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Lectin-Based Nanoprobes Functionalized with Enzyme for Highly Sensitive Electrochemical Monitoring of Dynamic Carbohydrate Expression on Living Cells

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A dual-functionalized nanoprobe was designed for highly sensitive and selective in situ evaluation of carbohydrates on living cells by integrating the specific carbohydrate recognition and enzymatic signal amplification of proteins on Au nanoparticles. A nanoscaffold of nanohorns functionalized with arginine-glycine-aspartic acid-serine tetrapeptide was also prepared on an electrode surface for cell capture and enhancing the electrical connectivity. Combined with the nanoprobe and peptide-nanohorns, a highly sensitive electrochemical strategy was developed for cytosensing, which showed a detection limit down to 15 cells, broad dynamic range, acceptable rapidity, and low cost. The proposed method was further used for monitoring of dynamic variation of carbohydrate expression on cancer cells in response to drugs, which obviated the destruction or labeling of cells and the covalent tagging of lectin and enzyme. The one-pot conjugation of three components was very convenient and could be extended for preparation of other lectin-functionalized nanoprobes. Further development of this technique would contribute considerably to meeting the challenges in comprehensive understanding of the glycomic codes.

Gold nanoparticles (AuNPs), owing to their unique properties, have been widely employed to couple with biomolecules for development of biological nanoprobes.[1−3] These hybrid bionanostructures elegantly incorporate the superior physical and chemical features of AuNPs with the highly selective catalytic and recognition properties of biomolecules.[1] Indeed, these nanoprobes have been used to develop various sensing and imaging platforms[4−9] and have contributed significantly to applications of bioanalytical technology in various areas including medical diagnostics, environmental monitoring, and antiterrorism.[10]

Current Au nanoprobe-based biosensing platforms include colorimetry,[6,7,11] light scattering,[12] electrochemical,[13] surface-enhanced Raman scattering,[14] mass spectrometry,[5] and surface plasmon-based methods.[15] Among these platforms, electrochemical methods have received increasingly considerable attention due to their operational simplicity, flexibility, low cost, and acceptable sensitivity.[16] Thus, electrochemical nanoprobes based on AuNPs have been actively developed for the analysis of proteins and DNA sequences.[17,18] However, the nanoprobes suitable for electrochemical carbohydrate sensing, especially in situ cell surface carbohydrate monitoring, have been rarely reported. Only the carbohydrate-modified AuNPs have been developed for impedimetric detection of the glycan−lectin interaction.[19]

Carbohydrates, a class of intricate and informative biomacromolecules, exist on all eukaryotic cell surfaces. Abnormal alteration in cell surface carbohydrate expression is associated with a variety of diseases, especially cancers.[20,21] Therefore, sensitive

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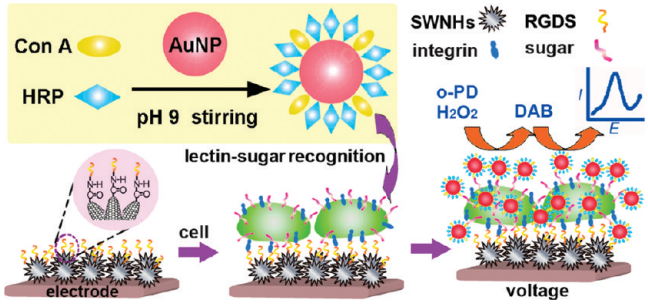
Scheme 1. Scheme of Nanoprobe Assembly and Electrochemical Strategy for in Situ Detection of Mannose Groups on Living Cells[a]



[a] Abbreviations: Con A, concanavalin A, a mannose group specific lectin; HRP, horseradish peroxidase; AuNP, gold nanoparticle; SWNHs, single-walled carbon nanohorns; RGDS, arginine-glycine-aspartic acid-serine tetrapeptide; *o*-PD, *o*-phenylenediamine; DAB, 2,2′-diaminoazobenzene.

analysis of carbohydrates on living cells in various events is keenly desirable for basic science advancement and clinical diagnostics.[22−28] Introducing the nanoprobes into the electrochemical carbohydrate assay can obviate the need of existing methods for cell lysis, cell labeling, or complicated instrumentation. Thus, the design of novel nanoprobes by integrating nanotechnology with multiple functions including specific recognition, biocatalysis, and signal amplification is urgent for deciphering the carbohydrate codes on cells.

