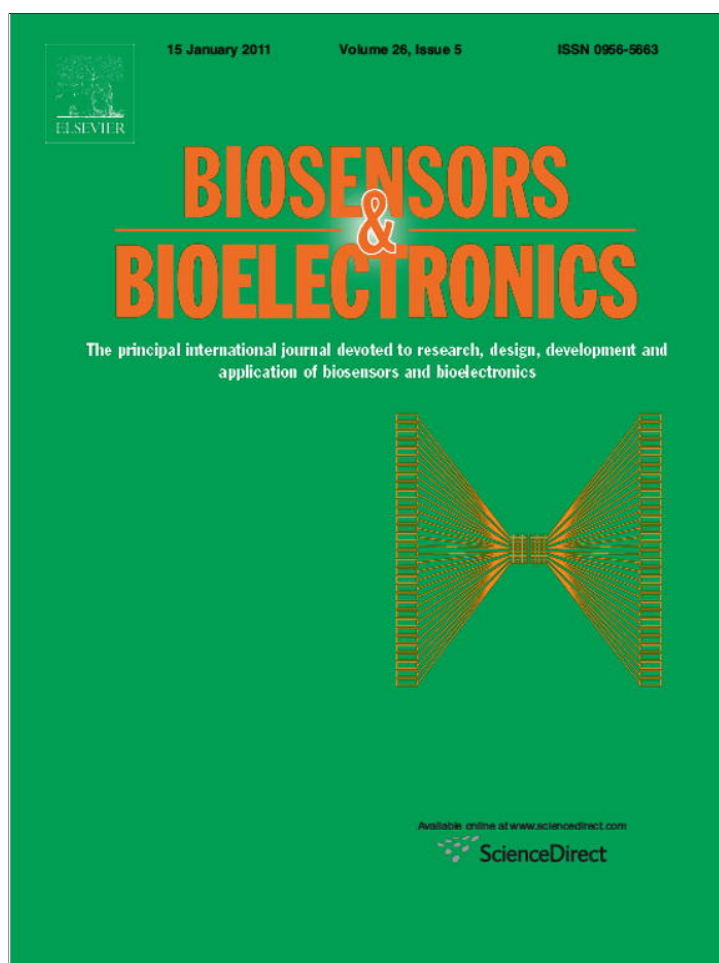


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Electrochemiluminescent biosensing of carbohydrate-functionalized CdS nanocomposites for in situ label-free analysis of cell surface carbohydrate

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ARTICLE INFO

Article history:

Received 29 August 2010

Received in revised form 12 October 2010

Accepted 25 October 2010

Available online 31 October 2010

Keywords:

Cytosensing

Electrochemiluminescence

Biosensors

Carbohydrate

CdS quantum dots

Lectin

ABSTRACT

A facile electrochemiluminescent (ECL) strategy for in situ label-free monitoring of carbohydrate expression on living cells was designed by integrating the specific recognition of lectin to carbohydrate with a carbohydrate-functionalized CdS nanocomposite. The mercaptopropionic acid-capped CdS quantum dots were firstly immobilized on carbon nanotubes modified electrode and then functionalized with carbohydrate using mannan as a model on the surface. The carbohydrate-functionalized CdS nanocomposite showed high ECL sensitivity and good stability, and could be used for competitive recognition to concanavalin A with the target cells in solution, which led to a change of ECL intensity due to the resistance of concanavalin A. The change depended on both the cell number and the expression level of cell surface carbohydrate. A wide linear response to cells ranging from 2×10^3 to 1×10^7 cells mL⁻¹ with a detection limit of 1.2×10^3 cells mL⁻¹ was obtained. The proposed biosensor could be used to in situ evaluate cell surface glycan, and the average number of mannose moieties on single living BGC cell was detected to be 8.7×10^7 . This sensitive strategy was further used for facile monitoring of dynamic carbohydrate expression on living cells in response to drugs. The proposed method could be further expanded to high-throughput detection with the addition of more specific glycan–lectin pairs to the repertoire.

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1. Introduction

Mammalian cell surfaces are covered with a dense and complex layer of carbohydrates, which may be the most abundant and diverse natural biopolymers (Ohtsubo and Marth, 2006). These cell surface carbohydrates play important roles in a myriad of biological events, including cellular adhesion, signal transduction, immune response and pathological processes (Ohtsubo and Marth, 2006; I.J. Chen et al., 2007; Marth and Grewal, 2008). More importantly, aberrant expression of carbohydrates on cell surface is often associated with a variety of diseases, especially cancers (Gorelik et al., 2001; Dube and Bertozzi, 2005; S. Chen et al., 2007). Therefore, sensitive analysis of carbohydrates on living cells has become an important subject not only for understanding their roles in disease development but also for early diagnosis.

