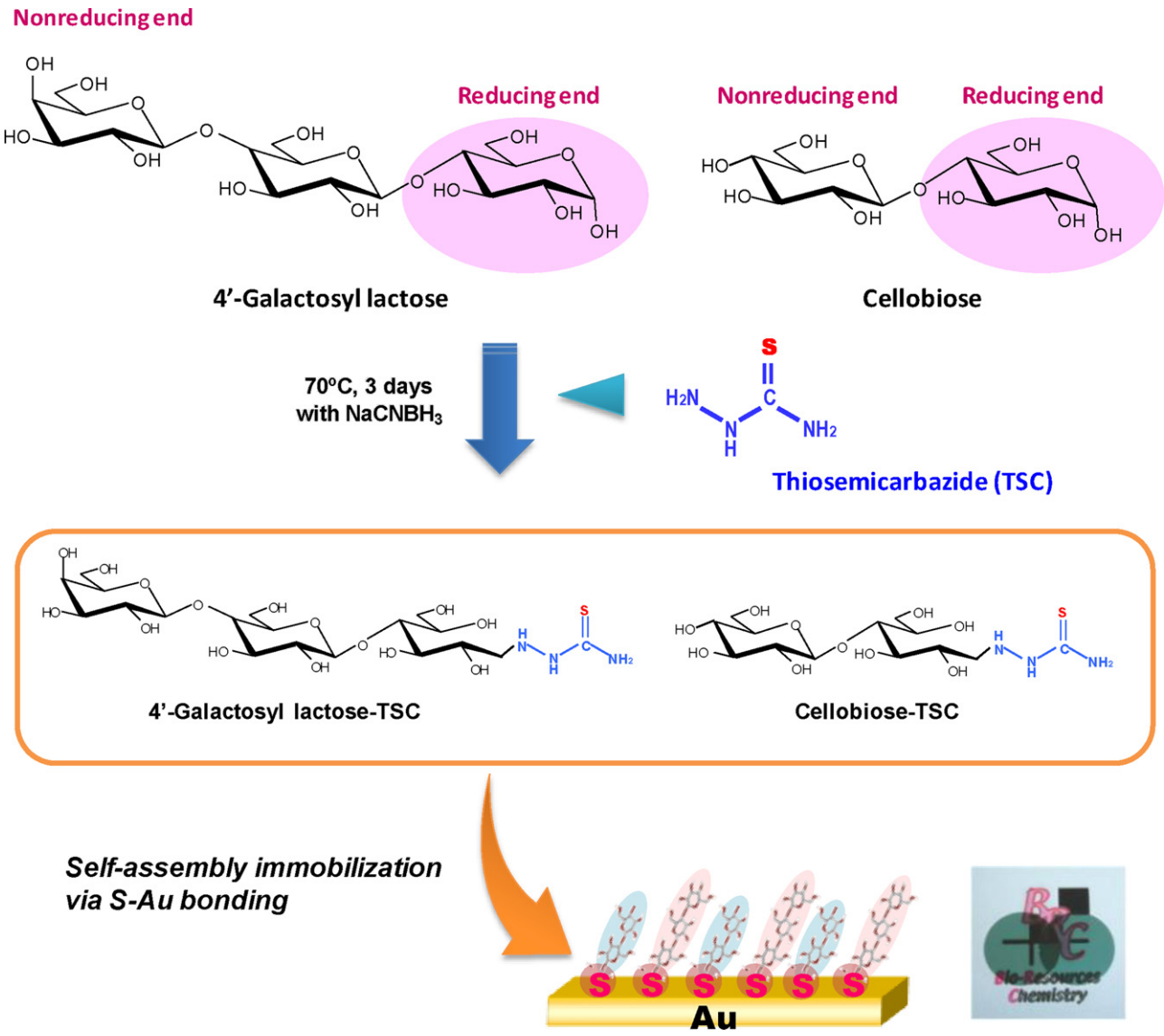

Fig. 1. Schematic illustration of TSC-labeling and self-assembly immobilization of GL and CL on a gold substrate. The photograph is an optical image of carbohydrate-SAMs (glass plates). (For interpretation of the references to color in the text, the reader is referred to the web version of the article.)

by rinsing with PBS. The residual cells adhering to the substrates were treated with trypsin–EDTA for enzymatic separation then the detached cells were counted using a counting chamber ($n > 3$). Microscopic images of cell adhesion and morphology were acquired with a Leica DMI 4000B (Leica Microsystems Co. Ltd., Germany) microscope. The cellular assays were carried out at least three times.

