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Competition-based transfer of carbohydrate expression information from a cell-adhered surface to a secondary surface†

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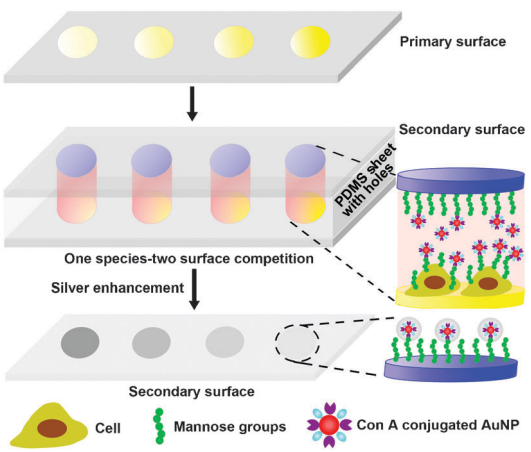
An information transfer strategy was developed for the visualization of carbohydrate expression by the competition of a primary cell-adhered solid surface with a carbohydrate assembled surface as an artificial secondary surface for one species. The strategy could be effectively utilized for *in situ* monitoring of dynamic carbohydrate expression on an adhesive cell surface.

A surface or interface in nature is an important assembly format for multiple biological molecules. The construction of surfaces or interfaces provides a basis for exploring ligand–receptor interactions, protein activities, multivalence expression and for screening libraries of potential drugs.[1,2] Correspondingly, it is crucial to develop facile and sensitive strategies for analyzing the composition of surfaces and studying the thermodynamics and kinetics of binding events that occur at the interface. Current techniques for probing a certain surface are mainly derived from spectroscopic/physical techniques,[1] which are useful for understanding the molecular-scale order at the interface. However, the quantitative study of biological recognition capability of an artificial solid surface is still a big challenge. Moreover, these methods rely on the use of sophisticated equipments, which limits their practical applicability. Thus it can be expected that the interfacial study will greatly require and benefit from the development of new quantitative methods, particularly largely instrumental-free ones.

In this work, a novel strategy for probing the surface composition is reported by selectively transferring the biological information from a primary cell-adhered solid surface to a carbohydrate assembled surface as an artificial secondary surface. This approach is based on the competition between the two surfaces for specifically binding the receptor species. Through detection of the binding extent on the secondary surface, the information of the ligand expression on the primary surface can be obtained. This strategy presents significantly expanded advantages and distinctly new possibilities over the previously reported suspension assay.[3] It is extremely useful for interrogation of the biomolecular recognition at the complex biological interface, particularly when the primary surface is hard to investigate with a desired sensitivity or when no detection method currently exists. Meanwhile, it can greatly increase the detection throughput, and allow the quantitative study of the natural state of adhered cells without disturbing cellular physiological activities or requiring a major instrument.

Considering that the cell membrane consists of complex biomacromolecule assemblies, it is difficult to study certain fundamental aspects of the cell surface in detail, especially in the case of carbohydrates.[4a] The broad range of membrane-bound carbohydrates participate in many important processes in living organisms.[4b] In particular, variations of glycan expression are associated with certain disease states.[5] Although various methods, especially fluorescence-based techniques, have been developed to monitor the dynamic change of carbohydrate expression on the cell surface,[6] they generally rely on the utility of sophisticated instruments.

To demonstrate the capability of the proposed strategy, as shown in Scheme 1, a human gastric gland carcinoma cell (BGC-823)-adhered glass slide was chosen as the model primary surface, and the mannose expression information on the cell surface was extracted and transferred to a secondary



Scheme 1 Schematic representation of mannose information transfer from a cell-adhered primary surface to a mannose-presenting secondary surface and visualization of cell surface carbohydrate expression.

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competitive glass slide presenting a mannose monolayer. Concanavalin A (Con A), a type of lectin exhibiting highly specific binding affinity for mannose, was thus chosen as the receptor[7] that could be recognized and competitively bound by the two surfaces. Ca^{2+} and Mn^{2+} were used to maintain the biological activity of Con A during recognition.[7] For tracing the competition result, gold nanoparticles (AuNPs) were used to carry Con A (ESI†), which could catalyze silver deposition and allow visualization and scanometric readout[8] of the mannose information. To increase the stability of the nanocomposite and reduce non-specific adsorption, bovine serum albumin (BSA) was also introduced to AuNPs.[9] The obtained nanoprobe, Con A and BSA co-functionalized AuNP, was demonstrated by UV-vis spectra and an electrophoresis image (Fig. S1 in ESI†), which integrated the specific recognition capability of lectin, non-specific blocking function of BSA and the signal amplification ability of AuNPs.