Recently, lectin-based biosensors or arrays have attracted considerable interest in selective analysis of carbohydrates on living cells (Zheng et al., 2005; Hsu et al., 2006; Tao et al., 2008). Lectins are a class of proteins with highly specific carbohydrate recognition ability (Lis and Sharon, 1998; Xie et al., 2009). These lectin-based analytical systems include fluorescent, electrochemical and scanometric detection (Tao et al., 2008; Cheng et al., 2008; Ding et al.,

2010). The fluorescent methods generally involve the fluorescent labeling of cells, leading to disturbance of the cellular nature and the interference of cell autofluorescence. Moreover, the surface immobilization of lectins could lead to partly loss of their biological activity. Focusing on these problems, our previous works developed several electrochemical (Cheng et al., 2008, 2009; Ding et al., 2008) and scanometric (Ding et al., 2010) methods for in situ monitoring of cell surface carbohydrates. However, the developed electrochemical methods required the immobilization of cells on electrode and/or the labeling of lectins with nanoparticles or enzymes, both of which resulted in steric effect on the interaction between lectins and cell surface carbohydrates, and thus lowered the analytical performance. In order to avoid this effect, this work used carbohydrate-functionalized CdS quantum dots (QDs) to design an electrochemiluminescent (ECL) strategy for in situ label-free analysis of cell surface carbohydrate.

ECL detection possesses a series of advantages such as simplified optical setup, low cost, wide range of analyte, excellent selectivity, low background noise and high sensitivity (Richter, 2004). Since the first work on the ECL of silicon QDs was reported in 2002 (Ding et al., 2002), the ECL phenomena of CdS (Ding et al., 2006; Jie et al., 2007; Wang et al., 2009), CdSe (Jiang and Ju, 2007; Huang and Zhu, 2009), and CdTe (Liu and Ju, 2008) in the presence of different co-reactants such as oxygen, hydrogen peroxide or peroxydisulfate have been used for the development of ECL biosensors. Herein, the mercaptopropionic acid (MPA)-capped CdS quantum dots were

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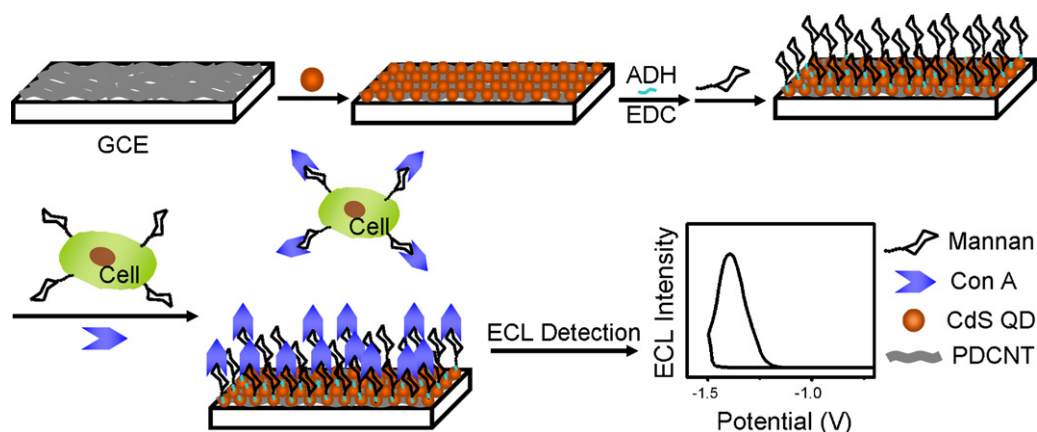


Fig. 1. Schematic representation of biosensor fabrication and competitive ECL assay.

firstly immobilized on carbon nanotubes modified electrode via a layer-by-layer method. As shown in Fig. 1, the immobilized CdS QDs were then functionalized with carbohydrate, using mannan as a model, and the resulting QDs showed a stable ECL behavior. Upon the competitive recognition of the immobilized mannan to concanavalin A (Con A) with mannan-expressed cell surface, the ECL intensity decreased due to the resistance of Con A. The change could respond to the cell amount and the expression extent of cell surface carbohydrate. The proposed method could further be used to monitor the dynamic variation of mannose expression on living cells. This strategy has potential applications in clinical diagnosis and elucidation of carbohydrate functions on living cells.