2.5. Induced P450 activity assay

The typical biological response of cultured HepG2 cells was investigated by an induced cytochrome P450 1A1 (CYP1A1) activity assay. After incubation of HepG2 cells for 24 h, 1 mL of induction reagent (1 μM 3-methylcholanthrene in DMEM) was poured into the culture medium and incubated at 37 °C for 3 days. The incubation media was replaced with fresh media every 24 h. After 3 days of incubation, CYP1A1 activity was measured by the ethoxyresorufin *O*-deethylase (EROD) assay (Lee, Cuddihy, Cater, & Kotov, 2009; Yoshiike & Kitaoka, 2011). The incubation medium was then replaced by 8 μM resorufinethylether (500 μL) and 40 μM dicumarol in DMEM. After incubation for 3 h, the amount of resorufin produced in each sample was measured by detecting the fluorescence intensity at 580 nm (excitation: 535 nm) using an F-3010 (Hitachi Ltd., Japan) spectrofluorometer. This enzymatic assay was performed at least three times.

3. Results and discussion

3.1. Self-assembly immobilization of 4′-galactosyl lactose and cellobiose on a gold surface

The formation of carbohydrate-SAMs from GL-TSC and CL-TSC was achieved through the spontaneous chemisorption of carbohydrate S-derivatives on an Au substrate as illustrated in Fig. 1. The QCM monitoring was performed to elucidate the delicate adsorption behavior of carbohydrate-TSCs on the Au surface of the QCM sensor chip. Fig. 2 shows the frequency variation profiles with time after the stepwise injection of TSC-modified or TSC-free oligosaccharides. The arrows indicate the repeated injections of each sample up to a plateau, the quasi-equilibrium level. For GL-TSC and CL-TSC, the QCM frequency immediately dropped after the first injection, and gradually decreased on subsequent sample

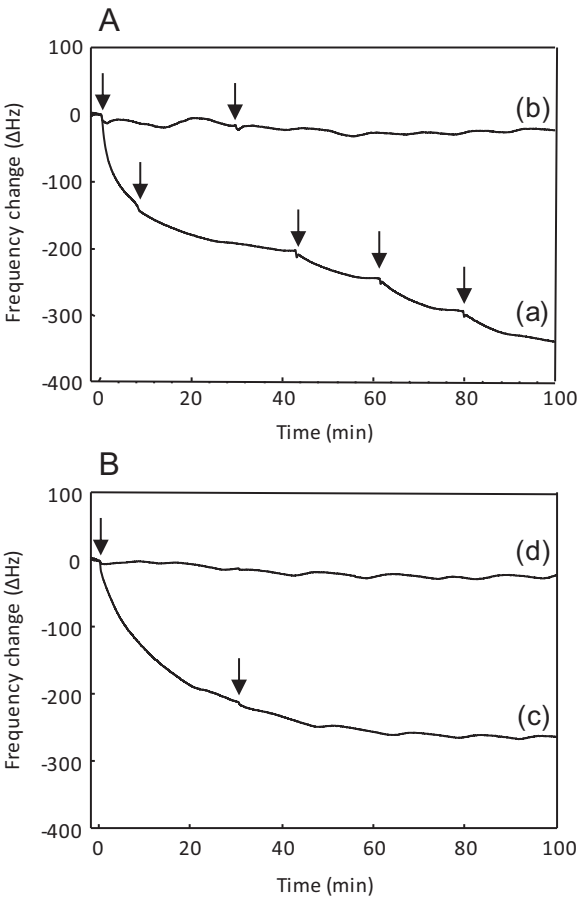

Fig. 2. QCM profiles for spontaneous chemisorption on a gold surface: (A) GL and (B) CL; with (a and c) or without (b and d) TSC-labeling. The arrows indicate the sample injection.

injections. By contrast, negligible frequency changes were observed without TSC-labeling, suggesting that neither TSC-free GL nor TSC-free CL were adsorbed onto the Au surfaces. A similar adsorption behavior for sugar-TSCs was observed for the mixture of GL-TSC and CL-TSC (data not shown). These results clearly indicated that the carbohydrate-TSCs strongly interacted with the Au surfaces of QCM sensor chips, possibly through a σ—π coordinate bond between the S and Au atoms (Ning, Xie, Xing, Deng, & Yang, 1996). The sugar densities of GL-SAM and CL-SAM surfaces were estimated to be 2.3 and 1.9 chains nm^{-2}, respectively, according to our previous study (Yoshiike & Kitaoka, 2011). The precise sugar densities for the hybrid SAMs of chito-/cello-hexaose were calculated from N/C atomic ratios determined by X-ray photoelectron spectroscopy (XPS). In this study, almost the same elemental compositions of GL-TSC and CL-TSC detected by XPS analysis could not allow us to conclude the sugar densities of hybrid SAMs (data not shown). At any rate, only reducing ends of sugar-TSCs with a relatively high density were fixed on the Au surfaces whereas nonreducing ends of oligosaccharides were presumably directed outward, making them favorable for biological interactions.

