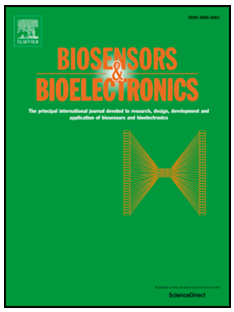

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Credit Author Statement

We confirm that the manuscript has been read and approved by all named authors. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Lizhen Chen designed and performed the experiments, and wrote as well as revised the manuscript.

Jie Wu performed some experiments including the detection of glycans on cell surface.

Feng Yan designed the OECT and the detection scheme.

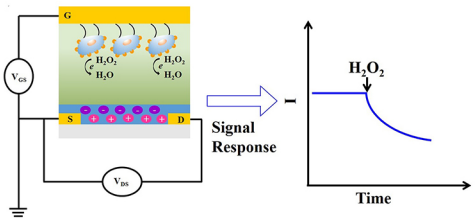
Huangxian Ju wrote the manuscript and discussed the strategy with Lizhen Chen.

Huangxian Ju provided the funds for financially supporting this work.

Signed by all authors as follows:

Lizhen Chen, Jie Wu, Feng Yan, Huangxian Ju

Date: Nov 25, 2020



A facile strategy for quantitative sensing of glycans on cell surface using organic electrochemical transistors

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Abstract: This work designed a facile sensing strategy for quantitation of glycans on cell surface using organic electrochemical transistors (OECTs). The sensing strategy was performed by covalently binding target cells on mercaptopropionic acid modified gate electrode for the recognition of horseradish peroxidase (HRP) labeled lectins to specific glycans on cell surface, which led to sensitive channel current signal responding to the captured HRP and thus the expression of cell surface glycans. The quantitation of glycans on cell surface was achieved by using glycan modified microspheres to simulate the cells, on which the amounts of glycans were detected with enzyme linked immunosorbent assay. The proposed sensing strategy had been used for the detection of mannose and galactose on HeLa cells with Concanavalin A and peanut agglutinin as the specific lectins, which showed 1.37×10^8 mannose and 1.13×10^8 galactose on each cell, respectively. By treating HeLa cells with exoglycosidases, the mannose and galactose expression levels showed the same changes as those detected with flow cytometric analysis, indicating the practical application of the OECT-based biosensor in cell surface glycan expression analysis.

Keywords: Organic electrochemical transistor; Glycan analysis; Cell-based biosensor; Lectin.

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1. Introduction

Glycans are generally expressed on cell surface proteins and influence the physiological functions of proteins. The glycosylation of proteins involved various cellular biological processes, including cell growth, differentiation, proliferation, cell-cell communication, immune response, progression of cancer and intracellular signaling events (Chen et al., 2018; Krasnova and Wong, 2016; Xiao et al., 2016). Abnormal expression of glycans on cell surface can indicate certain disease status such as inflammation and cancerization (Chen et al., 2007; Fuster and Esko, 2005). Therefore, many sensing strategies for glycan analysis have been developed using different detection technologies such as surface-enhanced Raman spectroscopy (Lin et al., 2013), mass spectrometry (Aizpurua-Olaizola et al., 2018), fluorescence imaging (Chen et al., 2013) and electrochemical methods (Chen et al., 2013; Chen et al., 2014; Cheng et al., 2009). Especially, as the quantitative analysis of glycan on cell surface can realize precise tracking of the expression change of glycan and accurate assessment of disease status, it is important to develop the convenient approaches for quantitative detection of glycans on cell surface (Chen et al., 2013).

In recent years, organic thin film transistors (OTFTs), as the new generation of the sensing platform, have attracted great interests due to their easy fabrication and low cost (Khan et al., 2010; Someya et al., 2010). Organic electrochemical transistors (OECTs), as one type of OTFTs, can operate in liquid electrolytes with stable performance under low operating voltages (<1 V) (Rivnay et al., 2018). They are usually consisted of drain, source and gate electrodes with conducting polymer to serve as the channel connecting drain and source electrodes, and have been used for design of chemical and biological sensors by immobilizing the recognition elements on the gate electrode. The most commonly used conducting polymer is poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate)

