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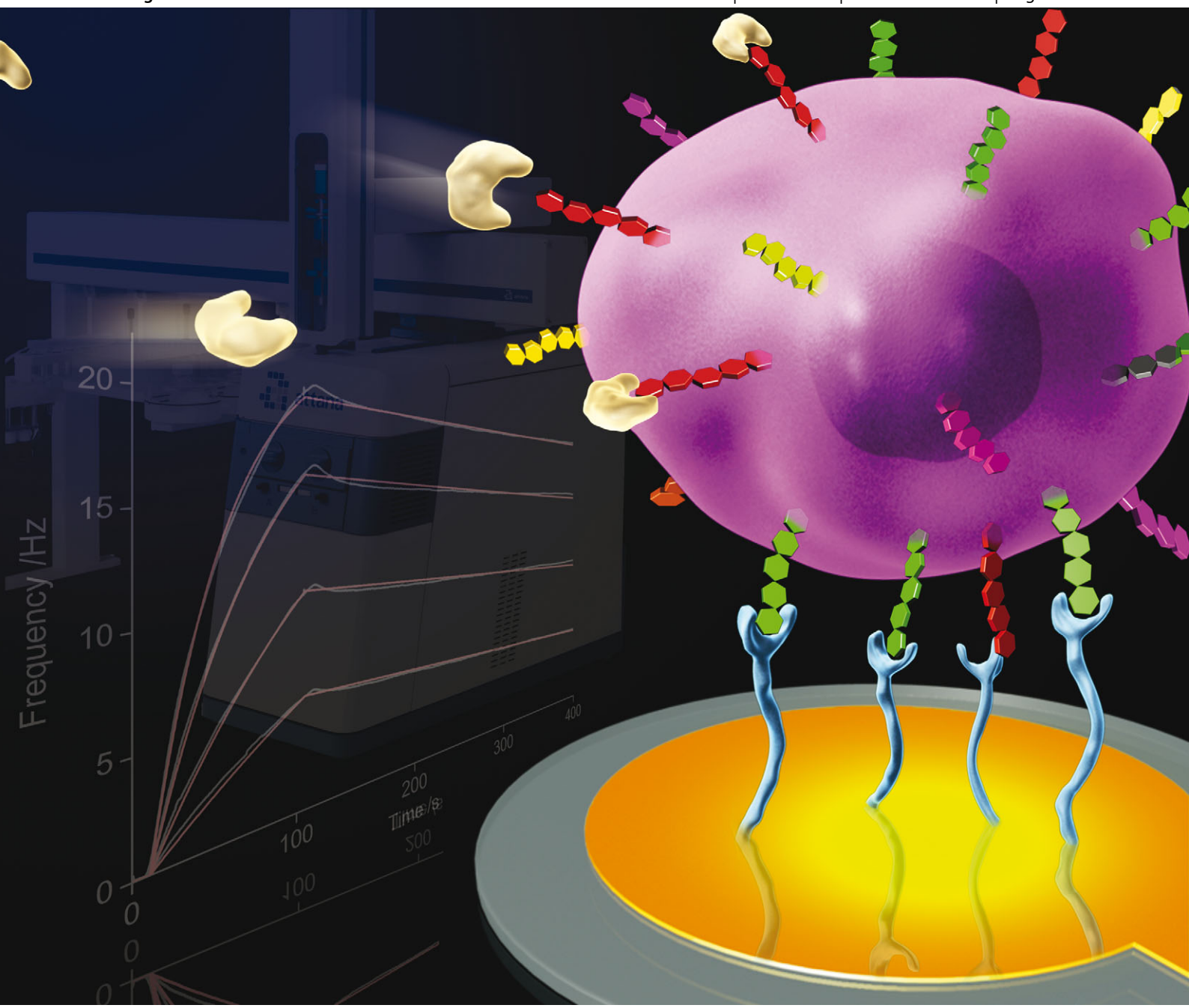

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A suspension-cell biosensor for real-time determination of binding kinetics of protein–carbohydrate interactions on cancer cell surfaces†

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A novel lectin-based suspension-cell biosensor for label-free determination of binding kinetics of protein–carbohydrate interactions on cancer cell surfaces using QCM is described. This cell-biosensor facilitates evaluation of glycosylation in real time on suspension cancer cell surfaces, where binding events take place, more closely mimicking a native environment compared with traditional biosensors.

Most membrane proteins on cell surfaces are glycosylated,[1] which have a variety of carbohydrate chains attached. Alterations in glycosylation patterns have been associated with the development and progression of specific diseases.[2] So far, many glycosylated proteins (glycoproteins) have been identified as biomarkers for various diseases, such as lymphoma cancer and breast cancer.[3] Changes in glycosylation patterns on cancer cell surfaces detected by carbohydrate binding proteins (lectins) have been reported to play a crucial role in cancer growth and metastasis formation.[4] In addition, the extracellularly extended carbohydrate structures on cell surfaces have been found to be of essential importance in many biological processes,[5–7] such as cell recognition and communication through specific interactions.