2. Experimental

2.1. Materials and reagents

Con A, bovine serum albumin (BSA), mannan, MPA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW: 200,000–350,000) were purchased from Sigma–Aldrich Inc. (USA). Adipic dihydrazide (ADH) and cadmium chloride ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$) were purchased from Alfa Aesar China Ltd. Swainsonine (SW) was from Merck KGaA (Darmstadt, Germany). Fluorescein lectin kit I containing fluorescein isothiocyanate (FITC)-labeled Con A was purchased from Vector Laboratories Inc. (USA). Multiwalled carbon nanotubes (CNTs, purity $\geq 98\%$, diameter 20–40 nm) were purchased from Nanoport Co. Ltd. (Shenzhen, China). Phosphate buffered saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4 and 1.41 mM KH_2PO_4 . All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water ($\geq 18 \text{ M}\Omega$, Milli-Q, Millipore).

CNTs were firstly treated with 3:1 $\text{H}_2\text{SO}_4/\text{HNO}_3$ in sonication for 4 h to obtain carboxylic group-functionalized CNTs. The resulting dispersion was filtered and washed repeatedly with water until pH was about 7.0. The oxidized CNTs were further functionalized with PDDA according to the reported method (Lai et al., 2009). The collected PDDA-functionalized CNTs (PDCNTs) were redispersed in water to a concentration of 1.0 mg mL^{-1} .

2.2. Apparatus

Electrochemical impedance spectroscopic (EIS) analysis was performed with an Autolab PGSTAT12 (Ecochemie, BV, The Netherlands) in 10 mM pH 7.4 PBS containing 5 mM $\text{K}_4\text{Fe}(\text{CN})_6$, 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.1 M KCl using the three-electrode sys-

tem. The electrochemical and ECL measurements were carried out on a MPI-A multifunctional analytical system (Xi'an Remex Analytical Instrument Ltd. Co.) with a conventional three-electrode system comprised of platinum wire as auxiliary, Ag/AgCl (saturated KCl solution) as reference, and a modified glassy carbon electrode (GCE) as working electrodes. UV–vis absorption spectrum was recorded with UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co., Japan). Photoluminescence (PL) spectrum was obtained on a Jasco FP 820 fluorometer (Jasco Co., Japan). Flow cytometric analysis was performed on a FACS Calibar flow cytometer (Becton Dickinson, USA). Scanning electron microscopic (SEM) images were obtained using a Hitachi S-4800 scanning electron microscope (Japan).

2.3. Cell line and cell culture

The BGC cell line was kindly provided by Affiliated Zhongda Hospital of Southeast University, Nanjing, China. BGC cells were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin ($100 \mu\text{g mL}^{-1}$), and streptomycin ($100 \mu\text{g mL}^{-1}$) at 37°C in a humidified atmosphere containing 5% CO_2 . The cells in the exponential growth were collected and separated from the medium by centrifugation at 1000 rpm for 5 min, and then washed thrice with sterile 0.01 M pH 7.4 PBS. The sediment was re-suspended in 10 mM pH 7.4 PBS to obtain a homogeneous cell suspension. Cell number was determined using a Petroff-Hausser cell counter (USA). SW-treated BGC cells were obtained by incubating the cells in a culture medium containing $2 \mu\text{g mL}^{-1}$ SW for 72 h.

2.4. Preparation of MPA-capped CdS QDs

The water-soluble CdS QDs were prepared using MPA as stabilizing agent according to a method similar to that reported previously (Ding et al., 2006). Briefly, 86 μL of MPA was added to 20 mL of 20 mM CdCl_2 solution. The pH of the solution was adjusted to 10 using 1 M sodium hydroxide. Then, 20 mL of 20 mM thioacetamide aqueous solution was added with extensive stirring in air for 30 min. After refluxed at 80°C for 10 h, the formed CdS colloid was dialyzed exhaustively against water overnight at room temperature to obtain CdS QDs solution. Finally, the product was condensed by ultrafiltration at 10000 rpm for 10 min, and the upper phase was decanted and kept at 4°C .

2.5. Preparation of the ECL biosensor

The glassy carbon electrode (GCE) with 5-mm diameter was polished to a mirror using 1.0, 0.3 and $0.05 \mu\text{m}$ alumina slurry