(PEDOT:PSS) owing to its stability, biocompatibility and high conductivity (Bernards and Malliaras, 2007; Inal et al., 2017; Nerdes et al., 2007). According to the physics of OECTs, the reaction on gate/electrolyte and channel/electrolyte interfaces can change the effect gate voltage of the transistors, which sensitively changes the drain-source channel current. Thus, a series of OECT-based biosensors have been reported through the redox reaction of electroactive molecules, such as glucose (Tang et al., 2011), lactate (Braendlein et al., 2017), uric acid (Liao et al., 2015), dopamine (Yang et al., 2018) and acetylcholine (Kergoat et al., 2014). Interestingly, some OECT-based biosensors have also been developed for the detection of electro-inactive molecules or biomacromolecules such as sialic acid (Chen et al., 2020), cortisol (Parlak et al., 2018), DNA (Lin et al., 2011), protein (Fu et al., 2017), bacteria (He et al., 2012), cell (Gu et al., 2016) and antibody (Kim et al., 2010). In order to extend the application of OECTs, our group previously prepared a lectin modified OECT biosensor along with the aptamer labeled nanoprobe for the detection of mannose on cell surface (Chen et al., 2018), however, the glycan on single cell surface could not be quantified because the signal was corresponding to the reaction between cell-aptamer rather than cell-lectin. In this work, a mercaptopropionic acid (MPA) modified OECT biosensor along with horseradish peroxidase labeled lectin (HRP-lectin) probe was proposed to design a facile sensing strategy for the quantitation of glycans on cell surface.

The sensing strategy firstly covalently immobilized target cells on the gate interface with a simple incubation of the cells on MPA modified gate electrode that was activated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS), and then used corresponding HRP-lectin to recognize the target glycan on cell surface, which introduced the HRP catalyzed reduction of hydrogen peroxide (H_2O_2) on gate electrode surface to produce the current signal for sensing of the glycan (Scheme 1). To achieve the quantitation of cell surface glycan, glycan modified

microspheres were used to simulate cells for obtaining the calibration curve. As a proof-of-concept, mannose and galactose on HeLa cells were respectively used as the model glycans to demonstrate the practicability of the proposed biosensing strategy. This work provided a new detection technique for the analysis of cell surface glycan expression. Although the sensing strategy detected mannose and galactose on HeLa cells using Con A and PNA as signal lectins, it could be extended conveniently for the quantitative analysis of other glycans or targets on cell surface by using their specific recognition elements, for example, using *Sambucus nigra* agglutinin and wheat germ agglutinin for the detection of N-acetylneuraminic acid and N-acetylglucosamine.

Preferred position for Scheme 1

2. Experimental

2.1. Materials and reagents

MPA, EDC, NHS and 30% wt H_2O_2 were brought from Sa'en Chemical Technology Co., Ltd. (China). Concanavalin A and peanut agglutinin conjugated with HRP (HRP-Con A, HRP-PNA) and fluorescein iosthiocyanate (FITC-Con A, FITC-PNA), bovine serum albumin (BSA) and exoglycosidases such as α-mannosidase and β-galactosidase were obtained Sigma-Aldrich Inc. (USA). NHS functionalized magnetic microspheres (NHS-MMPs) were purchased from Enriching Biotechnology Co., Ltd. (China). N-Acetyl-D-galctosamine, morpholineethanesulfonic acid (MES) and mannosamine hydrochloride were purchased from Aladdin Biotechnology Co., Ltd. (China). Phosphate buffered saline containing 2.7 mM KCl, 136.7 mM NaCl, 1.42 mM KH_2PO_4 and 8.72 mM Na_2HPO_4 (PBS, pH 7.4), cell culture medium containing 100 μg mL^{-1} penicillin and streptomycin (Dulbecco's modified eagle medium, DMEM), fetal bovine serum (Gibco,

Australia) and trypsin were bought from KeyGEN Biotechnology Co., Ltd. (China). HeLa cell line was provided by KeyGEN Biotechnology Co., Ltd. The commercial enzyme linked immunosorbent assay (ELISA) kits for mannose and galactose quantitation were purchased from Jin Yibai Biological Technology Co., Ltd (China).

Au target (5N, 99.999% purity) and Cr target (4N, 99.99% purity) were obtained from Zhongnuo Advanced Materials Technology Co., Ltd. (China). Dodecyl benzenesulfonic acid (DBSA), 3-glycidyloxypropyltrimethoxysilane (GOPS) and glycerin were bought from J&K Scientific Ltd. (China). Poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS, Clevios^{TM}, PH 1000) was supplied by Heraeus (Germany). All aqueous solutions were prepared using ultra-pure water (≥18 MΩ, Milli-Q, Millipore).

2.2. Apparatus

The behaviours of OECTs were recoreded with Keysight B1500A semiconductor analyzer (USA). Princeton Parstat MC 1000 electrochemical workstation (USA) was used to obtain the electrochemical impedance spectra (EIS). The Cr/Au layer was deposited on glass substrate through Kurt J. Lesker PVD75 E-beam evaporation system (USA). Atomic force microscopic (AFM) image was obtained with an atomic force microscopy (BRUKER Dimension Icon, Germany). The oxygen plasma treatment was accomplished with Harrick plasma cleaner PDC-002 (USA). KW-4A spin-coater was supplied by Beijing SETCAS Electronics Co., Ltd (China). The flow cytometric analysis was performed on Beckman Coulter FC500 flow cytometer (USA).