A mannan self-assembled monolayer as the secondary surface was prepared at a defined location on a glass slide by the organosilane method using adipic dihydrazide (ADH) as the linker.[10,11] The successful immobilization of mannan was verified using atomic force micrographs (Fig. 1a and b) and fluorescent images (Fig. S2 in ESI†). After incubation with the nanoprobe, the mannan assembled surface showed evenly distributed round particles, indicating effective binding of the nanoprobe to the mannan surface (Fig. 1c and d).

The specific recognition between the mannan spot and nanoprobe was verified by scanometric detection (Fig. 2). A control experiment was performed using methyl mannopyranoside to replace mannan to treat ADH. No silver signal could be detected on control spots, which were therefore utilized for quality control of the surface chemistry as well as the generation of background signal (*vide infra*). Compared with control spots, the incubation of mannan spots with the nanoprobe produced grey spots due to the specific recognition of Con A to mannan and the catalyzed deposition of silver by AuNPs. The optimal concentration of mannan for preparation of the secondary surface was 0.2 mg mL^{-1} (Fig. S3a in ESI†).

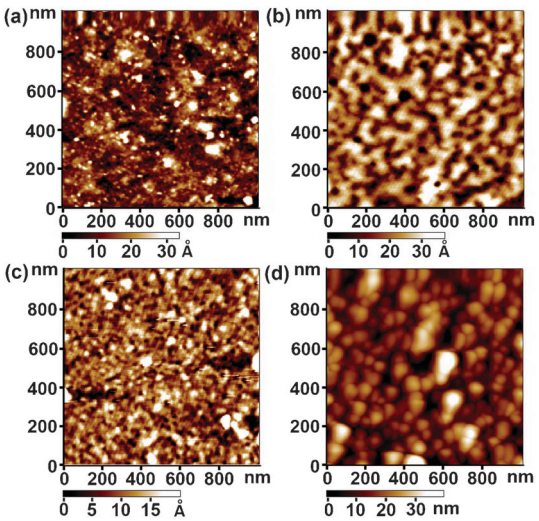

Fig. 1 Topographic images of epoxy- (a) and mannan/ADH/epoxy- (b) coated silicon chips, blocked mannan/ADH/epoxy-coated chip (c) and chip (c) after incubation with nanoprobe (d).

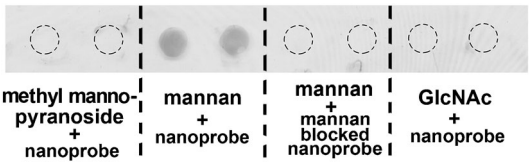

Fig. 2 Demonstration of the specific recognition between the surface-immobilized mannan and nanoprobe.

After the nanoprobe was blocked by mannan, or the mannan immobilized on the surface was displaced by *N*-acetylglucosamine (GlcNAc), the incubation and silver deposition steps did not produce a grey signal, indicating the selectivity of the nanoprobe toward carbohydrate.

For the preparation of the cell-adhered primary surface, an artificially prepared poly(dimethylsiloxane) (PDMS) sheet with an array of holes at defined locations[12] was covered on the glass slides to control the location of cell adhesion. After cell seeding and adhesion, the cell-adhered slide was directly used as the primary surface for information transfer. At a cell concentration of 5×10^6 cells mL^{-1}, 95.2% of cells in 20 μL could adhere on the slide. This design was of great significance for cell surface component detection that it enabled the study of the original surface state of adhered cells without trypsin treatment.[13]

The array-based competition was carried out using a sandwich set-up. The nanoprobe filled in the wells could be competitively bound by the two surfaces. The optimal concentration of nanoprobe was 14 nM (Fig. S3b in ESI†). Upon the increase of cell amount adhered on the primary surface, the silver signal of the corresponding mannan spot decreased (Fig. 3), which was attributed to the competition of the carbohydrates on cells with the surface-confined mannan to bind the nanoprobe. The greyscale data were also presented using a multicolor scale for more distinct visualization (Fig. 3b). The calibration curve showed a linear relationship between the relative greyscale intensity (I) and the logarithm of cell concentration in a wide range down to 5×10^4 cells mL^{-1} with a correlation coefficient R of 0.95, which was one order of magnitude more sensitive than the competition-free method with a range down to 5×10^5 cells mL^{-1} (Fig. S4 in ESI†).