This work developed a novel nanoprobe by integrating the functions of both specific recognition of carbohydrates and dual signal amplification of an enzyme on AuNPs through incorporation of proteins (Scheme 1). The specific recognition was achieved using lectins, a class of natural nonimmune proteins that can specifically recognize sugar epitopes,[29] as the recognition elements for gaining insight into the biologically relevant surface-accessible glycan motifs by selecting mannose and concanavalin A (Con A, a lectin) as an initial proof-of-concept recognition pair. Mannose presents in the form of mannose oligosaccharides on the cell surface, and its expression is closely related to such important biological processes as tumor growth and metastasis. The signal amplification was achieved with enormous loading of horseradish peroxidase on the AuNPs.[30] This nanoprobe could be used for highly sensitive detection of cells and in situ electrochemical monitoring of cell surface carbohydrates by coupling with the efficient capture of cells on electrodes modified with arginine-glycine-aspartic acid-serine tetrapeptide-functionalized single-walled carbon nanohorns (RGDS-SWNHs). The electrochemical signal was produced from the reduction of enzymatic reaction product on the modified electrode, and the capture of cells was due to the specific binding of cell surface integrin to the immobilized RGDS[31] and the adsorption of cells on the nanostructured surface. The enhanced sensitivity could be attributed to the combination of a dual signal amplification based on enzymatic catalysis, high surface-to-volume ratio of AuNPs for protein loading, the high molar ratio of enzyme to lectin on AuNPs, and the improved electrical connectivity based on SWNHs. K562 cell line, a human chronic myelogenous leukemia line, was chosen as a model to demonstrate the feasibility of the proposed strategy. This approach was further used to monitor dynamic variation of carbohydrate expression on living cells in response to drugs, using swainsonine (SW), a mannosidase II inhibitor, as the model.[32]

EXPERIMENTAL SECTION

Materials and Reagents. Con A, bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), HRP labeled Con A (HRP-Con A), and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Inc. (U.S.A.). $AuCl_3HCl·4H_2O$ (Au% > 48%), *o*-phenylenediamine (*o*-PD), and H_2O_2 was obtained from Shanghai Chemical Reagent Co., Ltd. (China). SW was from Merck KGaA (Darmstadt, Germany), and RGDS tetrapeptide was obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). SWNHs were obtained from Prof. Iijima's group (Japan Science and Technology Agency). Fluorescein-labeled Con A was from Vector laboratories (Burlingame, U.S.A.). Phosphate buffer saline (PBS, pH 7.4) containing NaCl (136.7 mM), KCl (2.7 mM), Na_2HPO_4 (8.72 mM), and KH_2PO_4 (1.41 mM) was used as incubation buffer. Phosphate buffer solutions with the marked concentrations and pHs were used as electrochemical assay buffer. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore).

SWNHs were dispersed in 30% HNO_3 and then refluxed for 24 h at 140 °C to obtain carboxylic group-functionalized SWNHs. After centrifugation, the sediment was washed with water until the pH reached 6.0 and then dispersed in water (0.5 mg mL^{-1}).

Cell Culture and Cell Treatment. K562 cell line was kindly provided by the Affiliated Zhongda Hospital, Southeast University, Nanjing, China. K562 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal calf serum (10%, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. The cells in exponential growth phase were collected and separated from the medium by centrifugation at 1000 rpm for 3 min and then thrice washed with a sterile pH 7.4 PBS. The sediment was resuspended in incubation buffer (0.01 M, pH 7.4) containing 1 mM Ca^{2+} and 1 mM Mg^{2+} to obtain a homogeneous cell suspension. Here, the divalent cations Ca^{2+} and Mg^{2+} were used to ensure the effective binding between cell surface integrins and RGDS on electrodes. Cell number was determined using a Petroff-Hausser cell counter (U.S.A.). SW-treated

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K562 cells were obtained by incubating the cells in a culture medium containing SW (2 μg mL^{-1}) for 4 days.[32]

Preparation of Nanoprobe. AuNPs with a diameter of 20 nm were prepared by a modified citrate-reduction method:[33] after trisodium citrate solution (1%, 5 mL) was added to a boiling and rapidly stirred $HAuCl_4$ solution (1 mM, 100 mL), the mixture was kept boiling and stirred for 20 min and cooled to room temperature to obtain AuNPs. The prepared AuNP solution (1.9 nM) was stored at 4 °C.