To thoroughly understand these interactions, novel and efficient methods are needed for fast and reliable recognition tests. To date, several means have been exploited to analyze carbohydrate–protein interactions, for example, enzyme-linked lectin assays (ELLAs),[8] X-ray crystallography,[9] mass spectrometry (MS),[10] nuclear magnetic resonance (NMR),[11] array technologies[12–15] and biosensors.[16,17] Among these, biosensors based on quartz crystal microbalance[18,19] (QCM) and surface plasmon resonance[20,21] (SPR) technologies have become increasingly popular due to the facilitated means of operation coupled with high performance in real time without the need for any labelling procedures, which can also be used for analysis of association and dissociation phases of a complex, enabling the study of binding kinetics, as well as providing insights into potential drug candidates.

However, the traditional biosensors (or approaches) are limited to using isolated and purified target glycoproteins from cells and then immobilizing the targets on sensor surfaces, where the binding kinetics data do not present the biological contexts and do not reflect their native structures and functions in cells accurately. To determine the binding kinetics of the glycoproteins in their native environments, recent studies have been concerned with measuring the interactions directly on intact cell surfaces. Examples of these studies include adherent cells grown on a poly-L-lysine coated gold surface to test the binding kinetics of membrane glycoproteins,[22] and captured on an antibody immobilized surface[23] based on SPR technologies. Previous studies have also utilized QCM cell biosensors to monitor protein–carbohydrate interactions in real-time by growing adherent cells on a polystyrene coated surface.[24,25] Compared with the adherent cells, the suspension cells are naturally live in suspension, which cannot be attached or grown onto a surface. Therefore, to develop a suspension-cell biosensor for monitoring the binding kinetics of protein–carbohydrate interactions on the suspension cell surfaces is highly desirable.

Here, we report an approach in which the suspension cells were captured onto a QCM chip surface in a simple and straightforward way *via* interactions of the cell surface carbohydrates and Concanavalin A (Con A). The affinity of interaction between lectins and the cells was investigated using the fabricated suspension-cell biosensors. To the best of our knowledge, this is the first report in which the suspension-cell QCM biosensor was fabricated and exploited for evaluating the binding kinetics of suspension-cell surface glycosylation.

In our experiments, firstly, a LNB-carboxyl QCM chip was inserted into an Attana cell biosensor. The carboxyl groups on the chip surface were activated by EDC–sulfo-NHS *in situ*, then Con A was covalently bound onto the carboxyl sensor chip surface *via* amine coupling, where the residual activated carboxyl groups were blocked with ethanolamine ((i) and (ii) in Scheme 1). Secondly, the Con A coated chip was withdrawn and the suspension cancer cells (human acute myelocytic leukemia cell line,

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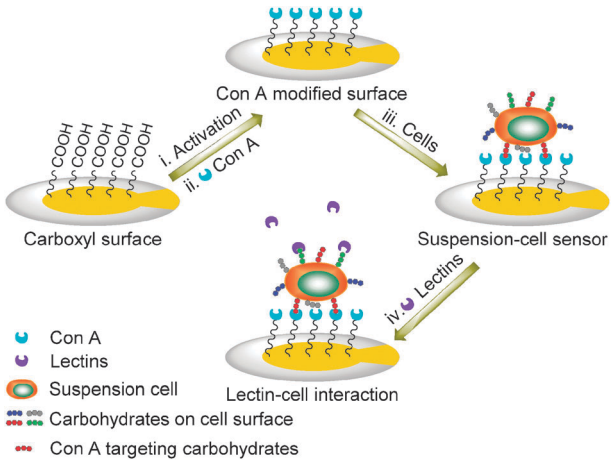

Scheme 1 Procedure for suspension-cell biosensor fabrication and interaction of protein-cell surfaces: (i) the carboxyl groups were activated by EDC–sulfo-NHS; (ii) Con A was covalently bound to the surface *via* amine coupling, then blocking the residual activated carboxyl groups by ethanolamine; (iii) suspension cells were immobilized by interaction between Con A and glycans on the cell surface. (iv) Lectins injected interacted with surfaces of immobilized suspension cells.

