Communication

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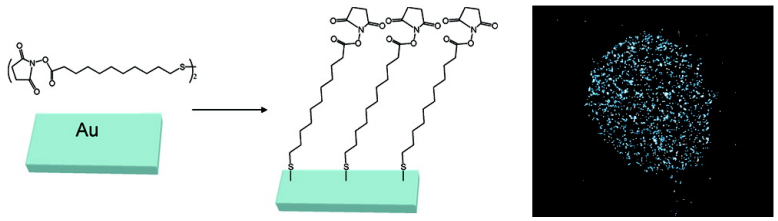

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Lectin Arrays for Profiling Cell Surface Carbohydrate Expression

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Cell surface carbohydrates play critical roles in many fundamental cellular processes, such as cell−cell communication, cell−extracellular matrix interactions, and immune response modulation.[1,2] Different cell types vary in the nature of their surface carbohydrate expression, and a single cell type may change its surface carbohydrate expression profile during cell growth and differentiation processes.[2] Altered carbohydrate expression patterns have also been observed on tumor cells,[3] and may play a role in the escape of tumor cells from immune surveillance as well as metastasis.[3,4] Effective tools for the identification of cell surface carbohydrate expression are essential to the study of these and other phenomena.

Lectins are proteins of nonimmune origin that can recognize and bind to specific carbohydrate structural epitopes. Because of their ability to recognize saccharide motifs, lectins are invaluable tools to interpret the sugar codes on cell surfaces.[5] Two recent studies have described the use of lectin arrays to analyze the carbohydrate motifs expressed on glycoproteins.[6,7] We present here a strategy for profiling cell surface carbohydrate expression patterns by evaluating cell-binding patterns on lectin arrays. The approach is demonstrated using a small array of six commercially available lectins with well-characterized binding specificities (Table 1). As over 100 lectins have been described in the literature,[8,9] the approach could readily be extended to provide a more comprehensive analysis.

The chemistry employed for lectin array fabrication is diagrammed in Figure 1. An N-hydroxy succinimidyl (NHS) ester alkyl disulfide was used to form a self-assembled monolayer with amine-reactive surface functionalities on gold thin film substrates.[10] These substrates were chosen due to their high degree of chemical homogeneity and amenability to a wide variety of chemical modifications. Different lectins were spotted onto the substrate and allowed to react for 3 h in a humid chamber. The chip was then incubated for 30 min in a solution of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) to minimize nonspecific binding, followed by rinsing in PBS.

The attachment of two of the lectins, ConA and WGA, to the surface was demonstrated in antibody binding experiments. Both ConA and WGA were deposited on two gold chips. After BSA blocking, biotinylated-anti-ConA and biotinylated-anti-WGA were added to one of the two chips, respectively, followed by washing with PBS. The chips were then incubated with fluorescein-labeled streptavidin for 30 min, washed with PBS, and scanned with a fluorescence imager. As shown in Figure 2A, the chip treated with anti-ConA showed a fluorescence signal only from the ConA spots, and the chip treated with anti-WGA showed a fluorescence signal only from the WGA spots. These results demonstrated the effective immobilization of the two lectins on the surface. Additional results from the ovalbumin and cell-binding experiments shown below indicate that the other four lectins were also present on the surface.

The retention of specific binding activity in the surface-bound lectins was evaluated using the glycoprotein chicken ovalbumin.

Table 1. Carbohydrate Specificities of the Lectins[a]

Lectins	Carbohydrate Specificity
Concanavalin A *(ConA)*	Manα(1,6) / Manα(1,3) > Manα(1,6)/Manβ(1,4)
Wheat Germ Agglutinin *(WGA)*	GlcNAc oligomers
Ulex Europaeus Agglutinin I*(UEA)*	Fucα(1,2)Gal
Ricinus Communis Agglutinin I*(RCA)*	Galβ(1,4)GlcNAcβ1
Griffonia Simplicifolia Lectin II *(GSL-II)*	GlcNAc on non-reducing terminus
Sambucus Nigra Lectin *(SNA)*	Siaα(2,6)Gal/GalNAc

[a] This table lists carbohydrates for which the lectins have the highest binding affinity. More detailed specificity information can be found in refs 8 and 9. Man: mannose; GlcNAc: *N*-acetylglucosamine; Gal: galactose; GalNAc: *N*-acetylgalactosamine; Fuc: fucose; Sia: sialic acid.

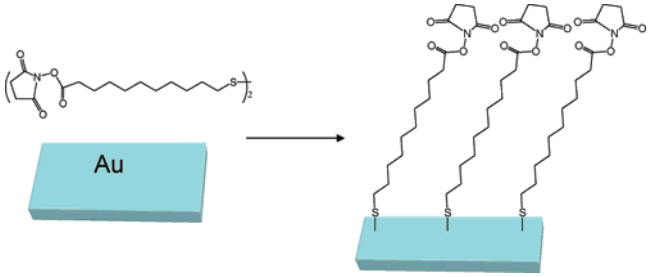

Figure 1. Surface fabrication: self-assembled NHS−ester monolayer on a gold substrate.