As shown in Scheme 1, the nanoprobe (4.7 nM) was prepared by adding HRP (10 mg mL^{-1}, 40 μL) and Con A (5 mg mL^{-1}, 24 μL) to a 8:3 H_2O−diluted AuNP solution (11 mL). After being adjusted to pH 9.0, the mixture was incubated for 30 min at 25 °C with gentle stirring and concentrated to 1 mL by centrifugation (15 000*g*, 20 min, 4 °C), washing, and resuspension with incubation buffer. To obtain the optimized nanoprobe, different ratios of HRP to Con A were used for preparation of the nanoprobes.

Biosensor Preparation. SWNHs (0.5 mg mL^{-1}, 6 μL) were dropped on a bare glassy carbon electrode (GCE) and dried in a desiccator to obtain SWNHs/GCE, which was then immersed in a solution containing EDC (2 mg mL^{-1}) and NHS (5 mg mL^{-1}) for 0.5 h. After the activated SWNHs/GCE was thoroughly rinsed with water, RGDS (1 mg mL^{-1}, 10 μL) was immediately dropped on its surface and incubated for 2 h to yield an RGDS-SWNHs/GCE. Following a rinse with incubation buffer, K562 cell suspension (10 μL) at a certain concentration was dropped on the RGDS-SWNHs/GCE and incubated at 25 °C for 2 h. After carefully rinsing with PBS to remove the noncaptured cells, the obtained cells/RGDS-SWNHs/GCE was used for subsequent assays.

Nanoprobe-Based Electrochemical Analysis. The cells/RGDS-SWNHs/GCE was first immersed in BSA (2%) solution for 30 min to block the nonspecific binding sites of nanoprobe on electrode surface and then incubated with nanoprobe solution (10 μL, containing 0.5 mM Ca^{2+} and 0.5 mM Mn^{2+} for the remaining binding activity of Con A) at room temperature for 1 h.[26,27] The nanoprobe could specifically bind to the mannose groups on cells captured on the modified electrode (Scheme 1). After carefully washing with incubation buffer, the electrode was immersed in a degassed PBS (0.2 M, pH 7.0) containing H_2O_2 (8.0 mM) and *o*-PD (10 mM) to catalyze the oxidation of *o*-PD by H_2O_2. After 2 min, an electrochemical measurement was performed to obtain the reduction signal of the catalytic product, 2,2′-diaminoazobenzene (DAB).

In order to verify the signal amplification of the nanoprobe, Con A-HRP (10 μg mL^{-1}, 10 μL, which was an optimal amount to obtain the maximum response at the electrode[27,28]) was employed to replace the nanoprobe to perform the same electrochemical analysis.

Monitoring of Dynamic Carbohydrate Expression in Response to SW. The RGDS-SWNH/GCEs were incubated with SW-treated and untreated K562 cells (1×10^6 cells mL^{-1}, 10 μL) for 2 h, respectively. After carefully rinsing with PBS (0.01 M, pH 7.4), the obtained electrodes were subjected to the nanoprobe-based electrochemical analysis.

Demonstration of HRP and Con A on Nanoprobe. After centrifugation of the nanoprobe (15 000*g*, 20 min, 4 °C), the sediment was collected and dissolved by sodium dodecyl sulfate (SDS) reducing buffer and then heated at 100 °C for 5 min. The resultant sample solution was loaded into wells of SDS-polyacrylamide gel for electrophoresis separation with a run time of 100 min at 100 V. All the equipment assembly, solution preparation, and operation process were according to the Mini-PROTEAN 3 Cell Instruction Manual (Bio-Rad, U.S.A.). After the electrophoresis was completed, the gel was stained with Coomassie blue and analyzed by the SensiAnsys gel imaging analysis system (Peiqing, Shanghai, China).

Control Experiment Using Flow Cytometry. K562 cells were collected by centrifugation (1000 rpm, 6 min) at room temperature. After being washed with cold incubation buffer, they were resuspended and added (1×10^7 cells mL^{-1}, 50 μL) to fluorescein-labeled lectin solution (20 μg mL^{-1}, 450 μL) containing Ca^{2+} (1 mM) and Mn^{2+} (1 mM). After incubation for 30 min, the cells were collected by centrifugation (1000 rpm, 6 min), washed with PBS, resuspended in 500 μL of PBS, and assayed by flow cytometry. Unlabeled K562 cells were used as the negative control for estimation of autofluorescence.