3.2. Lectin-binding behavior onto GL/CL hybrid nanolayers

The specific affinity of lectins for GL/CL hybrid SAMs was investigated by QCM monitoring of lectin adsorption behavior. Fig. 3 shows the frequency variations of GL/CL hybrid SAMs at each equilibrium state after separately adding two types of lectins, ConA and RCA_{120}, which have specific binding affinity for glucose and galactose residues, respectively. The addition of ConA lectin decreased

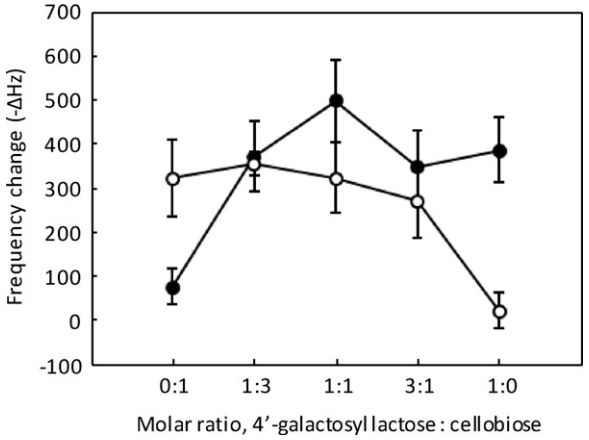

Fig. 3. QCM profiles for lectin-binding behavior onto carbohydrate-SAMs with various molar proportions of GL and CL. ConA (open symbols) and RCA_{120} (closed symbols).

the QCM frequency, *i.e.* increasing adsorption, with an increase in the CL ratios of hybrid SAMs. The addition of RCA_{120} lectin, however, resulted in a unique adsorption behavior. Strong affinity for the GL/CL hybrid SAMs was observed when the SAMs were prepared with a molar ratio of GL:CL = 1:1. In fact, the affinity for the equimolar hybrid SAMs was significantly higher than pure GL-SAM. This curious phenomenon may be attributed to the galactose density on the hybrid SAMs because the RCA_{120} lectin possesses multiple binding sites. In our previous study, we estimated the galactose residues on the lactose-modified cellulose paper by measuring the amount of RCA_{120}-FITC lectin adsorbed on the surface (Egusa et al., 2009). RCA_{120} has two binding sites for galactose in one tetra-complex protein (Wang et al., 2011), and thus could bind one or two residues per RCA_{120}-FITC molecule. In a similar manner, the optimum affinity of RCA_{120} for GL/CL hybrid SAMs would involve spatial matching between binding sites and galactose moieties on the hybrid SAMs. By contrast, ConA (tetramer) has four binding sites (Weatherman, Mortell, Chervenak, Kiessling, & Toone, 1996). However, there was no specific adsorption behavior found, and the proportional adsorption depended on the CL ratios. There is much room for further investigation with regard to the relationship between the type of lectin and its affinity for hybrid glyco-SAMs. At any rate, the results suggested that the combination of bioactive GL and CL spacer provided different biological responses.

3.3. HepG2 cells adhesion to hydrophilic glyco-nanolayers

Bioactive galactooligomers were successfully immobilized on the Au surfaces at various sugar ratios by the combined use of cellobiose as a spacer according to our established protocol (Yoshiike & Kitaoka, 2011). The as-designed glyco-nanolayers were expected to provide favorable growth environments for mammalian cells that recognize galactose residues (Uchida et al., 2001) and to stimulate biological responses (Gotoh et al., 2004), which should prove promising in various tissue engineering applications. To investigate the influence of galactosyl residues on cellular response, *i.e.* cell attachment and enzymatic activity, a cellular assay using human liver carcinoma cells (HepG2) was performed. It has recently been reported that the HepG2 cells possess a galactose-recognition receptor (Ise et al., 2010), and thus we anticipated a sensitive cellular response to the GL-SAMs. Fig. 4 shows the microscopic images of HepG2 cells cultured on each substrate. The sugar-SAMs prepared in this study were sufficiently transparent (light blue, Fig. 1), making them suitable for continuous monitoring of cell growth behavior. It is apparent from Fig. 4 that all HepG2 cells