2.3. Glycan modification of MMPs

The NHS-MMPs were collected by magnet from purchased dispersion, and washed twice with absolute ethyl alcohol. 1 mL of mannosamine hydrochloride or N-acetyl-D-galctosamine (1 mg mL^{-1}) in reaction buffer (0.1 M MES, 0.15 M NaCl, pH 6.0)

was mixed with NHS-MMPs for 2 h at room temperature. The modified MMPs were separated and mixed with 1 mL blocking buffer (0.1 M MES, 0.5% BSA, 0.15 M NaCl, pH 6.0) for another 1 h at room temperature. Finally, the mannosamine or N-acetyl-D-galctosamine modified MMPs were separated, washed with 1 mL washing buffer (0.05 M Tris-HCl, 0.15 M NaCl, pH 7.2) for three times, and dispersed in washing buffer, which were stored at 4 °C prior to use. The amounts of mannose and galactose groups on MMPs were calculated from their corresponding standard curves (R=0.99, Fig. S1) which were obtained with the ELISA kits under the guideline of operation instruction, respectively.

2.4. Cell culture and treatment

HeLa cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. The adherent cells were treated with tryspin and collected by centrifugation at 1000 rpm for 5 min. The cell concentration was confirmed using a Countess™ II cell counter (USA). The homogeneous cell suspension was dilliuted with PBS in certain concentration. For examining the change of glycan expression on cell surface, the cells were treated with culture medium containing 0.5 U mL^{-1} exoglycosidases at 37 °C for 1 h.

2.5. Device fabrication

The OECTs were prepared on the glass substrate using the reported method (Chen et al., 2018). In brief, the desired configuration of OECT was obtained by a shadow mask and the metal layers of Cr/Au were deposited on the glass with controlled thickness through the E-beam evaporation system. The Cr and Au layers were 10 and 100 nm thick, which were confirmed by AFM (Fig. S2). The size of gate electrode was 3×3 mm and the channel width and length were 0.2 and 6 mm, respectively, the distance between gate electrode and

channel was 2 mm (Chen et al., 2020). The conducting polymer was consisted of PEDOT:PSS mixed with 5% v/v glycerin, 0.1% v/v DBSA and 1% wt GOPS. The devices were treated with oxygen plasma and spin-coated with conducting polymer solution, following by an annealing process at 120 °C for 1 h under vacuum. In this work, the OECTs could be prepared in batch for disposable sensing of glycans on cell surface.

2.6. Modification of gate electrode and detection of glycans on cell surface

The bare Au gate electrode was firstly treated with fresh Piranha acid (H_2SO_4/H_2O_2=3:1 v/v) and then dropped with 10 μL of MPA (100 μM) to introduce the carboxyl functional group on gate interface at 37 °C for 2 h. 10 μL EDC/NHS solution (10 mM) was then dropped on gate electrode to activate the carboxyl group at 4 °C for 4 h.

To detect the amount of specific glycan on cell surface, 10 μL of homogeneous cell suspension was dropped on gate electrode to react at 37 °C for 1 h, in which the cells could be covalently immobilized on gate electrode via the reaction of -NH_2 group on the cells with activated carboxyl group on gate electrode, and 10 μL of HRP-lectin was then incubated on the gate interface for specific recognition of lectin to cell surface glycan at 4 °C for another 1 h. Afterwards, the channel current was detected in PBS containing 0.1 mM H_2O_2 at the gate voltage of 0.9 V and the drain-source voltage of 0.05 V.

2.7. Flow cytometric analysis

For flow cytometric analysis of the glycan expression, 1×10^6 HeLa cells suspended in 1 mL sterile pH 7.4 PBS were incubated with 10 μg mL^{-1} FITC-labeled lectins (ConA, PNA) at room temperature for 30 min (Cheng et al., 2009). The cells were collected by centrifugation at 1000 rpm for 5 min, washed twice with PBS, and re-suspended in 1 mL PBS. Unlabeled cells were used for the estimation of auto fluorescence.

3. Results and discussion

3.1. Biosensing strategy for detection of cell surface glycans

The MPA modified gate electrode was firstly activated with EDC and NHS, which led to quick convent binding of -NH_2 group on target cell surface with activated -COOH on the modified electrode (Fig. S3). HRP-Con A and HRP-PNA could thus be conveniently conjugated to electrode surface through the specific recognition of lectins to mannose and galactose on cell surface, respectively (Scheme 1B). Moreover, the amounts of cell surface mannose and galactose could be changed by the highly specific cleavage of α-mannosidase and β-galactosidase (Table S1), respectively. Reasonably, the amounts of HRP-lectins captured on the gate interface were related to the expression levels of glycans on cell surface. Although the biological recognition on gate interface could not produce any electron transfer to influence the drain-source channel current, the HRP-catalyzed reduction of H_2O_2 on gate/electrolyte interface changed the effect gate voltage (V_g^{eff}) of the transistor, and thus the channel current (Chen et al., 2018) (Scheme 1C, Fig. S4), which led to a simple OECT-based biosensing strategy for the detection of cell surface glycans.

