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Electrogenerated chemiluminescence biosensing for the detection of prostate PC-3 cancer cells incorporating antibody as capture probe and ruthenium complex-labelled *wheat germ agglutinin* as signal probe

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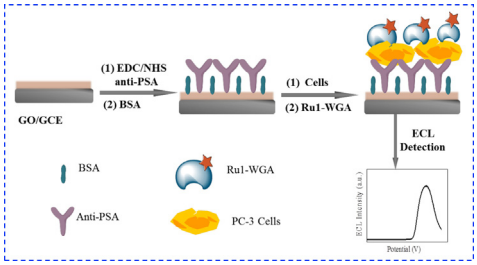
HIGHLIGHTS

- A novel biosensor was developed for the detection of prostate cancer cells.
- The selectivity of the biosensor was improved using antibody as capture probe.
- The biosensor showed the low extremely detection limit of 2.6×10^2 cells mL^{-1}.
- The ruthenium complex-labelled WGA can be transported in the cell vesicles.

GRAPHICAL ABSTRACT



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ABSTRACT

A highly selective and sensitive electrogenerated chemiluminescence (ECL) biosensor for the detection of prostate PC-3 cancer cells was designed using a prostate specific antibody as a capture probe and ruthenium complex-labelled *wheat germ agglutinin* as a signal probe. The ECL biosensor was fabricated by covalently immobilising the capture probe on a graphene oxide-coated glassy carbon electrode. Target PC-3 cells were selectively captured on the surface of the biosensor, and then, the signal probe was bound with the captured PC-3 cells to form a sandwich. In the presence of tripropylamine, the ECL intensity of the sandwich biosensor was logarithmically directly proportion to the concentration of PC-3 cells over a range from 7.0×10^2 to 3.0×10^4 cells mL^{-1}, with a detection limit of 2.6×10^2 cells mL^{-1}. The ECL biosensor was also applied to detect prostate specific antigen with a detection limit of 0.1 ng mL^{-1}. The high selectivity of the biosensor was demonstrated in comparison with that of a lectin-based biosensor. The strategy developed in this study may be a promising approach and could be extended to the design of ECL biosensors for highly sensitive and selective detection of other cancer-related cells or cancer biomarkers using different probes.

1. Introduction

Development of analytical methods for highly sensitive, selective and fast detection of cancer cells and biomarkers is of

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great significance to early diagnosis and therapeutic assessment of cancers. The most common methods for cancer diagnosis rely on technologies that are based on the paraffin fixation of tissues with visual inspection of cell morphology by a pathologist. In addition, cancer biomarkers involving DNA, RNA and proteins used in cancer diagnosis analyses recovered from paraffin-embedded tissues are significantly more degraded than those recovered from fresh tissues [1]. Therefore, within the context of improving the recovery rates of cancer, studies on the fast, sensitive and accurate detection of cancer cells and biomarkers are of great importance to early diagnosis. Currently available methods include polymerase chain reaction, flow cytometry, and cytopathological examination [2,3]. Nevertheless, these methods have some disadvantages, including the requirement for sophisticated instrumentation and qualified personnel combined with additional procedures to enrich target cells or express fluorescent protein in the cells, which in turn, result in increased cost, labour, and time [4].

In recent years, biosensor-based methods for the detection of cancer cells and cancer biomarkers have received much attention because of their simplicity, speed and flexibility [5]. Extensive efforts have been devoted to improving the sensitivity and selectivity of the biosensors, including employment of novel biological recognition molecules, highly sensitive techniques, and design of the immobilisation methods. The recognition molecules used mainly include antibodies, nucleic acid aptamers and antimicrobial peptides, which have an affinity for the epitopes present on the cell surface. Antibodies have been widely used as they have strong binding strength and high selectivity, in addition to many commercially available candidates being available. However, they have some limitations, such as challenging production in vivo [6] and decreases of immune reactivity after being labelled with large signal molecules. Aptamers and antimicrobial peptides suffer from possible drawbacks, such as a low specific selectivity and a limited number of commercially available candidates [7]. Lectins, a group of proteins extracted from plants or animals, can strongly bind to specific carbohydrate moieties on the surface of cells and are particularly interesting candidates as molecular recognition elements because of their ease of production and intrinsic stability [8]. They have been applied to design biosensors for the detection of cancer cells or bacteria [9–12]. In our previous work [9], we reported an electrogenerated chemiluminescence (ECL) biosensor for the detection of *Escherichia coli* employing *Concanavalin* A (*Con* A) as a recognition element, which demonstrated a much lower detection limit of 127 cells mL^{-1}. However, this lectin-based biosensor suffered from poor specificity because *Con* A bound with a panel of carbohydrate motifs. Therefore, a reasonable choice of recognition molecules needs to be made in the design of biosensors for the detection of cancer cells.