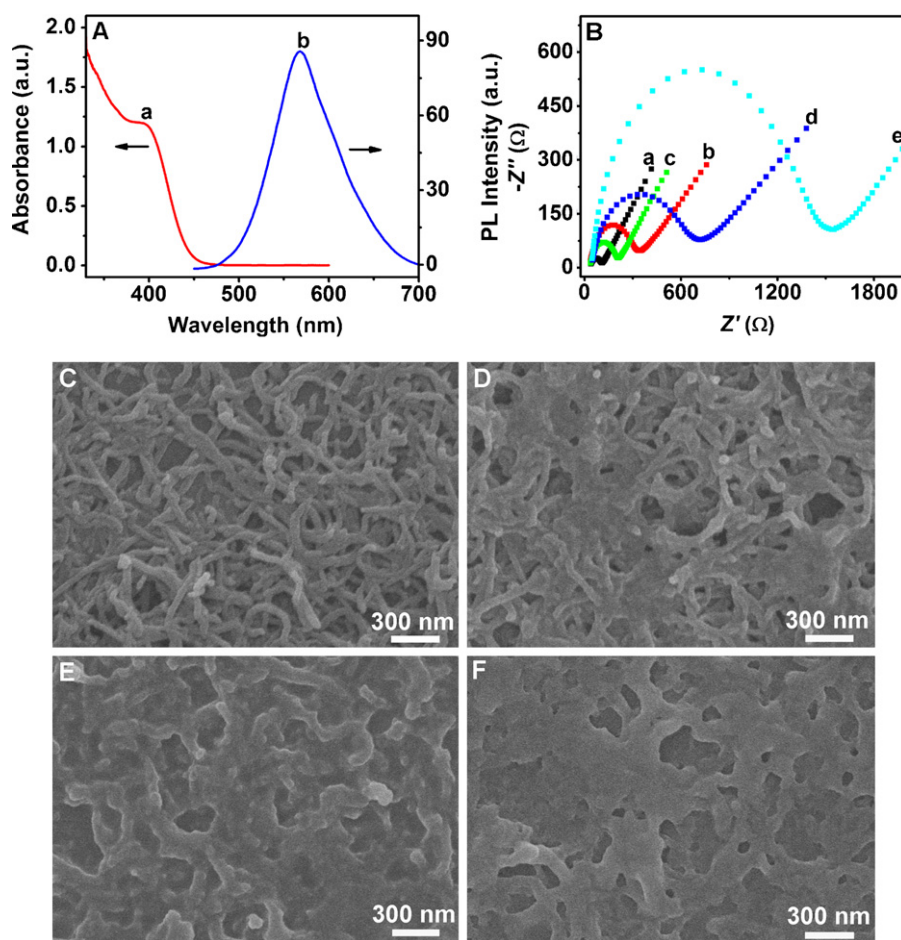


Fig. 2. (A) UV-vis (a) and PL (λ_{ex} : 418 nm) (b) spectra of QDs in pH 7.4 PBS, (B) EIS of PDCNTs (a), QDs/PDCNTs (b), ADH/QDs/PDCNTs (c), mannan/QDs/PDCNTs (d) and Con A/mannan/QDs/PDCNTs modified GCEs (e) in 10 mM pH 7.4 PBS containing 0.1 M KCl and 5 mM $\text{K}_4\text{Fe}(\text{CN})_6/5 \text{ mM } \text{K}_3\text{Fe}(\text{CN})_6$, and SEM images of (C) PDCNTs, (D) QDs/PDCNTs, (E) mannan/QDs/PDCNTs, and (F) Con A/mannan/QDs/PDCNTs.

(Beuhler) followed by rinsing thoroughly with deionized water. After successive sonication in 1:1 nitric acid, acetone and deionized water, the electrode was rinsed with water and allowed to dry at room temperature. As shown in Fig. 1, 15 μL of 1.0 mg mL^{-1} PDCNTs solution was dropped on the pretreated GCE, which was dried in a desiccator and then immersed in 10 μM QDs solution for 2 h. After the modified electrode was washed with ethanol and dried with a N_2 stream, a mixture of 3 mg mL^{-1} EDC and 2 mg mL^{-1} ADH in dimethyl sulfoxide was dropped onto its surface. After reaction for 5 h, the substrate was washed with ethanol, dried with a N_2 stream and then coated with 1 mg mL^{-1} mannan in pH 5.2 PBS, which was incubated overnight at 25 $^\circ\text{C}$ in a closed environment for mannan binding. The mannan-functionalized electrode was then soaked in 1% BSA solution for 30 min to block the surface, and rinsed with 50 mM pH 7.4 Tris-HCl buffer containing 0.1% Tween 20 and 10 mM pH 7.4 PBS, respectively.

2.6. Competitive binding and ECL detection

The mannan modified electrode was placed in a mixture of 0.5 μM Con A, 0.1 mM Ca^{2+} , 0.1 mM Mn^{2+} and desired amount of cells under gentle shaking at 37 $^\circ\text{C}$ for 1 h. Then the electrode was taken out, and washed twice with 50 mM pH 7.4 Tris-HCl buffer containing 0.1 M NaCl and 0.1% Tween 20, and twice with 10 mM pH 7.4 PBS.

After the competitive binding, the ECL signal was detected in 0.1 M pH 7.4 PBS containing 0.1 M $\text{K}_2\text{S}_2\text{O}_8$ and 0.1 M KCl. The cyclic scan was performed in the potential range of 0 to -1.5 V . The ECL

emission window was placed in front of the photomultiplier tube (detection range from 300 to 650 nm) biased at -500 V .