3.2. Characterization of gate electrodes

The typical transfer curve of the OECTs was tested in PBS. The newly made OECT device showed one order of magnitude change of channel current when the gate voltage varied from 0 to 1.2 V (Fig. 1A). The transconductance (g_m), which can be exacted from the differentiation of the channel current against gate voltage, reached the maximum value of 0.75 mS at the gate voltage of 0.9 V. Coincidentally, the optimized gate voltage for the detection of cell surface glycans also occurred at 0.9 V (Fig. S5). To eliminate the effect of OECT device difference on test repeatability, the detection signal was defined as $\Delta I/I_0$. I_0 represented the steady channel current before the addition of H_2O_2, and ΔI was the channel

current change upon addition of H_2O_2 for 500 s, at which the channel current decreased gently to reach the plateau.

Preferred position for Fig. 1

The EIS were used to characterize the detection process of cell surface glycans with the modified gate electrode. After the gate electrode was modified with MPA, the electron-transfer resistance (R_{et}) increased to ~1.0 kΩ owing to the repulsion between $[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$ and negatively charged carboxylate group of MPA. After the cells were incubated on the activated MPA-gate electrode, the R_{et} increased greatly to ~4.2 kΩ due to the poor electrical conductivity of cells (Fig. 1B). The specific recognition of HRP-Con A to mannose on cell surface further increased the R_{et} to ~4.9 kΩ. These results demonstrated the cell capture and lectin recognition processes for performing the biosensing strategy.

3.3. Quantitative detection of mannose on cell surface

To obtain the best performance of the biosensing strategy, the concentration of HRP-Con A used for recognition of cell surface mannose was firstly optimized. With the increasing HRP-Con A concentration, the current change increased and reached a maximum value at 60 μg mL^{-1} (Fig.2A and B), indicating the saturation binding of cell surface mannose. Thus 60 μg mL^{-1} HRP-Con A was used for detection of cell surface mannose, at which the current signal increased with the increasing cell concentration used for covalent capture (Fig.2C). The plot of current change versus the logarithm of cell number ranging from 500 to 50000 showed a linearity with the equation of $\Delta I/I_0 = -0.0474 + 0.0363 \log[N_{cell}]$ (Fig.2D). Meanwhile, the change of effective gate voltage (ΔV_g^{eff}) exacted from the transfer curve of the device with HRP-Con A-Cell-MPA modified gate electrode showed linear increase with

the increasing cell concentration (Fig.S6A).

Preferred position for Fig. 2

To achieve the quantitation of mannose on cell surface, mannosamine modified MMPs were prepared for obtaining the calibration curve, on which the amount of mannose group was detected to be 3.30×10^8 per MMP. Considering to the possible difference in the mannose group amounts on cells and the modified MMPs, the concentration of HRP-Con A used for recognition of mannose group on the MMPs was also optimized to be 80 μg mL^{-1} (Fig.3A and 3B). After the mannosamine modified MMPs were covalently captured on MPA modified gate electrode and then incubated with 80 μg mL^{-1} HRP-Con A solution, both the current signal and ΔV_g^{eff} linearly increased with the increasing amount of MMPs from 1000 to 10000, which provided two standard curves for the quantitative detection of cell surface mannose (Fig.3C, Fig.3D and Fig.S6B). With these standart curves, the amount of mannose expressed on each HeLa cell was detected to be 1.37×10^8, which located in the range of 3.5×10^7 to 3.0×10^{10} on different cells reported in previous works (Chen et al., 2013, Chen et al., 2015, Din et al., 2008, Han et al., 2011, Xue et al., 2010).

Preferred position for Fig. 3

3.4. Quantitative detection of galactose on cell surface

Similarly, the amount of galactose expressed on HeLa cells could be detected by using MPA modified gate electrode to capture N-acetyl-D-galctosamine modified MMPs, on which the amount of galactose group was 2.73×10^8, and HRP-PNA to recognize the galactose group on captured cell and MMP surfaces. The optimized HRP-PNA concentrations were 60 (Fig.S7A and S7B) and 80 μg mL^{-1} (Fig.S8A and S8B), respectively. With the recognition of HRP-PNA to galactose group, both the channel current change and

ΔV_g^{eff} value linearly correlated with the amount of HeLa cells from 500 to 50000 (Fig.S6C, Fig.S7C and Fig.S7D) and MMPs from 1000 to 10000 (Fig.S8C), respectively. Thus, both the channel current-based and ΔV_g^{eff}-based standard curves could be obtained for quantitative detection of cell surface galactose (Fig.S8D and Fig.S6D). On HeLa cell surface, the amount of galactose was detected to be 1.13×10^8.