To tackle the specificity issues of the lectin-based assays, some assays have been created using antibodies as capture probes and labelled lectins as signal probes for the detection of cancer biomarkers. Chen et al. reported [13] a fluorescence sandwich assay to detect the proteins of MUC1 and CEA using an antibody microarray to capture targets followed by detection with phycoerythrin-labelled lectins, respectively. Li et al. reported [14] an ECL method for the detection of prostate specific antigen (PSA) and membrane metallo-endopeptidase using antibodies as capture probes and ECL agent-labelled lectins as signal probes. These works demonstrated that the approach is promising in terms of making use of the potential advantages of antibodies and lectins.

Graphene oxide (GO) is a promising nanomaterial for the design of biosensors owing to its unique characteristics, such as facile surface modifications, extraordinary structures and electrical properties, as well as good biocompatibility [15]. Guo et al. [16] reported an ECL biosensor for thrombin based on covalently coupling the aptamer to a GO-adsorbed glassy carbon (GC) electrode and quantum dots (QDs)-labelled aptamer as the signal probe, demonstrating that GO can enhance the ECL intensity of QDs. Jung et al. [17] reported a photoluminescence immunoassay for the detection of pathogens involving the covalent immobilisation of the antibody on a GO-coated glass surface. Recently, Zhang et al. reported [18] an electrochemical impedance method for the detection of leukaemia K562 cells combining GO and poly-L-lysine on the substrate for the adhesion of the target cells, and they found that the assembled film displays improved immobilisation capacity for living cells and a good biocompatibility for preserving the activity of the living cells. These works demonstrated that GO is a promising nanomaterial for the fabrication of the sensing platforms of biosensors.

The detection techniques employed by biosensors for the detection of cancer cells involve fluorescence [19], voltammetry [10], impedimetry [20,21], and ECL [4,22,23]. Among these detection techniques, ECL is very promising because of its high sensitivity and ease of control [24,25]. Nie et al. [4] reported on an ECL biosensor fabricated by covalently coupling oligonucleotide probes on a conducting polymer modified GC electrode and using gold nanoparticles as a carrier for ruthenium complexes and oligonucleotide probes, with a detection limit of 300 cells mL^{-1} Ramos cells. Wu et al. [23] developed a hybrid bipolar electrode ECL biosensor for mucin-1 on MCF-7 cells. Chen et al. [12] reported an ECL biosensor for the detection of K562 cells, with a detection limit of 600 cells mL^{-1}. Han et al. [22] developed an ECL competitive biosensor using mannan-functionalised CdS quantum dots as the ECL probe, with a detection limit of 1.2×10^3 cells mL^{-1} for K562 cells. These works demonstrated that ECL biosensors have a low detection limit. However, the sensitivity and selectivity should be improved. To the best of our knowledge, an ECL biosensor incorporating an antibody as a capture probe and ruthenium complex-labelled lectin as a signal probe for the detection of cancer cells has not been reported.