K562, and human acute lymphocytic leukemia cell line, Jurkat cells) were captured onto the surface ((iii) in Scheme 1). Finally, the cell biosensor was washed with PBS buffer to remove the unbound cells and assembled into the QCM system. The interactions between proteins (lectins) and cells were monitored using QCM in real-time by injecting lectin solution onto the sensor surface ((iv) in Scheme 1). The details of the fabrication of suspension-cell biosensors are provided in ESI.†

To evaluate cell coverage on the sensor surface, the nuclei were stained with Hoechst 33258 and visualised under a fluorescent microscope (Olympus BX53). The fluorescent image is displayed in Fig. 1, which indicates that the suspension cells have been captured on the Con A-coated surface evenly.

In order to test protein–carbohydrate interactions in real-time, the prepared suspension cell chips were docked into the Cell A200 QCM instrument and equilibrated under a continuous flow (20 μL min^{-1}) of running buffer. The measurements were initiated when the resonant frequency was stable (baseline drift < 0.2 Hz min^{-1}). The interactions between lectins and cell surface glycans were evaluated by injecting lectins in running buffer over the surfaces in a range of concentrations. The surface regeneration was performed between the interaction tests. Fig. 2 shows a typical cycle in which Wheat germ agglutinin (WGA) at 50 μg mL^{-1} was injected twice successively with a regeneration

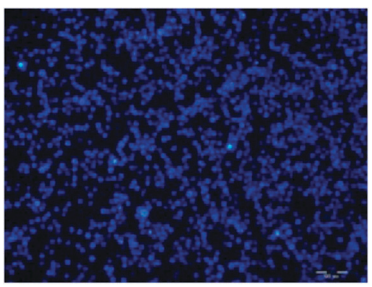

Fig. 1 After *ex situ* cell capture, the fluorescent image was taken under a fluorescent microscope (Olympus BX53).

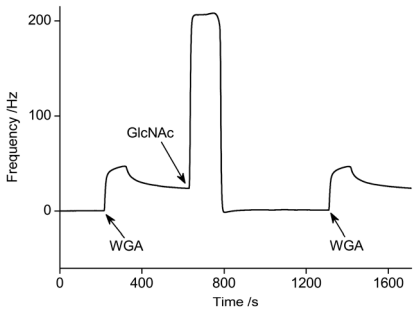

Fig. 2 Binding and regeneration of WGA to immobilized K562 cells, where the frequency shift was recorded during the interactions and regeneration.

step in between, with the regeneration solution being 300 mM *N*-acetylglucosamine (GlcNAc). The results implied good regeneration and reproducibility of the cell biosensor.

To evaluate the cell surface glycosylation, the lectin screening was performed with the sequential injections of lectins separated by regeneration steps in real-time, wherein a range of lectins, WGA, Con A, Soybean agglutinin (SBA), Peanut agglutinin (PNA) and Ulex europeus agglutinin I (UEA I), at 50 μg mL^{-1} were injected over Jurkat and K562 cell surfaces, respectively, and the corresponding monosaccharides (300 mM) were used as the regeneration solutions. The responses were simultaneously monitored by the Attana Cell A200 QCM biosensor and the maximal frequency shifts obtained for each lectin were recorded, and are summarized in Fig. 3a.

As can be seen, the different frequency shifts produced from the interactions between lectins and cancer cell surfaces resulted from the different glycosylation on cell surfaces. The biggest frequency shift (123 Hz) was obtained from interaction of

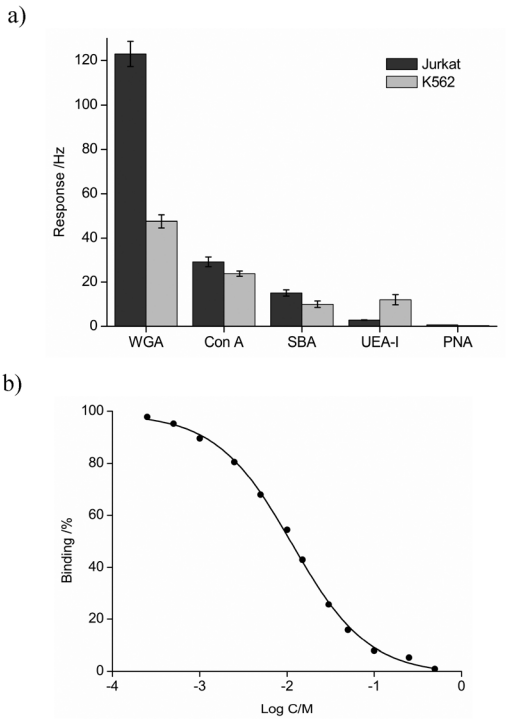

Fig. 3 (a) Lectins, WGA, Con A, SBA, UEA-I and PNA were injected over two different cell surfaces (Jurkat and K562 cells), respectively. The maximal frequency shift observed at the end of the injection phase was monitored; (b) competition plot of the interaction of WGA (50 μg mL^{-1}) with Jurkat cells using GlcNAc (0.25–495 mM) as the ligand.