3.5. Profiling of glycans on cell surface

To demonstrate the practicability of the proposed biosensing strategy, the changes of glycan expressions on cell surface upon exoglycosidase treatment were examined. After HeLa cells were treated with 0.5 U mL^{-1} α-mannosidase or β-galactosidase at 37 °C for 1 h, they were covalently captured on gate electrode surface for the detection of mannose and galactose, respectively. As shown in Fig.4A, the exoglycosidase treatments led to significant decrease of current signal change, indicating the specific cleavage of mannose and galactose from the cell surface. The results were consistent with those from the flow cytometric analysis (Fig.4B), indicating the good reliability and potential application of the proposed biosensing strategy in profiling of cell surface glycans.

Preferred position for Fig. 4

4. Conclusion

This work uses OECTs to develop a sensing strategy for analysis of glycan expression on cell surface. This strategy can be conveniently performed by covalently capturing target cells on gate electrode surface and then using corresponding lectin to specifically recognize cell surface glycan. Both the channel current and effective gate voltage changes show good relativity with the cell concentration used for the capture process. Through using mannosamine or N-acetyl-D-galctosamine modified MMPs to simulate the cells and HRP

labelled Con A or PNA to recognize the specific glycan, two quantitative methods are proposed for the detection of mannose and galactose on cell surface, respectively. These methods have been demonstrated by changing the glycan expression on cell surface with exoglycosidase treatment. This work extends the application of OECTs and provides a convenient and versatile sensing platform for the analysis of cell surface glycan expressions.

CRediT authorship contribution statement

Lizhen Chen: Conceptualization, Data curation, Writing - original draft. **Jie Wu:** Methodology, Project administration. **Feng Yan:** Resources, Formal analysis. **Huangxian Ju:** Funding acquisition, Writing - review & editing, Formal analysis, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.bios…..

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Figure captions

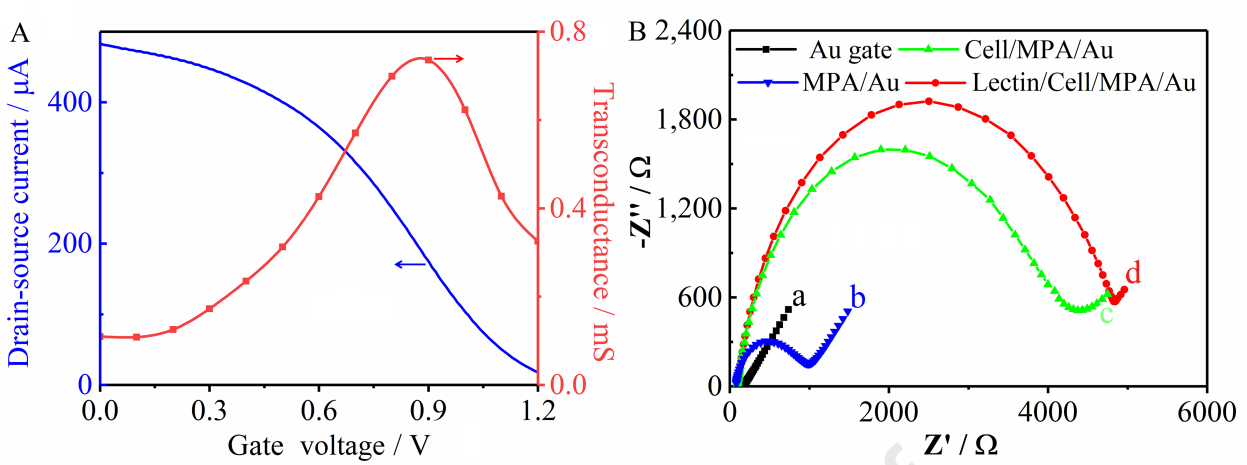
Scheme 1. (A) Schematic illustration of general device with MPA modified gate electrode. (B) Schematic for covalent immobilization of cells on gate modification and recognition of lectin to cell surface glycan. (C) OECT-based biosensing mechanism in PBS.

