VIP Glycome Analysis

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A Simple Electrochemical Cytosensor Array for Dynamic Analysis of Carcinoma Cell Surface Glycans**

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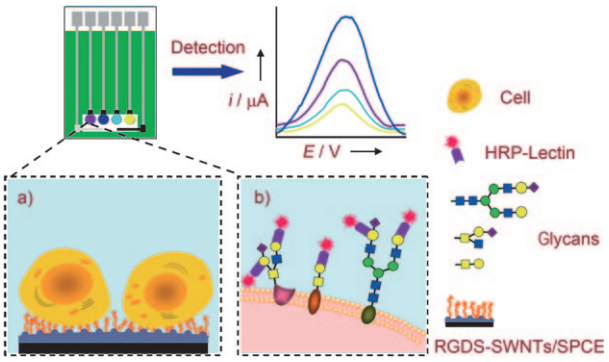
Glycosylation is one of the most common post-transcriptional modifications in eukaryotes. Glycans account for a large degree of cell-surface structural variations, and form complicated codes for cellular physiology.[1] Glycomics, which studies the structure and function of diverse glycans, is quickly becoming a driving force for deciphering various cellular pathophysiological processes and discovering potential biomarkers and therapeutic targets.[2] Dynamic changes in the glycosylation status on carcinoma cell surfaces have been observed to play important roles in oncogenic transformation, cell differentiation, and metastasis. Thus, it is urgent to develop sensitive, pragmatic, and high-throughput monitoring technology for analyzing these changes[3] and for glycomics studies.

Some methods, including mass spectrometry, high performance liquid chromatography, and chemical analysis, can be powerful for glycomics, but they are unsuitable for detection of glycans on the surfaces of living cells because of the associated destructive sample preparation methods.[4] Approaches based on lectin arrays open an avenue for high-throughput analysis of glycans on intact cells;[5] however, the reproducibility of lectin array data needs to be improved, and reliable quality-control methodology is also being developed.[3d] Electrochemical methods have attracted considerable attention in nondestructive analysis of living cells.[6] Two methods for electrochemical monitoring of cell-surface carbohydrates have been proposed based on the specific recognition of cell-surface glycans by lectins labeled with horseradish peroxidase (HRP) or quantum dots.[7] These methods can be used to evaluate the specific carbohydrate sites of single glycans on cell surfaces.

By integrating micromachining and nanotechnology, many exquisite electrochemical sensor arrays have been developed for simultaneous analysis of individual cellular functions and multimarkers.[8] On the basis of preliminary work on the evaluation of carbohydrate sites,[7a] we developed a disposable electrochemical cytosensor array for simultaneous multiple analysis of glycans on intact cell surfaces. Effective monitoring of the dynamic variation of glycans on cancer cell surfaces during both drug inducement and erythroid differentiation of human leukemic K562 cells demonstrated that the design strategy and the disposable cytosensor array combine to give a facile and powerful protocol for dynamic analysis of the carcinoma cell surface glycome.

The cytosensor array was prepared by using single-walled carbon nanotubes (SWNTs) functionalized with an arginine–glycine–aspartic acid–serine (RGDS) peptide to modify four working electrodes of screen-printed carbon electrodes (SPCEs, see the Supporting Information) for cell capture, which was achieved by exploiting the high affinity between cell-surface integrins and the RGDS tetrapeptides.[9a] Compared with a bare graphite electrode, the nanocomposite-modified electrode offered a more homogeneous surface for cell loading (Figure S1 in the Supporting Information), which enhanced the reproducibility of both the cytosensor arrays and the obtained signals. The biomimetic RGDS-functionalized interface preserves the activity of captured cells for subsequent detection. As shown in Scheme 1, glycans on cancer cell surfaces could be selectively conjugated to corresponding HRP lectins.[9b,c] In this study, four HRP lectins (concanavalin A (ConA), dolichos biflorus agglutinin (DBA), peanut agglutinin (PNA), and wheat germ agglutinin (WGA)) were used for simultaneous multiple analysis at four different working electrodes (Table S1 in the Supporting Information). The immobilized HRP, the amount of which



Scheme 1. Schematic representation of the electrochemical cytosensor array for cell-surface glycan analysis, and close-up illustrations of a) cells captured on RGDS-SWNTs/SPCE and b) HRP-lectin binding with cell-surface glycans.

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depended on the specific carbohydrate sites on the cell surface, produced characteristic electrochemical signals from enzyme catalysis in the solution containing 8.0 mM H_2O_2 and 10 mM *o*-phenylenediamine (*o*-PD; Figure S2 in the Supporting Information) for sensitive readout of cell-surface glycans.