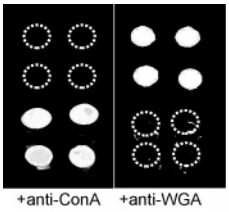

Figure 2A

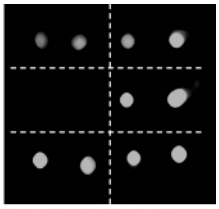

Figure 2B

Figure 2. (A) Antibody test. WGA (the top four spots) and ConA (the bottom four spots) were spotted onto two NHS-modified gold substrates. Biotinylated-anti-ConA was added to the left chip, and biotinylated-anti-WGA was added to the right chip. After incubation and washing, fluorescein−streptavidin was added to both chips. Only ConA showed a fluorescence signal on the chip treated with anti-ConA, and only WGA showed a fluorescence signal on the chip treated with anti-WGA. (B) Fluorescein-tagged chicken ovalbumin was added to a lectin array composed of six lectins. Each lectin was spotted in duplicate according to the pattern shown in Figure 3C. The observed binding pattern is in accordance with literature reports of the carbohydrate structure of chicken ovalbumin and the carbohydrate binding specificities of the lectins.

Chicken ovalbumin was labeled with fluorescein and incubated with the array of the six lectins shown in Table 1, followed by washing and fluorescence scanning. The observed binding pattern (Figure 2B) is in agreement with literature reports of lectin binding

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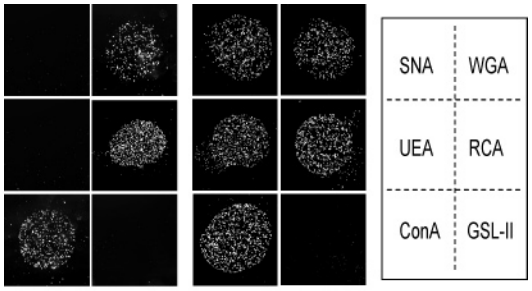

Figure 3A Figure 3B Figure 3C

Figure 3. BHK-21 cells and Caco-2 cells showed distinct binding patterns to the lectin array. The lectins were spotted according to the pattern in (C). BHK-21 cells bound to three of the lectins (A) while Caco-2 cells bound to every lectin except GSL-II (B).

specificities. Chicken ovalbumin is reported to have a single N-linked glycan at Asn392, which contains Man, GlcNAc, Gal, and a small amount of sialic acid.[11,12] ConA, WGA, RCA, GSL-II, and SNA recognize those sugars respectively (Table 1). Each of these lectins showed a fluorescence signal, while UEA, the lectin which binds exclusively to fucose, showed no fluorescence signal, reflecting the absence of fucose groups in chicken ovalbumin. In control experiments using fluorescently tagged streptavidin, which is a protein with no carbohydrate functionalities,[13] no fluorescence was detected from any of the lectins (data not shown). These results indicate that the surface-immobilized lectins retain their ability to specifically recognize and bind to carbohydrate structural motifs.

The lectin arrays described above were used to study cell surface carbohydrate expression. The thickness of the gold thin films employed in these experiments was 30 nm in order for the substrate to be sufficiently transparent for cell-binding observations using an inverted optical microscope. The cells employed were BHK-21, which is a fibroblast cell line derived from baby hamster kidney, and Caco-2, an adenocarcinoma cell line derived from human colon. Cells were cultured in medium to about 90% confluence and were then collected in medium after trypsin treatment. After centrifugation at 200*g* for 5 min, supernatant was removed and cell pellets were resuspended in PBS. After a second 5 min centrifugation, the supernatant was removed and cell pellets were resuspended to $\sim 10^6$ cells/mL in Dulbecco's PBS buffer containing 1.0 mM Ca^{2+} and 0.5 mM Mg^{2+}.

Lectin arrays were placed in the wells of a 6-well tissue culture plate, and 2 mL of cell suspension was added to each well to cover the lectin chips. The plate was then incubated on ice for 30 min, after which the chips were carefully taken out of the cell solution and placed into wells with Dulbecco's PBS buffer to wash off nonspecifically bound cells. After 2−3 washes, the chips were observed with an optical microscope. The two cell lines exhibited distinct patterns of binding to the lectin arrays; BHK-21 cells bound to only three of the lectins, ConA, WGA, and RCA, while Caco-2 cells bound to every lectin except GSL-II (Figure 3). On the basis of the lectins' binding specificities, as shown in Table 1, these results indicated that BHK-21 cells have mannose, galactose, and GlcNAc expressed on their surfaces, and no detectable expression of Siaα2,6Gal(NAc), Fucα(1,2)Gal, or nonreducing terminal GlcNAc. In contrast, Caco-2 cells express mannose, galactose, sialic acid, fucose, and GlcNAc epitopes on the cell surfaces, but lack nonreducing terminal GlcNAc. These inferences are in agreement with existing reports; mannose and galactose are expressed ubiquitously on mammalian cells.[2] Both BHK-21 cells and Caco-2 cells have been reported to express proteoglycans containing heparin sulfate, which has GlcNAc on the reducing terminus.[14,20] Many studies have observed increased fucose and sialic acid expression on several intestinal tumor cells,[15,17] and Caco-2, which was derived from a human colonic tumor, has been reported to bind to lectins which can specifically recognize sialic acid[15] and fucose.[16] Although there have been limited reports suggesting the expression of fucose and sialic acid on BHK-21 cells,[18,19] our results clearly show that the expression levels of these moieties, if they are expressed at all, are much lower than the levels found on the Caco-2 cells.

The present work provides a proof-of-principle demonstration of the direct qualitative profiling of cell surface carbohydrate expression patterns by simple microscopic observation of cell binding to lectin arrays. The approach described here may readily be expanded to encompass a wider variety of carbohydrate structural motifs by increasing the number of lectins or other specific ligands present in the array. The parallelism inherent in array technologies permits a wide variety of cell surface carbohydrate groups to be screened at one time, in marked contrast to the intrinsically serial nature of existing technologies, such as flow sorting or histological analysis.

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Supporting Information Available: Experimental details for the lectin array fabrication and cell-binding experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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