Apparatus and Characterization. The morphologies of AuNPs and Au nanoprobe were observed under a JEOL JEM-2100 transmission electron microscopy (TEM, Japan). The UV−vis spectra were obtained with a UV-3600 UV−vis-NIR spectrophotometer (Shimadzu, Kyoto, Japan). Flow cytometry was carried out on a FACS Calibur flow cytometer (Becton Dickinson, U.S.A.). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed at Mini-PROTEAN 3 cell (Bio-Rad, U.S.A.). Electrochemical impedance spectroscopic measurements were performed on a PGSTAT30/FRA2 system (Autolab, Netherlands) in PBS (10 mM, pH 7.4) containing $Fe(CN)_6^{3-/4-}$ (5 mM + 5 mM) and KCl (0.1 M) using a conventional three-electrode system with a modified GCE as the working electrode, platinum wire as the auxiliary electrode, and saturated calomel electrode as the reference electrode. The impedance spectra were recorded within the frequency range of 5×10^{-2}−10^5 Hz. The amplitude of the applied sine wave potential was 5 mV. Differential pulse voltammetric (DPV) measurements were performed on a CHI 730 electrochemical analyzer (CHI Co., TX) from −0.3 to −0.8 V with a pulse amplitude of 50 mV and a width of 50 ms.

RESULTS AND DISCUSSION

Detection Principle. A nanoprobe was first prepared by incubating the mixture containing AuNPs, Con A for recognition of mannose groups on living cells, and HRP for dual signal amplification (Scheme 1). In order to electrochemically evaluate the mannose expression on cell surface, K562 cells were captured on the RGDS-SWNHs modified electrode by the specific binding of cell surface integrin to RGDS and the adsorption of cells on the nanostructured surface. After incubation with the cell-captured electrode, the nanoprobe could specifically bind the mannose groups on cell surfaces. The HRP on the bound nanoprobe could then catalyze the oxidation of *o*-PD by H_2O_2, the enzymatic product was finally reduced on the electrode surface to produce a reduction peak. The peak current was directly related to the amount of HRP on the electrode and, thus, reflected the expression extent of mannose groups on K562 cells and the

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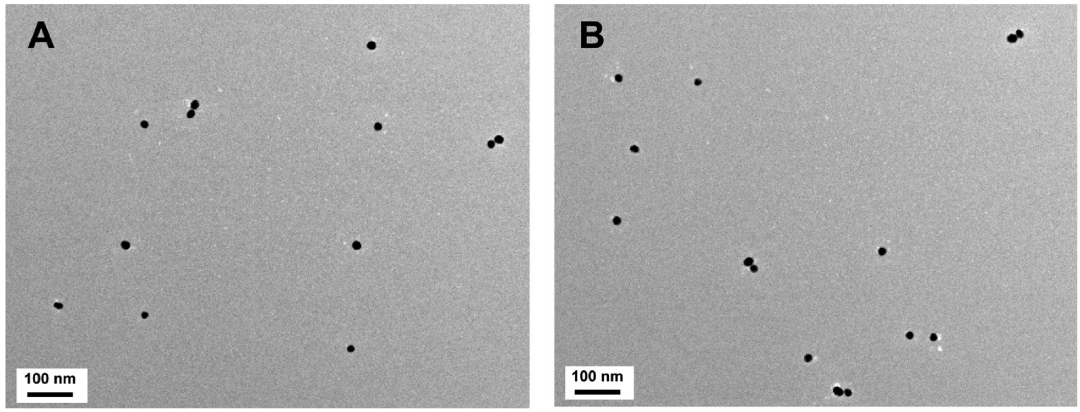

Figure 1. TEM images of (A) gold nanoparticles and (B) nanoprobes.