Owing to the facile information transfer capability of the competition strategy, the dynamic alteration of carbohydrate expression on living cells in response to drugs could also be investigated. Swainsonine (SW), a specific mannosidase II inhibitor, was chosen as the model drug, which could increase the expression of terminal high-mannose type glycans on the cell surface.[14] SW-treated BGC-823 cells could adhere on the primary surface with the same adhesion ratio as untreated cells. After treatment with SW for 2 days, the SW-treated cells showed a weaker silver signal than untreated cells at the same cell concentration, indicating that the SW-treated cells were more competitive toward the nanoprobe than untreated cells (Fig. 4). In the cell concentration range of 5×10^5 to 5×10^6 cells mL^{-1}, a statistically significant change ($p < 0.05$, one-way ANOVA) could be obtained between the two cell subsets, which could be observed in Fig. 3b and 4b. The mannose expression on SW-treated cells was estimated to increase by 93% compared with untreated cells, demonstrating

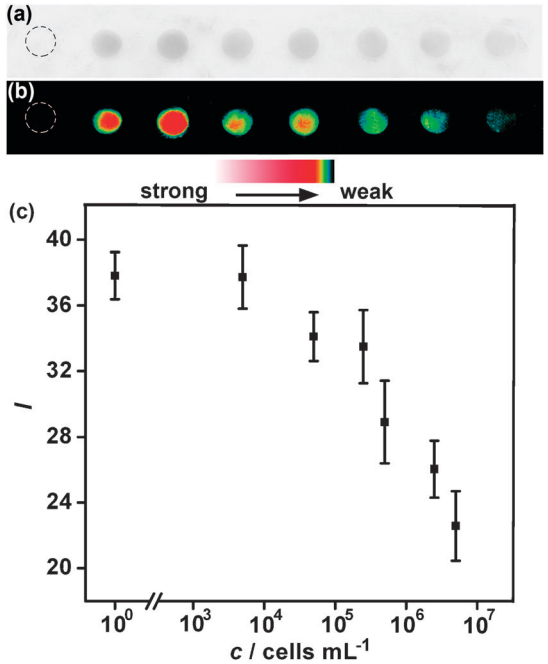

Fig. 3 (a) Greyscale and (b) color scanometric images of a carbohydrate-modified slide after competition with a BGC-823 cell-adhered slide for binding the nanoprobe. The leftmost spot, as circled, was the control spot. The spots from the second to the rightmost corresponded to 0, 5.0×10^3, 5.0×10^4, 2.5×10^5, 5.0×10^5, 2.5×10^6 and 5.0×10^6 cells mL^{-1}, respectively. (c) Plot of *I vs.* cell concentration.

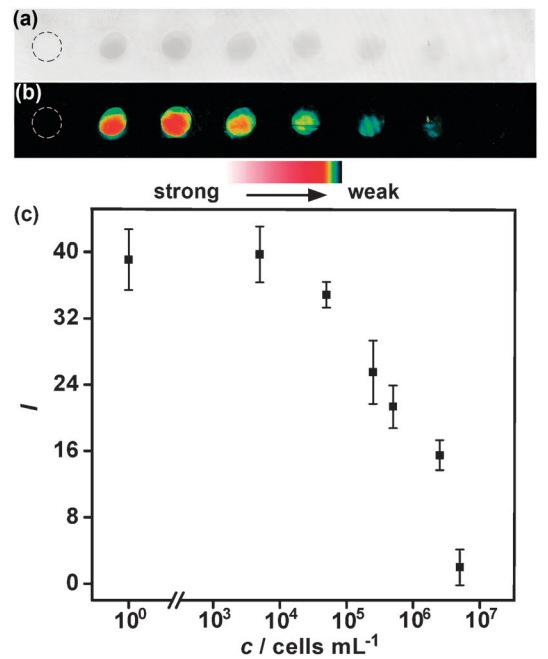

Fig. 4 (a) Greyscale and (b) color scanometric images of a carbohydrate-modified slide after competition with a slide adhered by SW-treated cells for binding to the nanoprobe. The leftmost spot was the control spot. The spots from the second to the rightmost corresponded to SW-treated cell concentration of 0, 5.0×10^3, 5.0×10^4, 2.5×10^5, 5.0×10^5, 2.5×10^6 and 5.0×10^6 cells mL^{-1}, respectively. (c) Plot of *I vs.* cell concentration.

the modification effect of SW on cell surface glycosylation. The observed change tendency was in good agreement with the flow cytometric assay using fluorescein-conjugated Con A to recognize BGC-823 cell surface mannose moieties.[15]

In conclusion, this work provides a novel protocol for selective extraction and transfer of biological expression information from a primary solid surface to an artificial secondary surface. By a competitive binding of receptor species to the two surfaces, the information transfer is achieved, which can then be facilely detected at the secondary surface. Using cell surface carbohydrate as the model analyte, the carbohydrate expression information on the cell-adhered primary slide could be transferred to the carbohydrate-assembled slide by the competition process using lectin-attached AuNPs as the nanoprobe. The AuNP-catalyzed silver enhancement led to the visualization of carbohydrate expression by a scanometric method. This information transfer pathway could increase the detection sensitivity and allow the biological species to be investigated at their original state. The scanometric method was used to monitor the dynamic change of the cell surface carbohydrate expression without using large instruments. This technology could be employed for studying other active components on the cell surface, and most importantly, it could be expanded to achieve information transfer between different substrates to better fit the detection requirement.

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