2.7. Flow cytometric analysis

BGC cells were collected by centrifugation at 1000 rpm for 6 min at room temperature. After the cells were washed with cold PBS, they were resuspended in PBS at a concentration of 1×10^7 cells mL^{-1} . Then, 50 μL cell suspension was then added to the mixture of 445 μL PBS and 5 μL 2 mg mL^{-1} fluorescein-labeled Con A. After incubation for 30 min, the cells were collected by centrifugation at 1000 rpm for 6 min, washed with PBS, resuspended in 500 μL PBS, and assayed by flow cytometry. Unlabeled BGC cells were used for estimation of autofluorescence.

3. Results and discussion

3.1. Characterization of MPA-capped CdS QDs

The formation of MPA-modified CdS QDs was characterized by PL and UV-vis spectra of 20-times dilution of the as-synthesized CdS QDs solution. The first UV-vis absorption peak occurred at 427 nm (curve a, Fig. 2A), from which the size of the resulting CdS QDs and the concentration of QDs solution could be estimated to be 3.1 nm and 76 $\mu\text{mol L}^{-1}$ from the adsorption peak and Peng's empirical equations (Yu et al., 2003). The PL spectrum (excited at 418 nm) of CdS QDs solution showed a relatively narrow emission with a maximum intensity at 567 nm (curve b, Fig. 2A). The sim-

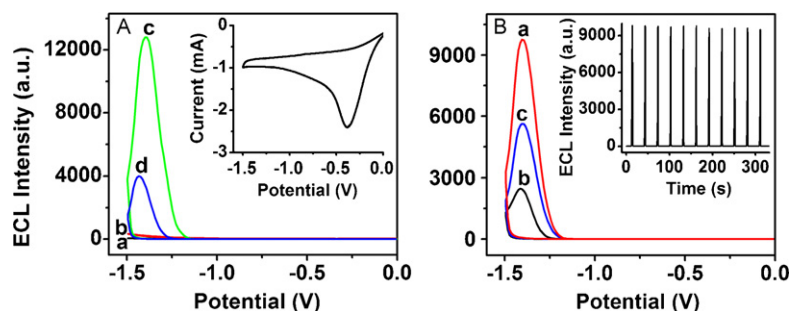


Fig. 3. ECL-potential curves of (A) bare (a), PDCNTs (b), QDs/PDCNTs (c) and QDs (d) modified GCEs, and (B) mannan/QDs/PDCNTs/GCE (a) and mannan/QDs/PDCNTs/GCE after incubation with $0.5 \mu\text{M}$ Con A in absence (b) and presence (c) of $1 \times 10^5 \text{ cells mL}^{-1}$ BGC cells. Insets: (A) cyclic voltammogram of QDs/PDCNTs/GCE, and (B) ECL emission from mannan/QDs/PDCNTs/GCE upon continuous cyclic scans between 0 and -1.5 V in 0.1 M pH 7.4 PBS containing 0.1 M KCl and 0.1 M $\text{K}_2\text{S}_2\text{O}_8$. Scan rate: 100 mV s^{-1} .

ilar PL excited wavelength and absorption wavelength indicated the emitter was the excited state of QD core (QDs^*) (Iwasaki et al., 2004).

3.2. Characterization of the biosensor fabrication

The electrode modified with PDCNTs composite film showed a small electron-transfer resistance (R_{et}) (curve a, Fig. 2B), indicating that the redox probe possessed good electron transfer kinetics at the PDCNTs composite film. After CdS QDs were assembled on the PDCNTs-modified electrode, the R_{et} slightly increased (curve b, Fig. 2B). Subsequently, the functionalization of the composite film with positively charged ADH slightly decreased the R_{et} owing to its attraction to negatively charged redox species (curve c, Fig. 2B), while an obvious increase in the resistance was observed when mannan was bound to the ADH/QDs/PDCNTs film (curve d, Fig. 2B). The increase in R_{et} was caused by the hindrance of mannan to the electron transfer. The further binding of Con A to the mannan immobilized electrode through recognition between lectin and carbohydrate produced a much higher R_{et} value (curve e, Fig. 2B), due to the resistance of the protein layer.