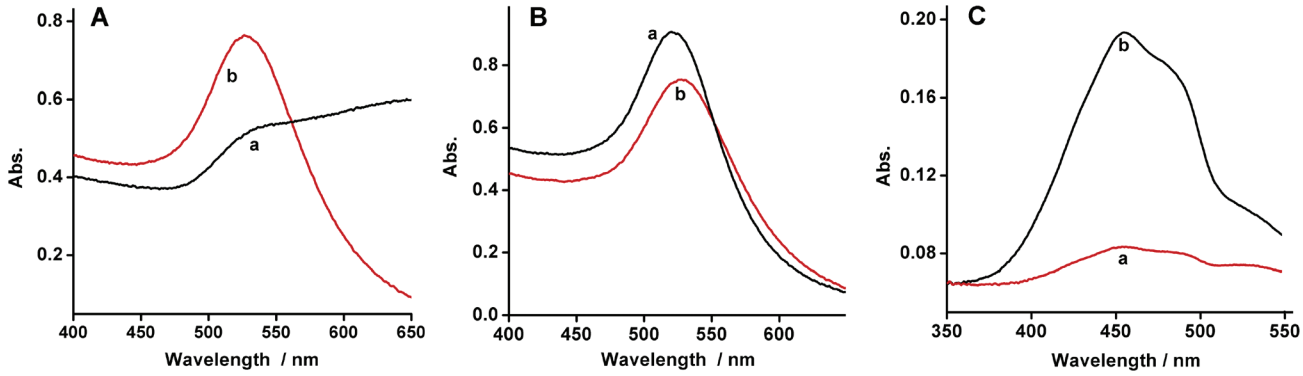

Figure 2. (A, B) UV−vis spectra of (a) 0.94 nM Au nanoparticles and (b) 0.79 nM nanoprobes in (A) incubation buffer and (B) H_2O, and (C) UV−vis spectra of 94 pM (a) Au nanoparticles and (b) nanoprobe after reaction with 8 mM H_2O_2 and 10 mM *o*-PD in 5 mL of 0.2 M, pH 7.0 PBS for 5 min. The reaction can be stopped by adding 50 μL of 3 M HCl.

amount of cells captured to the electrode, providing a strategy for monitoring mannose expression on living cells.

Preparation and Characterization of Nanoprobe. The nanoprobe was fabricated through incubation of AuNPs (20 nm, Figure 1), synthesized by the classical citrate reduction method, with Con A and HRP at pH 9 for 30 min under gentle stirring. The Con A can specifically recognize mannose groups on the cell surface.[29] This facile conjugation method made the HRP-to-Con A ratio on nanoprobe be easily adjusted by changing the ratio of the two proteins in the incubation step. The conjugation of proteins on AuNPs did not lead to aggregation of AuNPs as confirmed by the identical morphologies of transmission electron microscopic images of AuNPs before and after the conjugation step (Figure 1).

UV−vis spectroscopy was employed to demonstrate the successful conjugation of proteins. As shown in Figure 2A, AuNPs exhibited a salt-induced aggregation when mixed with PBS containing 136.7 mM NaCl,[13] which resulted in disappearance of their characteristic absorption peak around 520 nm and color change of the AuNP solution from red to blue. In contrast, the nanoprobe in PBS exhibited the same absorption peak and color as those in water (Figure 2B), indicative of the protection of proteins at the surface of AuNPs. To further demonstrate the existence of HRP, the nanoprobe was added to a mixture of *o*-PD and H_2O_2.[10,13] Upon the addition, the color of the mixture became quickly yellow, and the UV−vis spectrum showed an absorption peak of the oxidation product of *o*-PD at 457 nm (Figure 2C), which demonstrated a much more pronounced catalytic effect of the nanoprobe compared with AuNPs of the same concentration with the same reaction time. The catalytic effect could be attributed to the presence of HRP on the nanoprobe.

Capture of Cells on Nanoscaffold. SWNHs are typically composed of tubes of about 2−5 nm in diameter and 30−50 nm in length, which have closed ends with cone-shaped tips and associate to give rise to round-shaped aggregates of 100 nm in diameter. The oxidation treatment of SWNHs can produce abundant oxygen-rich groups extending radially at their cone ends for linkage with peptide.[34] Herein, the treated SWNHs were first coated on the surface of a GCE for binding of RGDS tetrapeptide in the presence of EDC and NHS. The RGDS tetrapeptide contains the minimal adhesion domain (RGD), which can selectively bind integrin receptors on cell surface.[31] The specific recognition between RGD peptide and cell surface integrin widely exists in cellular adhesion phenomena,[35] thus leading to the binding of RGDS with mutiple types of cells that have integrin on their surface.[28] The incorporation of RGDS with SWNHs offered a nanoscale analogue of extracellular matrix for cell anchorage. The tip-bound tetrapeptide facilitated the capture of K562 cells on the electrode surface, which could be verified by comparison of the cell capture ratio on SWNHs/GCE before and after RGDS coupling. At the cell concentration of 2×10^6 cells mL^{-1}, 51.7% of K562 cells in 10 μL could be captured on a RGDS-SWNHs/GCE, while only 35.3% of K562 cells could be captured on a

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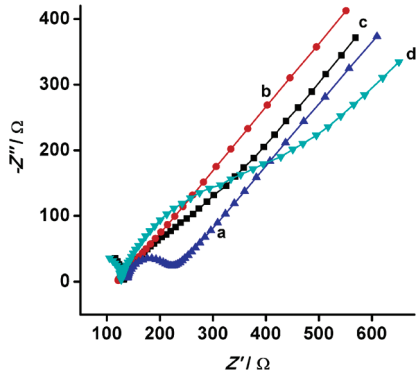

Figure 3. Electrochemical impedance spectra of (a) bare GCE, (b) SWNHs/GCE, (c) RGDS-SWNHs/GCE, and (d) cells/RGDS-SWNHs/GCE in 10 mM, pH 7.4 PBS containing 0.1 M KCl and 10 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$.