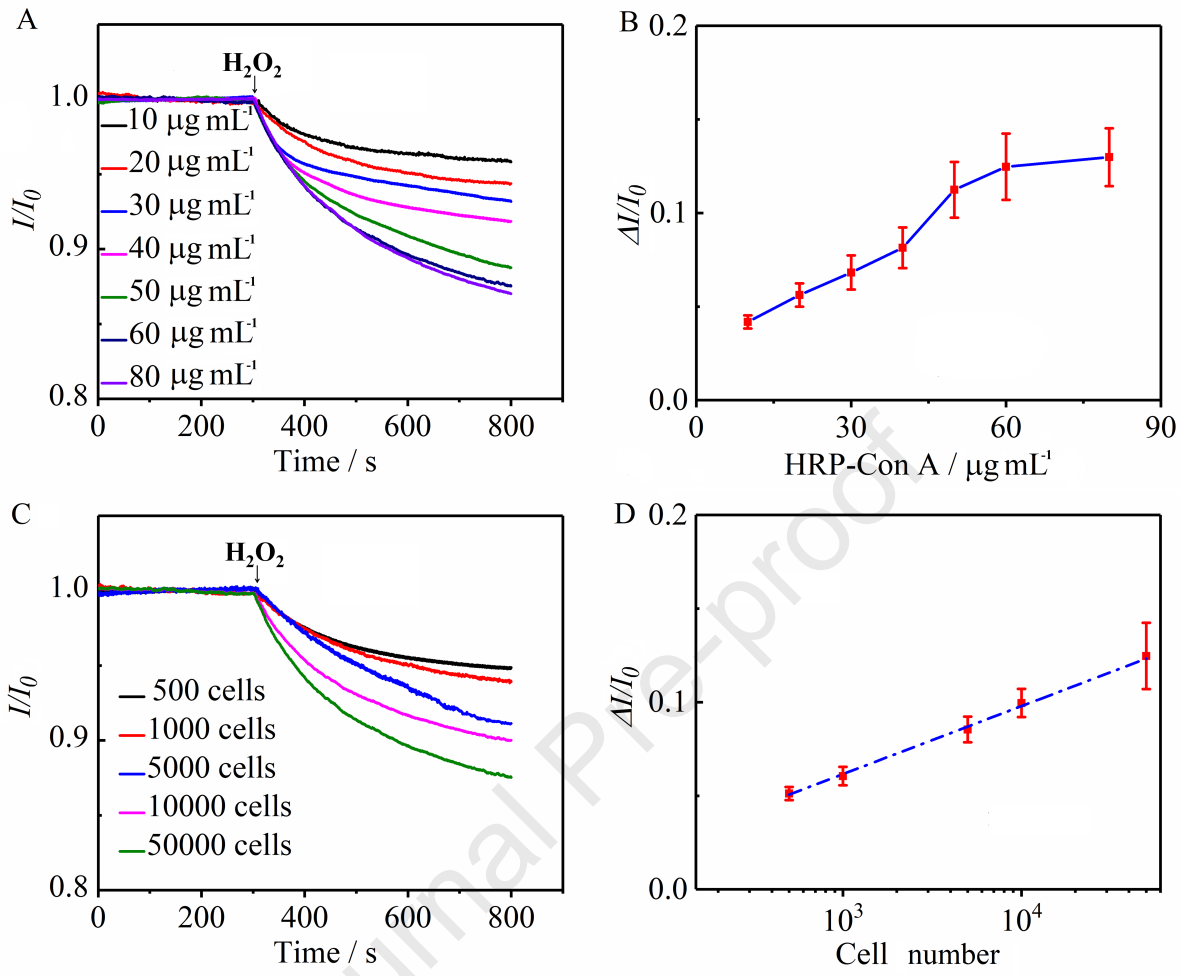
Fig. 1. (A) Transfer and transconductance curves of the newly made device in pH 7.4 PBS. (B) EIS of bare (a), MPA modified (b), Cell-MPA modified (c) and HRP-Con A-Cell-MPA modified (d) gate electrodes in 0.1 M KCl containing 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1). Frequency range: 100 kHz to 0.01 Hz; amplitude: 10 mV.