SEM was also used to characterize the stepwise fabrication process of the biosensor. As shown in Fig. 2C, the PDCNTs film displayed a well-dispersed structure in the form of small bundles or single tubes. The diameters of these bundles were 20–40 nm. As compared with the image of the PDCNTs film, a denser homogeneous structure could be observed for the QDs/PDCNTs film (Fig. 2D), indicating the binding of CdS QDs onto the PDCNTs surface through the electrostatic adsorption. As shown in Fig. 2E, after mannan was immobilized onto the QDs/PDCNTs film using ADH as a linker, the tube structure became plumper and the structure of film was completely changed, confirming that the mannan was bound successfully. This uniform nanostructure provided a significant increase of the effective electrode surface and was in favor of the recognition reaction between Con A and mannan. After incubation with Con A, the morphology of mannan-functionalized surface showed a larger particle size and became much thicker and rougher (Fig. 2F), indicating effective recognition of Con A to the immobilized mannan.

3.3. Electrochemical and ECL behaviors

In 0.1 M pH 7.4 PBS containing 0.1 M KCl and 0.1 M $\text{K}_2\text{S}_2\text{O}_8$ as co-reactant, the CdS QDs modified electrode showed a sensitive ECL emission starting at about -1.21 V with a peak at around -1.40 V , while the ECL emission of both bare GCE and PDCNTs/GCE was neglectable (Fig. 3A), indicating the ECL background was very low. The typical cyclic voltammogram of the QDs modified electrode showed a cathodic peak at -0.39 V (inset in Fig. 3A), which could

be attributed to the reduction of $\text{S}_2\text{O}_8^{2-}$ (Jie et al., 2007). The ECL intensity of the QDs/PDCNTs/GCE was about 3-fold that of QDs/GCE (curve d, Fig. 3A). The results indicated that the porous structure and better conductivity of the CdS QDs/PDCNTs film facilitated the ECL reaction, which could be used for sensitive ECL detection. The possible ECL mechanism was similar to those reported previously (Ding et al., 2002; Myung et al., 2002; Jie et al., 2007). Briefly, when the potential was scanned with an initial negative direction, the CdS QDs immobilized on the electrode were reduced to form the anion radical ($^-\text{CdS}^-$) by electron injection, and the reduction of $\text{S}_2\text{O}_8^{2-}$ at -0.39 V produced a strong oxidant, $^-\text{SO}_4^-$. The $^-\text{SO}_4^-$ could react with $^-\text{CdS}^-$, by injecting a hole into the highest occupied molecular orbital (HOMO), to produce an excited state of the QDs (CdS^*) to emit ECL signal.

After the mannan was bound to the QDs/PDCNTs film, the strong ECL emission was maintained (curve a, Fig. 3B) with an intensity decrease from 12,840 (curve c, Fig. 3A) to 9780 a.u. The ECL emission was very stable. Upon successive scanning between 0 and -1.5 V for 11 cycles, no obvious change was observed (inset in Fig. 3B). After incubated with Con A, the mannan modified electrode displayed an obvious decrease of ECL intensity (curve b, Fig. 3B). It could be ascribed to specific recognition between mannose moieties and Con A. The bound protein layer inhibited the ECL reaction of CdS QDs on the electrode, and thus decreased the ECL intensity. When BGC cells was added in Con A solution for competition with the mannan-modified electrode to bind Con A, the ECL signal of the resulting electrode increased. Moreover, the increase magnitude depended on the cell amount and the expression extent of cell surface carbohydrate. These results suggested that the designed interface could offer a suitable platform for development of ECL biosensor to monitor cell surface carbohydrates.

3.4. Optimization of Con A concentration and recognition time

The optimal concentration of Con A for recognition to the mannan modified electrode was important for accurate and sensitive reflection of the expression extent of carbohydrates on BGC cells. As shown in Fig. 4A, the ECL intensity decreased with the increasing concentration of Con A from 0.01 to $0.1 \mu\text{M}$, which was due to the fact that the amount of lectin at low concentration was insufficient for binding mannose moieties on mannan modified electrode. At the Con A concentration of $0.5 \mu\text{M}$, the ECL intensity reached a low plateau, indicating the saturation binding of Con A to mannan modified electrode. Therefore, the optimal Con A concentration for detection of cell surface carbohydrate was $0.5 \mu\text{M}$.

Recognition time was also an important parameter for the kinetic binding between lectin and carbohydrate. The ECL signals of mannan modified electrode were detected after different recognition times in presence (curve a, Fig. 4B) and absence (curve b,

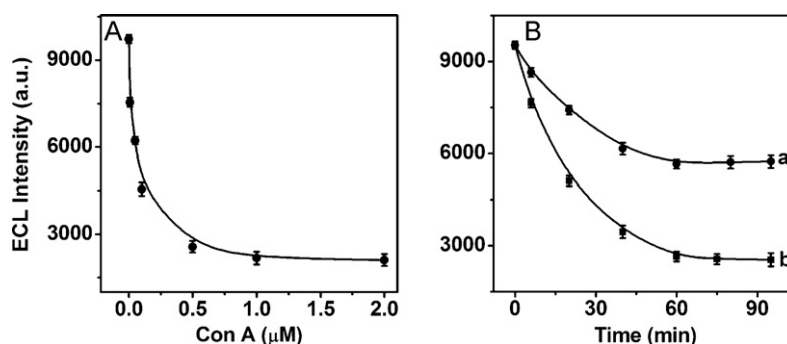


Fig. 4. Plots of ECL intensity vs. (A) Con A concentration for binding to mannan modified electrode with reaction time of 60 min, and (B) reaction time with 0.5 μM Con A in presence (a) and absence (b) of 1×10^5 cells mL⁻¹ BGC cells.