The optimal concentration of K562 cells used for capture of cells on the cytosensor array was 6×10^6 cells mL^{-1} (Figure 1a). Although the electrochemical response tended to the maximum value at a concentration of 2×10^6 cells mL^{-1}, an excess of cells relative to the RGDS tetrapeptide sites on each cytosensor was used to account for the expression variance of integrin at different phase K562 cells and the change in its binding capability with RGDS tetrapeptides upon cell treatment. With the standardized array preparation and the optimal cell concentration, 68.5% untreated K562 cells could be captured on the array from the added K562 cell suspension, while the percentage for differentiated K562 cells was nearly identical at 66.8%. Thus the number of cells captured on different cytosensors was almost the same, indicating formation of a noncompact cell monolayer.

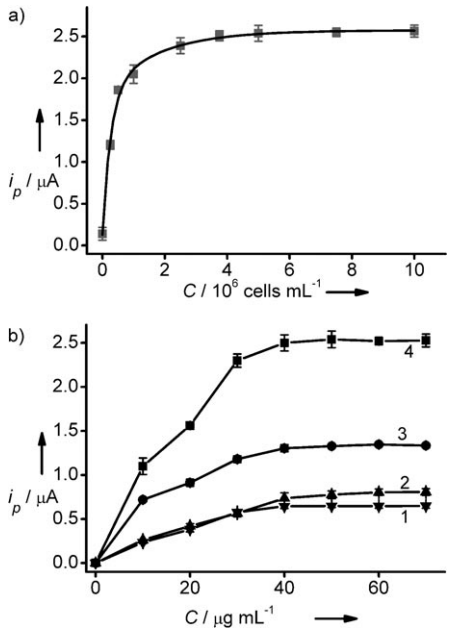

Figure 1. Dependences of differential pulse voltammetry (DPV) response on a) K562 cell concentration for capture before conjugation with 60 μg mL^{-1} HRP WGA, and b) HRP DBA (1), PNA (2), ConA (3), and WGA (4) concentrations after incubation with 6×10^6 K562 cells mL^{-1}.

After HRP lectins were incubated at RGDS–SWNTs/SPCEs, which were first treated with phosphate-buffered saline (PBS) and blocking solution the resulting electrodes showed very low enzyme-catalyzed electrochemical response (Figure 1a), indicating very little nonspecific adsorption of these HRP lectins on the array. This result confirmed the sensitivity of the designed protocol. With increasing concentration of HRP lectins, all responses at K562/RGDS–SWNTs/SPCEs treated with different HRP lectins increased and tended to stable values at similar mass concentrations of lectin (Figure 1b). The optimal concentration of HRP lectins was 60 μg mL^{-1}, which was also in excess relative to cell-surface glycans.

Under the optimal conditions, the DPV peak currents could be used to quantify the amount of HRP lectin conjugated on the captured K562 cells, which directly depended on the quantity of glycans on the cell surface with different lectin-binding specificities. As shown in Figure 2, K562 cells showed strong binding to WGA, moderate binding to ConA, a fairly weak interaction with PNA, and weak interaction with DBA. These findings indicate that the K562 cell surface expressed high abundance of WGA-recognizing glycan, moderate abundance of mannose oligosaccharides (ConA-recognizing glycan), and low abundance of PNA- and DBA-recognizing glycans (Table S1 in the Supporting Information). The flow cytometric analysis of lectins labeled with fluorescein isothiocyanate (FITC) binding to K562 cells confirmed these findings (Figure S3 in the Supporting Information). More importantly, this strategy could be used to obtain more sensitive profiles of glycans with low abundance, such as DBA-recognizing glycan, than flow cytometric analysis with FTIC-labeled lectins owing to the background autofluorescence of cells (curve a, Figure S3 in Supporting Information). The electrochemical detection hardly showed any background signal for HRP-lectin-free control (curve 1, Figure 2). The obtained glycome profile was also in agreement with reported data.[10a] Thus, the proposed strategy could accurately evaluate multiplicate glycan expression statuses on cancer cell surfaces using enzyme-generated electrochemical signals. The intraassay and interassay imprecision for detection of four glycans with the cytosensor arrays were examined using four working electrodes at the same array and five different arrays. The intraassay peak currents differed by less than 5.0% and interassay peak currents by less than 8.0% (Table S2 in the Supporting Information), showing acceptable reproducibility.

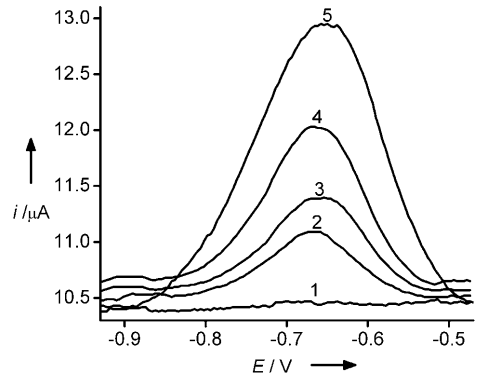

Figure 2. Typical DPV curves of HRP-lectin-bound glycans on K562 cells analyzed by the designed array. 1) HRP-lectin-free control, 2) HRP DBA, 3) HRP PNA, 4) HRP ConA, and 5) HRP WGA.