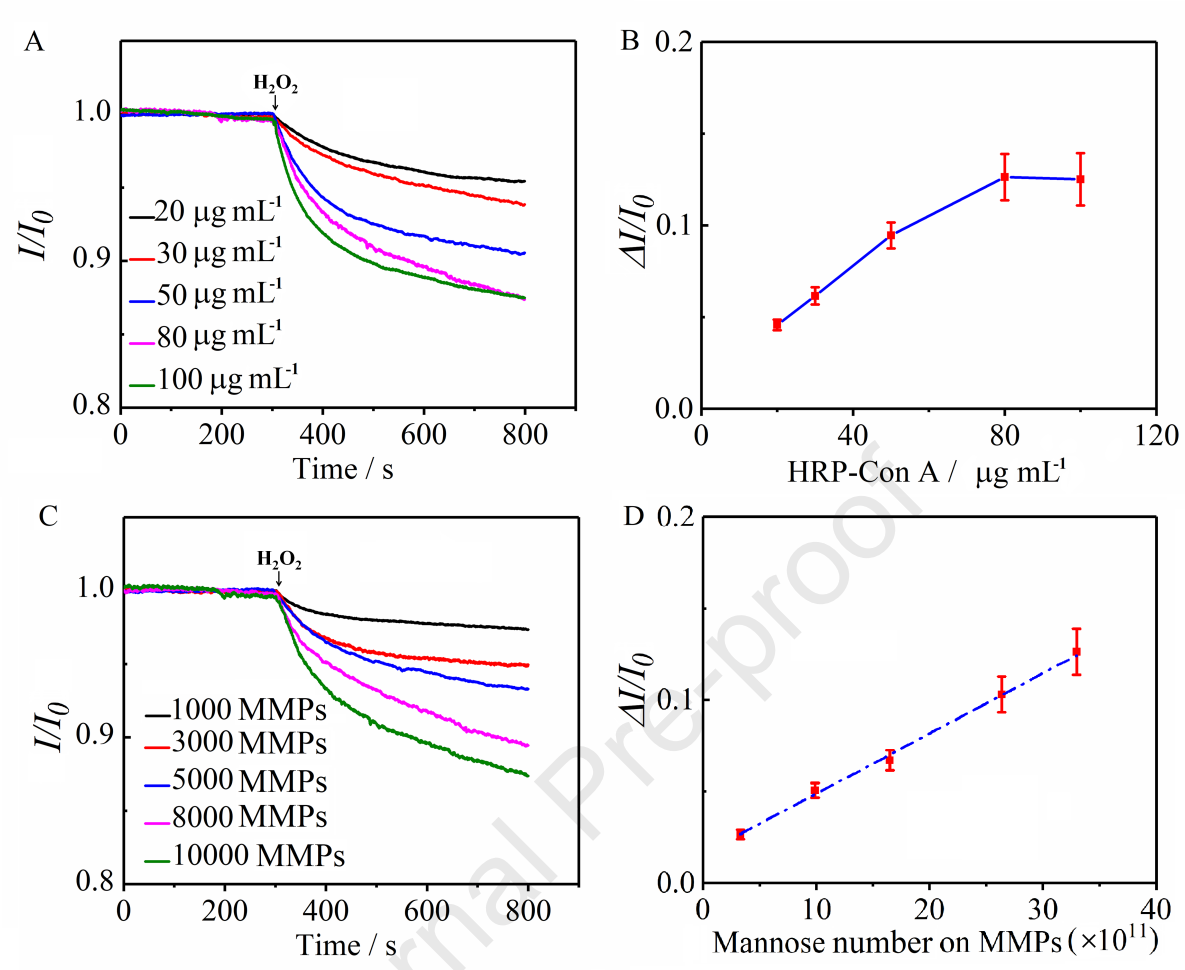
Fig. 2. (A, B) Optimization of HRP-Con A concentration for mannose detection on HeLa cells. (C) Normalized current responses to cell numbers (N_{Cells}) used for covalent immobilization on gate interfaces. (D) Plot of $\Delta I/I_0$ *vs* $\log(N_{Cells})$.

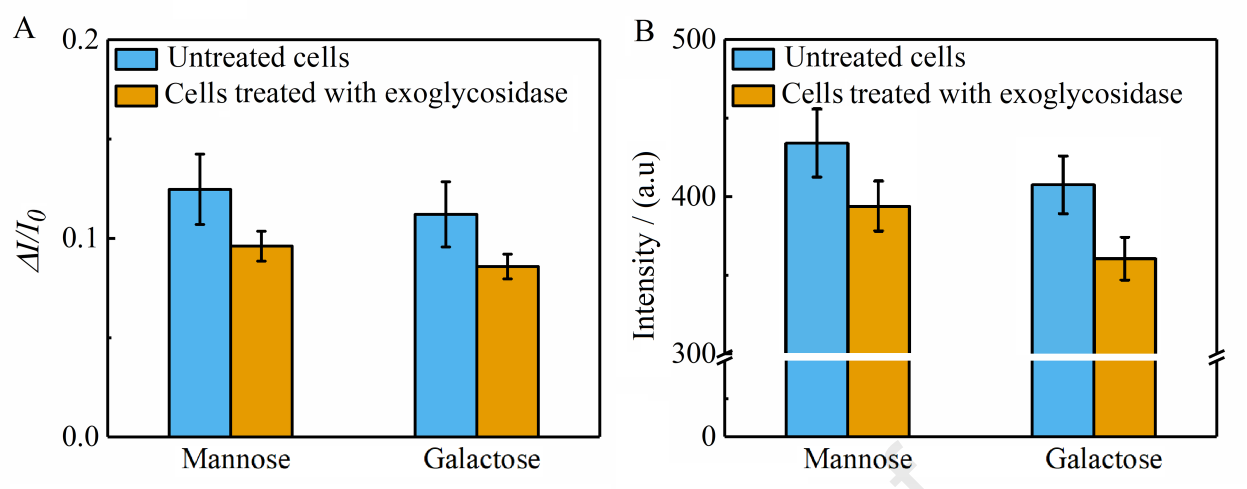
Fig. 3. (A, B) Optimization of HRP-Con A concentration for mannose detection on microspheres. (C) Normalized current responses to microsphere numbers used for covalent immobilization on gate interfaces. (D) Plot of $\Delta I/I_0$ *vs* mannose on MMPs