Fig. 4B) of 1×10^5 cells mL⁻¹ BGC cells in pH 7.4 PBS containing 0.5 μM Con A. With the increasing incubation time, the difference of ECL intensity between two cases increased and tended to a constant value at 60 min, indicative of the sufficient binding of Con A to cell-surface mannosyl groups. For ensuring the binding efficiency of BGC cells at low concentration, 60 min was chosen as the optimal recognition time.

3.5. Detection of BGC cells and evaluation of mannosyl groups on cell surface

The proposed ECL method exhibited sensitive response to BGC cells (Fig. 5A). Upon addition of BGC cells in the recognition solution containing 0.5 μM Con A, 0.1 mM Ca²⁺, and 0.1 mM Mn²⁺, the competitive binding of the active carbohydrate sites on BGC cells to the Con A decreased the amount of Con A bound to mannan modified electrode surface, and thus increased the ECL intensity. The calibration curve showed a linear relationship between the ECL intensity and the logarithm of cell concentration over the range from 2×10^3 to 1×10^7 cells mL⁻¹ with a correlation coefficient *R* of 0.997 (*n* = 7) (inset in Fig. 5A). The detection limit for cell concentration was calculated to be 1.2×10^3 cells mL⁻¹ at 3σ, which was comparable with that of 1000 cells mL⁻¹ at an impedance cytosensor for detection of K562 cells (Hao et al., 2007), and much lower than those of 6000 cells mL⁻¹ at an immunosensor chip for detection of *E. coli* O157:H7 (Ruan et al., 2002), and 1.0×10^4 cells mL⁻¹ at an immunosensor for detection of *Salmonella* species based on a quartz crystal microbalance (Wong et al., 2002). The low detection limit was attributed to the sensitive ECL detection, in situ label-free homogeneous competition and free of sample immobilization and separation.

In order to detect the quantity of those mannosyl groups on BGC cells, the linear relationships between ECL intensity and the amounts of cells and mannose were analyzed. As shown in Fig. 5B, at low cell concentration, the ECL intensity (*I*_{ECL}) showed a linear

increase with the increasing number of BGC cells (*n*) from 1000 to 8000 cells (*R* = 0.996). The linear regression equation was:

$$I_{ECL}(\text{a.u.}) = 4.03 \times 10^3 + 0.18n \quad (1)$$

On the other hand, when mannose was employed to replace BGC cells for the same experiment, a calibration curve between ECL intensity and the amount of mannose (*m*) was obtained (Fig. 5C). The plot of *I*_{ECL} versus *m* showed a linear calibration in the range of 0.1–2 pmol (*R* = 0.998). The linear regression equation was:

$$I_{ECL}(\text{a.u.}) = 2.69 \times 10^3 + 1.25 \times 10^3 m \text{ (pmol)} \quad (2)$$

Assuming that the mannose had the same binding kinetics as that at BGC cell surface, the average Con A binding capacity of single BGC cell could be calculated from Eqs. (1) and (2) to correspond to 8.7×10^7 molecules of mannose moieties. The result was slightly higher than the reported 5.3×10^7 mannose moieties (Cheng et al., 2008), but more veracious, because the latter suffered from the steric effect of cell immobilization and biological activity loss of enzyme-labeled lectin.

3.6. Monitoring of dynamic carbohydrate expression on cells in response to drug

The proposed ECL method could be used for not only evaluating the carbohydrate expression, but also dynamically monitoring its change on living cells. SW, a well-established mannosidase inhibitor (Srinivasan et al., 2009; Li et al., 2010), was chosen as the model drug to treat the cells for verifying the ability of dynamic monitoring. During treatment with SW over 72 h, the SW-treated cells revealed a progressively enhanced change of ECL intensity (ΔI_{ECL}) when untreated cells were used as a control (Fig. 6A). This result indicated greater expression of terminal mannose on SW-treated BGC cells. Compared with untreated cells, the mannose moieties of SW-treated BGC cells increased by 33.6% when treated for 72 h. This value was compatible with that of 38.5% obtained