The aim of this work is to develop a highly sensitive and selective sandwich ECL biosensor for the detection of cancer cells based on using an antibody as a capture probe and ruthenium complex-labelled lectin as a signal probe. The principle scheme of the designed ECL biosensor for the determination of cancer cells is demonstrated in Fig. 1. As a proof-of-principle, the prostate PC-3 cancer cell was chosen as a model cancer cell, and an anti-PSA antibody was employed as the recogniser and capture probe for cells via specific affinity to prostate membrane antigen on the cell surface. In addition, bis(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine-ruthenium (*N*-succinimidyl ester-bis(hexafluorophosphate) (Ru1)-labelled *wheat germ agglutinin* (WGA) lectin was employed as a signal probe, as WGA is easily obtained compared to an antibody, and WGA has an affinity for cells via the specific binding capacity to *N*-acetylglucosamine (GlcNAc) of *N*-glycans on the cell surface. Ru1 was used as the ECL tag because of its high ECL efficacy and regeneration on the electrode surface. The biosensor was fabricated by adsorbing GO onto a GC electrode and further covalently coupling the capture probe onto the surface of the GO/GC electrode. With a sandwich, after incubating with PC-3 cells and the signal probe, the biosensor shows strong ECL intensity with tripropylamine as the coreactant. The proposed ECL biosensor could sensitively detect the cancer cells and significantly improved the selectivity for different cancer cells compared with the lectin-based biosensors. Thus, these results prove the feasibility and reliability of analysing cancer cells via the proposed biosensor. In this paper, the characteristics and analytical performances of the biosensor for the detection of the PC-3 cells are presented.

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2. Experimental and methods

2.1. Reagents and apparatus

Bis(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine-ruthenium (*N*-succinimidyl ester-bis(hexafluorophosphate), M_w = 1014.66, with the cation abbreviated as Ru1, *wheat germ agglutinin* from *Triticum vulgaris wheat* (WGA, M_w = 36 KDa), *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropy)-*N*′-ethylcarbodimide hydrochloride (EDC) were purchased from Sigma–Aldrich (USA). Monoclonal mouse anti-human PSA antibody was obtained from Zhongshan Golden Bridge Biotechnology Co., Ltd. (China). Prostate specific antigen (PSA) from human semen was obtained from Fitzgerald Industries International, Inc. (USA). PC-3 cells were obtained from the Shanghai Institute of Cell Biology of the Chinese Academy of Sciences (China). *N*,*N*-dimethylformamide (DMF), sodium periodate, and tripropylamine (TPrA) were purchased from First Reagent Corporation of Shanghai (China). 4-(4-*N*-maleimidophenyl) butyric acid hydrazide hydrochloride (MPBH) was obtained from Pierce Inc. (Rockford, USA). Bovine serum albumin (BSA) was obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). Graphene oxide (GO) was prepared from natural graphite powders using a modified Hummers method [26,27].

In addition, 10 mM phosphate buffer saline (10 mM PBS, pH 7.40) consisting of 10 mM Na_2HPO_4/NaH_2PO_4 and 100 mM KCl was used to prepare WGA, PSA and anti-PSA solutions. Moreover, 10 mM PBS containing 1% Triton X-100 was used as washing buffer. All other reagents were of analytical grade. Millipore Milli-Q water (18 MΩ cm) was used throughout the experiments.

2.2. Apparatus

The ECL experimental set-up consisted of a CHI 440 workstation (Shanghai Chenhua Instruments, China) and an ultra-weak chemiluminescence analyser controlled by a personal computer with the BPCL program (Institute of Biophysics, Chinese Academy of Sciences, China). A commercial cylindroid glass cell was used as an ECL cell. A three-electrode system composed of a biosensor or a glassy carbon electrode (GC, 2 mm diameter) as working electrode, a platinum wire as counter electrode, and an Ag/AgCl (saturated KCl) as reference electrode was used. All of the potentials in this work are in comparison with the reference electrode. The ECL emission was detected with a photomultiplier tube (PMT) that was biased at −900 V.

Electrochemical impedance spectroscopic measurements were carried out on a CHI 660B electrochemical workstation in 5 mL of a 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 100 mM KCl. The impedance spectra were recorded within the frequency range of 0.1–100 KHz with a sampling rate of 12 points per decade. The amplitude of the applied sine wave potential in each case was 5 mV. The UV–vis adsorption was acquired with a UV–vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan).

The interaction between the signal probe of Ru1-WGA and PC-3 cells was evaluated using confocal microscopy to assess whether the signal probe bound cells. Live PC-3 cells were incubated with 20 μM signal probe at 37 °C, 5% CO_2 for 1 h. After incubation, the cells were washed thoroughly and fixed, and the cell membranes were stained using the signal probe. In addition, the nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI). Confocal images were obtained using a Leica TCS SP5 laser scanning confocal microscope (Leica, Germany).