SWNHs/GCE. The latter was due to the adsorption of cells on nanostructure surface, which was beneficial to the improvement of detection sensitivity. At a fixed binding time, the cell capture ratio of RGDS-SWNHs/GCE differed by less than 3.0%, suggesting good reproducibility for preparation of cells/RGDS-SWNHs/GCE. A trypan blue staining experiment showed that most of the captured cells were living (Figure S-1 in the Supporting Information), indicating the capability of RGDS-SWNHs to maintain the normal condition of cells.

The preparation of the RGDS-SWNHs modified GCE and the subsequent cell capture were verified by monitoring the change of electron-transfer resistance (R_{et}) of $[Fe(CN)_6]^{3-/4-}$ as redox probes at the electrode surface with electrochemical impedance spectra (EIS) (Figure 3). The coating of SWNHs on GCE significantly facilitated the interfacial electron transfer, exhibiting a straight line in the spectrum. The binding of the tetrapeptide to SWNHs slightly increased the R_{et}, and the capture of cells to the RGDS-SWNHs/GCE further hindered the access of the redox probes to the electrode, generating an obvious R_{et} increase. The highly conductive SWNHs could not only increase the surface area for cell capture but also greatly enhance the electrical connectivity, which would improve the sensitivity of cell surface carbohydrate detection.

Electrochemical Behavior. After incubating the cells/RGDS-SWNHs/GCE in nanoprobe solution, the nanoprobe could bind to the cells due to the specific recognition between lectins on AuNPs and corresponding carbohydrates on cell surfaces. Upon addition of *o*-PD and H_2O_2 into assay buffer, the DPV curve of the nanoprobe-bound electrode exhibited a reduction peak at −0.55 V (Figure 4A), which corresponded to the electrochemical reduction of the oxidation product of *o*-PD by H_2O_2 in presence of HRP.

A blocking experiment and a competitive experiment were performed to verify the specific recognition between Con A on AuNPs and mannose groups on the cell surface. The blocking experiment was performed by adding mannan, a mannose polysaccharide, in the nanoprobe solution to obtain mannan-blocked nanoprobe. When the cells/RGDS-SWNHs/GCE was incubated in the mannan-blocked nanoprobe solution, only a tiny signal was observed (Figure S-2, curve a, in the Supporting Information), indicating that the mannan-blocked nanoprobe could not bind to the cells. The competitive experiment used the mixture

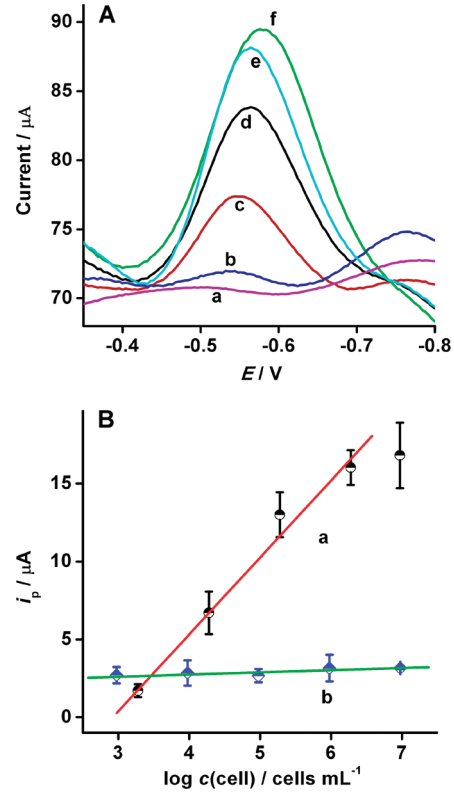

Figure 4. (A) DPV curves of nanoprobes/cells/RGDS-SWNHs/GCE obtained with K562 cell concentrations of 0, 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 1×10^7 cells mL^{-1} (from a to f) in 0.2 M, pH 7.0 PBS containing 8.0 mM H_2O_2 and 10 mM *o*-PD, and (B) plots of peak current vs logarithm of cell concentration using (a) nanoprobes and (b) HRP-Con A for recognition. The error bars represent the standard deviations calculated from six electrodes.

of Con A and the nanoprobe to incubate the cells/RGDS-SWNHs/GCE. The resulting electrode also showed a major decrease of the peak current (Figure S-2, curve b, in the Supporting Information), indicating the decrease in the amount of bound nanoprobe on electrode surface and efficient competition between Con A and nanoprobe. These results indicated the specific binding of Con A to cell surface mannose groups.