N-acetyl-glucosamine binding lectin WGA with the Jurkat cell surface, which could be explained by the binding of WGA to highly branched glycoconjugates at the surface of Jurkat cells. The binding of Con A and SBA was recorded with maximal frequency shifts of 29 Hz and 15 Hz, respectively, whereas very weak responses were produced with UEA I and PNA to the Jurkat cell surface. The binding of WGA, Con A, SBA and UEA-I to the K562 cell surface was recorded with maximal frequency shifts of 48 Hz, 24 Hz, 10 Hz and 12 Hz, respectively, whereas no response was measured with PNA. These results suggested that Jurkat and K562 cells expressed different levels of *N*-acetyl-D-glucosamine, D-mannose- and D-glucose-, *N*-acetyl-galactosamine-, and α-L-fucose-containing glycoconjugates at their surfaces. This measurement of the responses obtained for each of the lectins to cell surfaces enabled a real-time analysis of the lectin–cell interactions.

A competition experiment was carried out using *N*-acetyl-glucosamine as the ligand, which is a known ligand bound to WGA. The samples were prepared by mixing a stock solution of WGA (50 μg mL^{-1}) with the consecutively diluted carbohydrate ligand in the running buffer. All measurements were performed successively with regeneration steps in between. Increasing concentrations of the competitor (0.25–495 mM) resulted in a gradual decrease in binding of WGA to the cell surface, with reduced frequency differences as a consequence. The resulting competitive binding curve is displayed in Fig. 3b. The competitive binding data were subjected to non-linear regression analysis, and the EC_{50}-value (11.5 mM) was obtained.

To further measure the binding kinetics of cell surface glycans, the kinetics of lectin–cell interactions was determined for WGA and SBA with Jurkat and K562 cells, respectively. A typical example of the frequency shifts recorded from the SBA at 0.052–0.96 μM to the K562 cell surface is displayed in Fig. 4 along with the theoretical 1 : 1 fit produced using Evaluation software (Attana).[21] The association and dissociation constants (K_a = 7.56 × 10^4 M^{-1} s^{-1}; K_d = 3.89 × 10^{-4} s^{-1}) as well as the affinity constant (K_D = 5.15 nM) were obtained (Table 1). Moreover, the kinetics of WGA-K562, WGA-Jurkat, SBA-Jurkat cells were also evaluated, respectively; resulting kinetic data are presented in Table 1. The results clearly showed that the binding constants varied considerably depending on lectins and cells, which resulted from the different glycosylation on the suspension-cell surfaces.

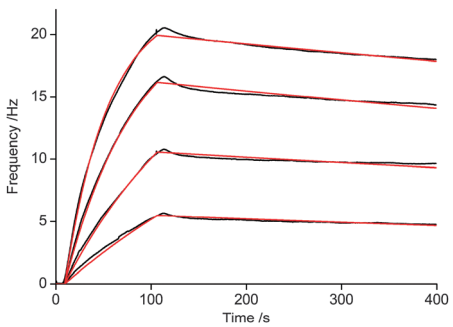

Fig. 4 Kinetic evaluation of the interactions between SBA and the K562 cell surface, SBA at 0.052–0.96 μM was injected and the responses were recorded (black lines). Theoretical 1 : 1 fit generated using Evaluation software (Attana) was overlaid (red lines), the association and dissociation constants, K_a and K_d, were determined to be 7.56 × 10^4 M^{-1} s^{-1} and 3.89 × 10^{-4} s^{-1}, respectively, which gave a K_D of 5.15 nM.

Table 1 The binding constants of SBA-K562, WGA-K562, SBA-Jurkat, WGA-Jurkat cells obtained using Evaluation software (Attana)

	K562			Jurkat		
Lectins	K_a (M^{-1} S^{-1})	K_d (S^{-1})	K_D (nM)	K_a (M^{-1} S^{-1})	K_d (S^{-1})	K_D (nM)
SBA	7.56 × 10^4	3.89 × 10^{-4}	5.15	4.44 × 10^4	5.31 × 10^{-4}	12
WGA	1.52 × 10^6	1.33 × 10^{-3}	0.87	1.06 × 10^6	2.99 × 10^{-3}	2.83

In conclusion, we have developed a novel label-free suspension-cell biosensor based on QCM technology to evaluate the interactions between protein and cell surface glycan in real time, where the suspension cancer cells were captured onto Con A-coated quartz crystals. This cell biosensor allows us to directly determine the binding kinetics in the native environment, providing insight into the nature of glycoconjugates exposed on cell surfaces, which may have impacts on the study of the biological activities of glycoproteins and the discovery of drugs, as well as on the diagnosis of diseases.

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