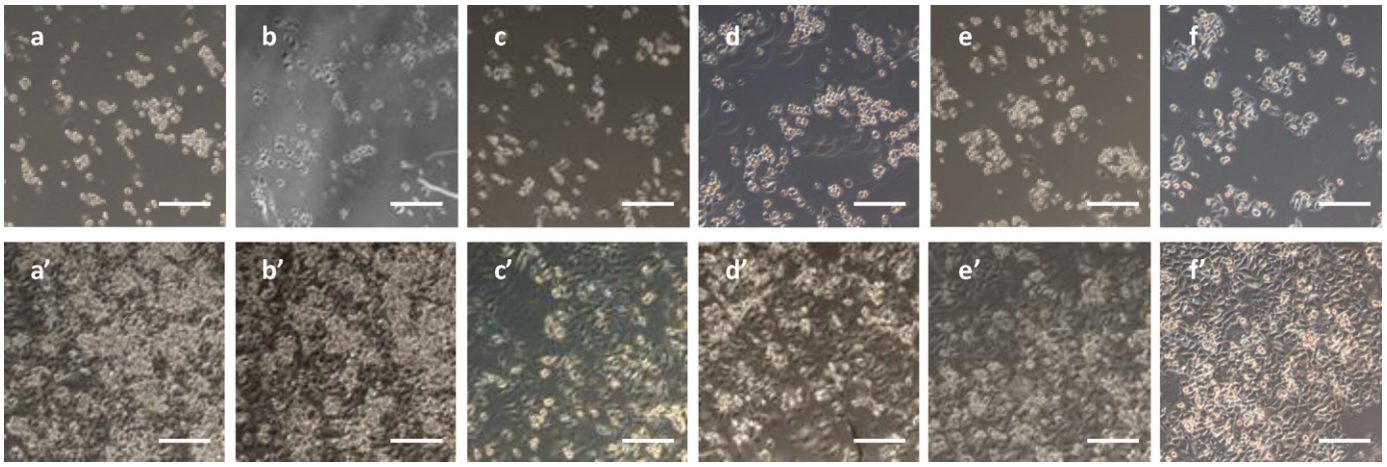

Fig. 4. Microscopic images of HepG2 cell morphology, after 24 h (a–f) and 96 h (a′–f′) incubation, on the hybrid nanolayers of GL and CL with varying molar proportions of GL: 0% (a and a′), 25% (b and b′), 50% (c and c′), 75% (d and d′) and 100% (e and e′). Control, TCPS (f and f′). The scale bars represent 300 μm.

grew well during the 96 h after cell seeding. The hybrid SAMs were very hydrophilic, as evidenced by their water contact angles of around 21–28°, as compared to hydrophobic TCPS (*ca.* 70°). In general, initial cell adhesion preferentially occurs on the hydrophobic substrates since growth factor proteins favorably adhere to hydrophobic surfaces. Thus, hydrophilic substrates are effective at preventing the cell attachment. However, when glyco-interfaces were employed, excellent cell adhesion was found for all hybrid SAMs. Thus, the carbohydrate-mediated interactions with cell surfaces possibly promoted the cell attachment. After incubation for 24 h, all the attached cells were similar in appearance. However, upon detailed examination with time, HepG2 cells were more softly adherent on glyco-layers with higher GL density, particularly obvious after 4 days of incubation. By contrast, extended culture of HepG2 cells resulted in a 2D monolayer on the TCPS plate. Cell morphology is closely involved in biological functions, and the organization of attached cells in direct contact with cell culture scaffolds is a key factor. In our previous study, multicellular HepG2 spheroids with a 3D form were observed on the glyco-SAMs with high chitohexaose density, leading to high cytochrome P450 activity (Yoshiike & Kitaoka, 2011). In this study, obvious 3D morphology for attached HepG2 cells was not observed; however the soft cell attachment to the hybrid SAMs, which is potentially required to initiate such a spheroid formation, was quite different from 2D flat cell morphology on the TCPS plate. Hence, the GL residues on the hybrid nanolayers contributed to biologically significant soft attachments *via* GlcNAc-recognition receptors on the HepG2 cells. By contrast, HepG2 cells tightly adhered to hydrophobic TCPS plates without a GL-recognition system *via* integrin-mediated adhesion. These results suggested that the GL ratios had a substantial influence on cellular responses such as cell adhesion.