Optimization of the HRP-to-Con A Ratio. To obtain a high sensitivity, the ratio of HRP to lectin used to prepare the nanoprobe was optimized by observing the change of the reduction peak current upon the increasing ratio (Figure 5). At a constant total protein weight, the peak current increased with the increasing molar ratio of HRP to lectin until 8:1, followed by a sharp decrease, which was due to the fact that the recognition protein on the nanoprobe was insufficient for binding to K562 cells. Thus 8:1 of HRP to Con A was chosen as the optimal ratio for the preparation of the nanoprobe.

The coexistence of HRP and Con A on the nanoprobe could be further verified by SDS-PAGE (Figure 5B). After being reacted with the SDS reducing buffer, the proteins on AuNPs were denatured and separated from the nanoparticles. The resultant polypeptides showed two distinct bands at around 30 and 40 kD, corresponding to Con A monomer and HRP, respectively, which demonstrated the presence of the two kinds of proteins on the nanoprobe.

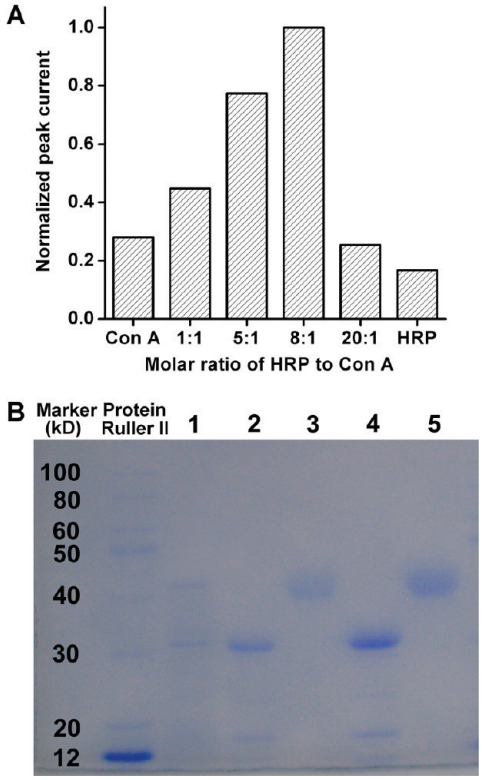

Figure 5. (A) Dependence of normalized DPV peak current for nanoprobe-catalyzed analysis on molar ratio of HRP to Con A used for preparation of nanoprobes. (B) Sodium dodecyl sulfate polyacrylamide gel electrophoresis stained with Coomassie blue. Wells: protein ruller II and Au nanoprobes, 5 μg of Con A, 5 μg of HRP, 10 μg of Con A, and 10 μg of HRP from (1) to (5).

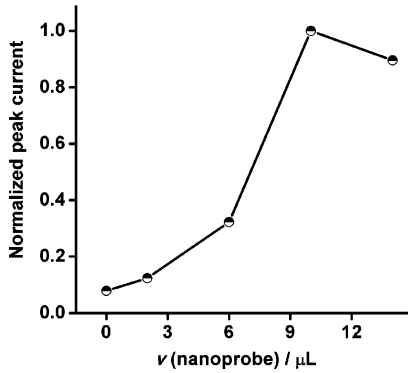

Figure 6. Dependence of normalized DPV peak current for nanoprobe-catalyzed analysis on volume of 4.7 nM nanoprobe solution used for incubation with the cell-captured electrode.

Electrochemical Detection. The amount of nanoprobe solution (4.7 nM) used for incubation with cells/RGDS-SWNHs/electrode was optimized. With the increasing volume of nanoprobe solution (4.7 nM), the peak current increased until the volume of 10 μL (Figure 6), which was used for cell recognition. With this amount, the DPV peak current increased linearly with the logarithmic value of the cell concentration over the range from 2.0×10^3 to 2.0×10^6 cells mL^{-1}, with a correlation coefficient R of 0.995 (Figure 4A). The limit of detection was 1.5×10^3 cells mL^{-1} at 3σ.