To further test the performance of the designed array, the glycan expression patterns on the surface of K562 cells treated with 3′-azido-3′-deoxythymidine (AZT) were simultaneously analyzed. The intracellular metabolite of AZT can inhibit

nucleotide sugar transport and significantly modify the glycosylation of proteins and lipids.[10] Compared with untreated K562 cells, the expression of WGA-recognizing glycan on AZT-treated K562 cells showed a increase of 16%, while the amount of PNA-recognizing glycan increased by 13%. As for the glycans corresponding to ConA and DBA, the changes were insignificant ($p < 0.05$; Figure S4 in the Supporting Information). The results indicated that the WGA- and PNA-recognizing glycans on the cells were sensitive to AZT treatment, in accordance with the results from other laboratories.[10]

In view of the high sensitivity, acceptable reproducibility, and the capability for multianalysis, the designed electrochemical cytosensor array was used for analyzing the dynamic change of the K562 cell-surface glycome during erythroid differentiation induced by sodium butyrate (NaBut). For this model experiment, K562 cells were gradually induced to differentiate into erythroid lineages by culturing in the presence of NaBut for 1–7 days, and the erythroid differentiation degree could be evidenced by benzidine staining (reported as the percentage of benzidine-positive cells (BPC)).[11] As shown in Figure 3, upon treatment the DPV

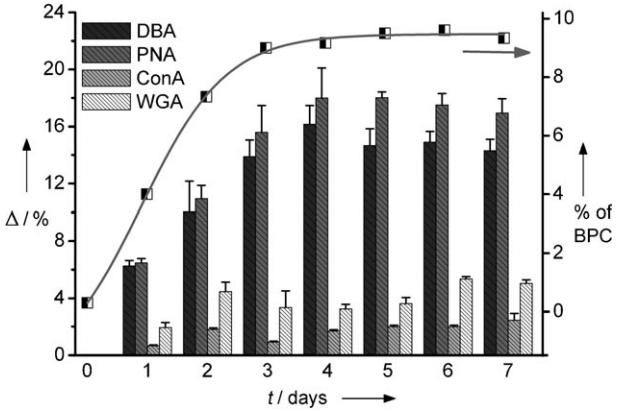

Figure 3. Glycan expression changes on K562 cells treated with NaBut from 1 to 7 days monitored with the designed arrays (columns; left axis), and percentage of benzidine-positive cells (BPC) during NaBut treatment (line and symbols; right axis).

signals for HRP PNA and HRP DBA progressively increased and tended to steady magnitudes after three days, while the DPV signals for WGA- and ConA-recognizing glycans showed small changes. Thus the expression of PNA- and DBA-recognizing glycans (O glycans)[12a] on erythroid-differentiated K562 cell surfaces significantly increased, while WGA- and ConA-recognizing glycans were expressed at relatively steady levels. This difference could be attributed to the increasing expression of glycophorin A on the erythroid-differentiated K562 cells, which contained 15 O glycans and a single N glycan.[5c,12b] The dynamic change of the degree of K562 cell-surface glycan expression was similar to the degree of erythroid differentiation. These results demonstrated that the designed array was competent for highly sensitive and high-throughput analysis of a dynamic glycome on cancer cell surfaces.

In summary, a facile electrochemical cytosensor array was successfully developed for dynamic analysis of a cell-surface glycome by integrating micromachining, nanotechnology, and specific recognition between biomolecules. The proposed array could implement multiplex evaluation of both the glycan expression profile on an intact cell surface and the dynamic changes in the glycome during drug treatment with high sensitivity and acceptable accuracy and reproducibility owing to the low background signal and low nonspecific adsorption. This facile cytosensor array has the potential to become a powerful and pragmatic tool to decode the cell-surface glycome and discover potential glycan biomarkers and novel therapeutic targets.

Experimental Section

A suspension of K562 cells at a given concentration (1 μL) was dropped on each RGDS–SWNTs/SPCE for cell capture at 37°C for 1 h. After rinsing with PBS, the obtained K562 cytosensor array was blocked with 3% bovine serum albumin (BSA) solution for 30 min. Different HRP lectins (1 μL) were then dropped on each cytosensor and incubated for 1 h to conjugate the lectins to the cell-surface glycans.[7a] After careful washing with 0.01M pH 7.4 PBS, the differential pulse voltammetric measurements were performed according to the procedure in the Supporting Information.

Details of K562 cell treatment, SPCE and RGDS–SWNTs/SPCE preparation, condition optimization, and flow cytometric analysis are described in the Supporting Information.

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