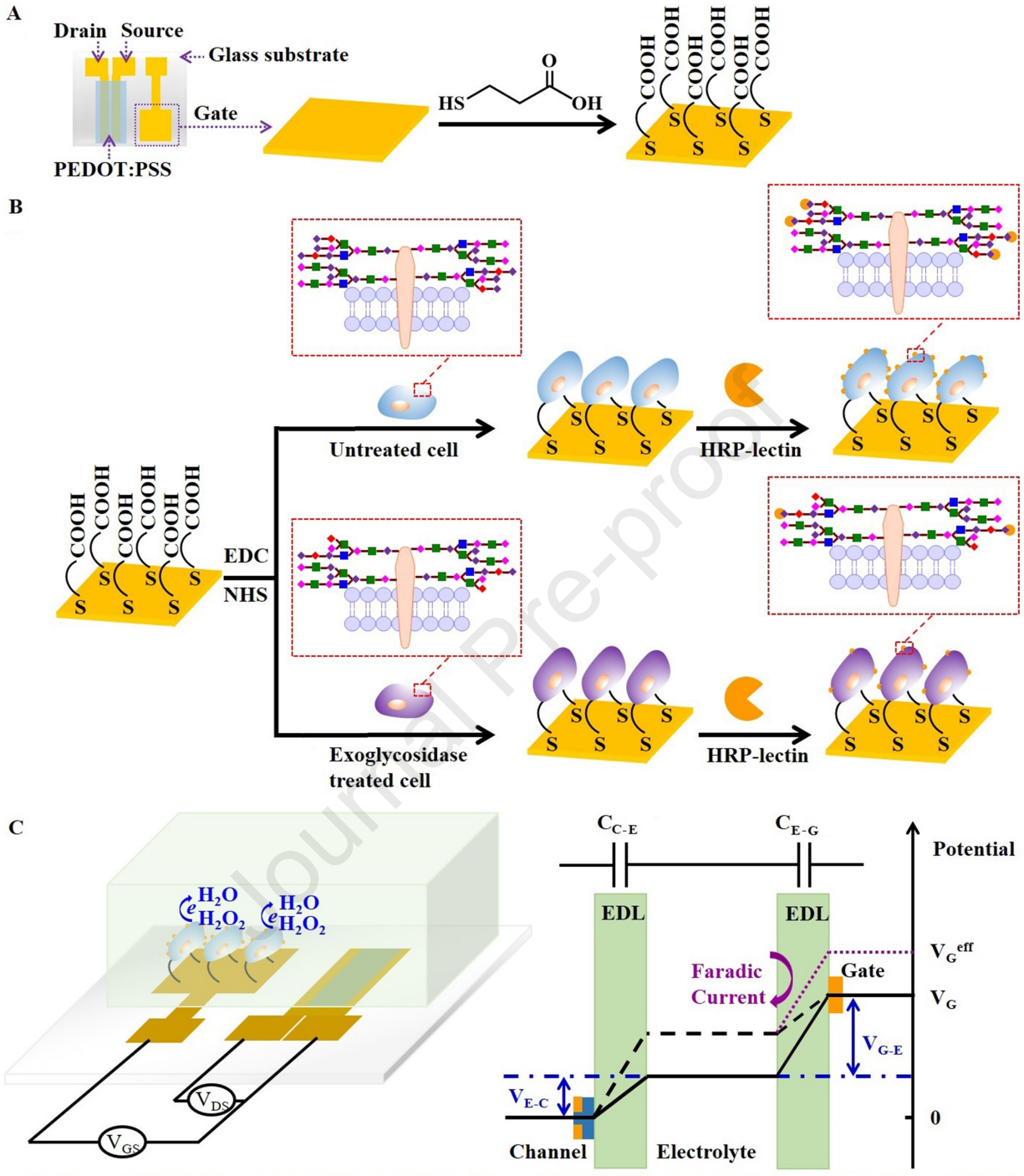
Fig. 4. (A) Normalized current and (B) flow cytometric analysis of mannose and galactose expression changes upon cleavage of mannosidase and galactosidase, respectively.










A
Drain
Source
Glass substrate
Gate
PEDOT:PSS
HS
O
OH
COOH
S
B
EDC
NHS
Untreated cell
HRP-lectin
Exoglycosidase
treated cell
HRP-lectin
C
H_2O
H_2O_2
e
V_{DS}
V_{GS}
C_{C-E}
C_{E-G}
Potential
EDL
EDL
Faradic
Current
Gate
V_G^{eff}
V_G
V_{G-E}
V_{E-C}
0
Channel
Electrolyte

Highlights

A facile biosensor was designed with organic electrochemical transistor for analysis of cell surface glycan expression.

The quantitative detection of cell surface glycans was achieved by using glycan modified microspheres to simulate the cells.

The amounts of mannose and galactose on HeLa cells were detected with the proposed biosensing strategy.

This strategy was demonstrated by changing cell surface glycan expression with exoglycosidase treatment.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.