Taking into account that 10 μL of K562 cell suspension was used for incubation, the presented strategy achieved the limit of

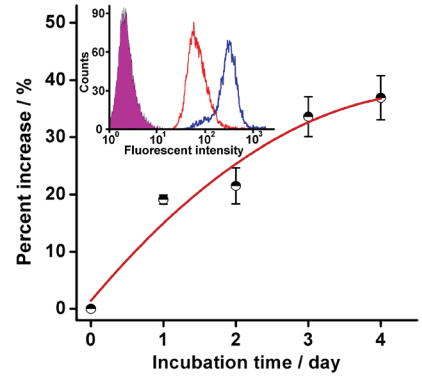

Figure 7. Time-dependent effect of SW on expression of Con A-binding cites on K562 cells. The percent increase (Δ%) was calculated as follows: $\Delta\% = [T/C - 1] \times 100\%$, where T and C are the peak currents obtained on SW-treated or control cells on GCE after incubation with nanoprobes. Data shown are from three independent sets of experiments with three replicate electrodes for each sample. Inset: flow cytometric analysis of Con A-binding sites on K562 cells after being treated with SW for 2 days (blue), untreated K562 cells (red) with fluorescein-labeled Con A, and autofluorescence of unlabeled K562 cells (purple).

detection of only 15 K562 cells, which was comparable with that of 6 BGC-823 and 8 K562 cells by HRP-tagged lectin[28] or quantum dot-tagged lectin[26] and much lower than that of 6×10^2 and 6×10^4 *E. coli* O157:H7 cells by immunosensors.[36,37]

In order to demonstrate the enhanced sensitivity of this nanoprobe-based strategy, the commercially available HRP-Con A was used to replace the nanoprobe for performing the same procedure. Under optimal concentration of HRP-Con A, the change of peak current was very slight upon the increase of cell concentration (Figure 4B, curve b). By comparison between the two slopes, the detection sensitivity of the proposed strategy was 34 times higher than that using simple HRP-Con A, verifying the advantages of the designed nanoprobe. The high sensitivity achieved by this work resulted from the combination of the dual signal amplification by the nanoprobe based on the enzymatic cycle, the enormous enzyme loading, and the enhanced electrical connectivity by SWNHs.

Monitoring of Dynamic Carbohydrate Expression on Cells in Response to SW. The high sensitivity and facility of the proposed strategy allowed it to be further used in evaluation of the dynamic alteration of carbohydrate expression on living cells in response to drugs, using swainsonine (SW) as a model. SW, a specific mannosidase II inhibitor, could increase the expression of terminal high-mannose type glycan on the cell surface.[32,38] SW-treated K562 cells could be captured by RGDS-SWNHs/GCE at the same cell capture ratio as untreated cells. During treatment with SW over 4 days, the SW-treated cells revealed a progressively increased response when untreated cells were used as a control (Figure 7). This result indicated the increase of terminal mannose on the cell surface and the modification effect of SW on cell surface glycosylation. To validate the observed change, the SW-treated K562 cells were stained with FITC-conjugated Con A and assayed using flow

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cytometry (inset in Figure 7), which also showed an increased binding to FITC-Con A compared with SW-untreated K562 cells. Compared with existing methods for monitoring dynamic glycan expression on cell surface, this strategy offered the following advantages: (1) the proposed method obviated the need for cell lysis[22] and cell labeling,[23] which could destruct and disturb the cellular nature, respectively; (2) the preparation of the nanoprobe did not involve covalent coupling,[24,26] which often requires more drastic conditions and leads to partial denaturation of proteins[39,40] and, thus, can maintain the biological activity of proteins; (3) the measurement of DPV peak current was more quantitative and facile than microscopy-based observation;[25] (4) the dual signal amplification coupled with enhanced electrical connectivity provided high sensitivity; (5) the method also obviated the need of complicated equipment, such as mass spectrometry[22] and flow cytometry,[24] thus providing the opportunity for miniaturization.

CONCLUSIONS

This work developed a novel lectin-based nanoprobe functionalized with enzyme for highly sensitive and selective in situ electrochemical evaluation of cell surface mannose expression. The nanoprobe could also be used for convenient detection of cell number with a broad range and low limit of detection by combination with a RGDS-SWNHs modified electrode for efficient cell capture and enhancing the electrical connectivity. The one-pot synthesis of the nanoprobe including the functionalization with the enzyme could be adapted to any other kind of available lectins, and this strategy could be expanded with the addition of more specific glycan−lectin pairs to the repertoire.[41] The high surface-to-volume ratio of AuNPs and the high molar ratio of enzyme to lectin led to a dual signal amplification for highly sensitive detection. Further development of the nanoprobes will evolve toward a very promising future for reliable diagnostics of glycan-relevant biomarkers for cancer and other diseases.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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