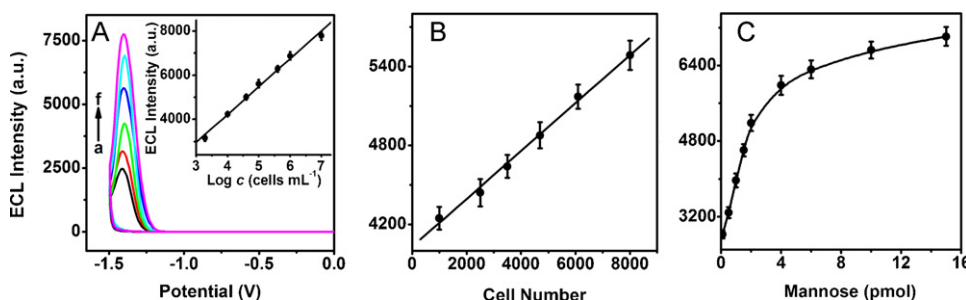


Fig. 5. (A) ECL–potential curves of mannan/QDs/PDCNTs/GCE after incubation with 0.5 μM Con A and 0, 2.0×10^3 , 1.0×10^4 , 1.0×10^5 , 1.0×10^6 , 1.0×10^7 cells mL⁻¹ BGC cells (from a to f), and plots of ECL intensity vs. amounts of (B) BGC cells and (C) mannose. Inset in (A): plot of ECL intensity vs. logarithm value of BGC cell concentration.

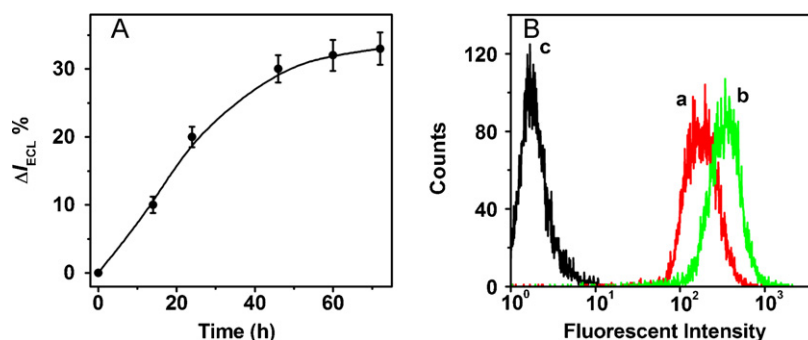


Fig. 6. (A) Change of BGC cell surface mannose moieties with SW treatment time, and (B) flow cytometric analysis of mannose moieties expressed on BGC cells before (a) and after (b) SW treatment for 3 days, and (c) autofluorescence of unlabeled BGC cells.

from flow cytometric analysis using FITC-labelled Con A for recognition (Fig. 6B), suggesting acceptable reliability of the proposed ECL method for evaluation of cell-surface carbohydrates.

3.7. Reproducibility and stability of ECL cytosensing assay

The reproducibility of the biosensor was estimated by determining 1.0×10^5 cells mL^{-1} with four biosensors made at the same electrode. Four measurements from the batch resulted in a relative standard deviation of 8.3%, indicating good reproducibility of the fabrication protocol. When the biosensor was stored in PBS at 4°C and then measured at intervals over several days, no obvious variation of ECL response was observed after two weeks of storage. After four weeks the response was still retained 92% of the initial response. These results indicated acceptable stability.

4. Conclusions

Carbohydrate-functionalized CdS QDs/PDCNTs nanocomposites and a novel ECL strategy for in situ label-free evaluation of cell surface mannose moieties have been constructed. This protocol integrates the specific interaction between lectin and the corresponding carbohydrate and the favorable ECL property of the CdS QDs/PDCNTs. This ECL strategy possesses high sensitivity, good stability, and acceptable reproducibility. Using mannan as a model, the proposed method can be used to evaluate the average amount of mannosyl groups on cell surface. This method obviates cell or lectin labeling, thus the biological activity of cells and protein can be maintained to the largest extent. Although this method is unspecific to the kind of mammalian cells, the designed biosensor can be expanded to specifically monitor the mannose expression on other kinds of cells without the cross-reactivity to other cell surface carbohydrates. Furthermore, the proposed strategy can be expanded for specific detection of other cell surface carbohydrates with the addition of more specific carbohydrate-lectin pairs to the repertoire (Liang et al., 2008), and this system can be used for analysis of dynamic changes in cell surface carbohydrates in response to drug. It could be anticipated that this method would become a powerful tool to decode the complex mechanisms underlying carbohydrate-related biological processes.

Acknowledgements

We gratefully acknowledge the National Science Funds for Creative Research Groups (20821063), the Major Research Plan

(90713015) and General Program (20875044) from the National Natural Science Foundation of China, and National Basic Research Program of China (2010CB732400).

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