3.4. Cellular response of HepG2 cultured on hybrid glyco-nanolayers

The mammalian liver plays very important roles in living systems including metabolic functions, detoxification, protein synthesis and production of various biochemicals (Bissell, Rizki, & Mian, 2003; Lee et al., 2009; Sutherland, 1988). Therefore, liver cells are commonly used for tissue engineering studies for *in vitro* cell culture applications. However, the major liver functions are frequently diminished when cells are cultured outside the host organism because of the presence of a flat 2D culture. It has been reported that 3D cultured liver cells can maintain their inherent biological functions that are crucial for biomimetic approaches (Bissell et al., 2003; Sutherland, 1988). In this study, the enzymatic activity of cytochrome P450, common in cellular detoxification, was investigated. Cytochrome P450 activity is a major parameter of cellular physiology in hepatic cell cultures (Lee et al., 2009). Hence, we measured the enzymatic activity of CYP1A1 by ethoxyresorufin-*O*-deethylase (EROD) assay after 96 h of culture. Fig. 5 shows the EROD response of HepG2 cells cultured on the hybrid nanolayers of GL and CL. The relative EROD activity, normalized for live HepG2 cells on the sugar-SAMs, was much higher than that for TCPS. In particular, 50% GL/50% CL-SAM demonstrated the highest enzymatic activity similar to the specific RCA_{120} affinity as shown in Fig. 3. These results indicated that the interaction of the cells with the hybrid SAMs, regulated by the amount of GL moieties on the cell scaffold, influences the cellular response. Immobilized GL moieties present bioactive galactose residues on the glyco-nanolayer surfaces, resulting in a direct interaction with the galactose-receptors on the cell surface. This then differentiates the cellular adhesion to the scaffold from the plastic plates. The accumulated sugar residues act as a bioregulator to stimulate the HepG2 cells, demonstrating the as-designed carbohydrate-SAMs can exhibit biological functions. Furthermore, these results indicated that the GL/CL ratios on the hybrid nanolayers influence the cellular response of HepG2 cells. 50% GL-SAM showed two-fold higher activity than pure GL-SAM, indicating that tailoring the glyco-density is important for regulating the cellular response. In summary, the HepG2 cell

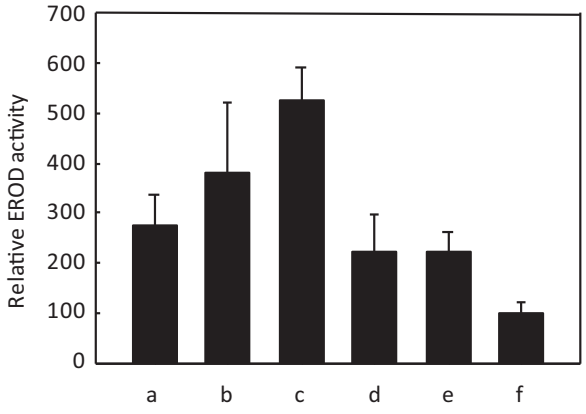

Fig. 5. Enzymatic response for the deethoxylation of ethoxyresorufin *via* cytochrome P450 CYP1A1 of HepG2 cells cultured on hybrid nanolayers of GL and CL with varying molar proportions of GL: 0% (a), 25% (b), 50% (c), 75% (d) and 100% (e). Control, TCPS (f).

culture assay demonstrated that galactose residues on the SAMs strongly influenced the biological response of HepG2 cells. These unique findings should provide a novel strategy and approach for controlling cell culture environments and cellular responses *via* fine-tuning of the surface assembly of various oligosaccharides.

4. Conclusion

Two oligosaccharides, 4′-galactosyl lactose (GL) and cellobiose (CL), were successfully self-assembled on a transparent Au substrate through site-selective S-derivatization of the sugar-reducing ends with TSC. The binding behavior of RCA_{120} lectin to hybrid SAMs was strongly influenced by the molar ratios of GL and CL, resulting in the highest affinity being for 50% GL/50% CL-SAM. By contrast, the binding of ConA lectin exhibited simple decreasing adsorption with a decrease in the CL ratio. The RCA_{120} lectin likely recognized the clustering state of GL molecules on the SAM surfaces. HepG2 cells adhered to hydrophilic hybrid SAMs as well as a commercial hydrophobic TCPS plates. CYP1A1 activity for detoxification was significantly higher on the hybrid SAMs, which closely depended on the molar ratios of two oligosaccharides. The architectural design of carbohydrate-based hybrid nanolayers *via* vectorial chain immobilization is expected to broaden the functional developments of glyco-decorated biointerfaces.

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