2.3. Synthesis of the signal probe (Ru1-WGA)

The signal probe (Ru1-WGA) was synthesised following a previously reported method [28]. Briefly, 4 mg of Ru1 (3.94 μmol) was dissolved in 100 μL of DMF. Then, 50 μL of the Ru1 solution (1.97 μmol) was added to 1.5 mL of 0.20 M carbonate buffer (pH 8.5) containing 2 mg of WGA (0.056 μmol) and stirred gently free of light for 6 h at 25 °C. The mixture solution was filtered by a dialysis bag (molecular weight cut-off = 3000). The filtrate was extensively dialysed against 500 mL changes of 10 mM PBS for six changes at 25 °C to separate unreacted Ru1 and was used as the signal probe solution and stored at 4 °C before use. The concentration of the signal probe solution was calculated on the basis of UV–vis spectrometry. The concentration of the signal probe solution collected was estimated to be 1.26×10^{-5} M on the basis of the amount of Ru1 in the signal probe according to the absorbance of Ru1 at 457 nm and WGA at 280 nm. The mole ratio of Ru1 to WGA in the signal probe was calculated to be 4:1 on the basis of the two absorbance values, including $A_{280\,nm}$ = 0.455 for WGA and $A_{457\,nm}$ = 0.110 for Ru1 as well as their respective molar extinction coefficient values, ε ($\varepsilon_{Ru1,\ 457\,nm} = 1.40 \times 10^4\ M^{-1}\ cm^{-1}$, $\varepsilon_{Ru1,280\,nm} = 5.36 \times 10^4\ M^{-1}\ cm^{-1}$, and $\varepsilon_{WGA,280\,nm} = 1.24 \times 10^5\ M^{-1}\ cm^{-1}$) [29,30].

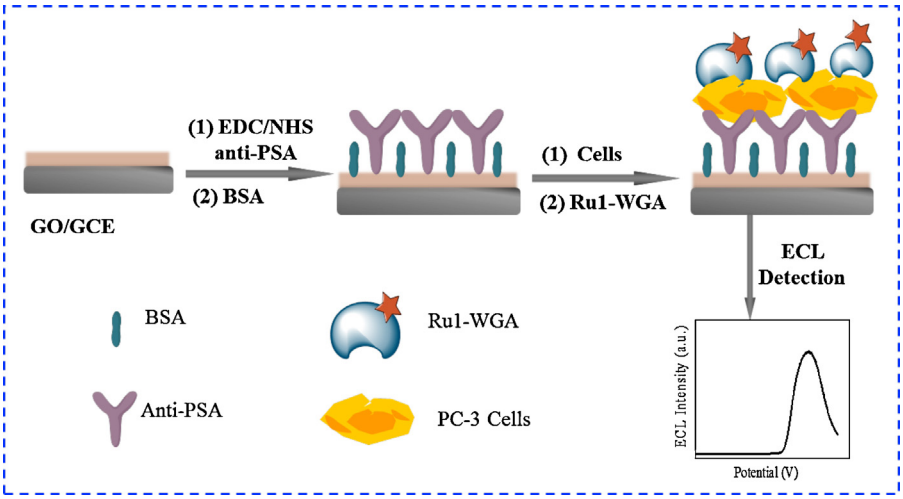

Fig. 1. Schematic diagram of the fabrication of the ECL biosensor and the detection of cancer cells.

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2.4. Culture and collection of cells

PC-3 cells were cultured in 6 mL of Ham's F-12 medium (GIBCO, Grand Island, USA) supplemented with 7% foetal bovine serum (Zhejiang Tianhang Biological Technology Co., Ltd., China) and 2 mM L-glutamine (Life Technologies, Gaithersburg, MD, USA) in an incubator (5% CO_2, 37 °C). All cells were collected and separated from the medium by centrifugation at 1000 × *g* for 5 min and then washed twice with sterilised 10 mM PBS (pH 7.40). The sediments were resuspended in 10 mM PBS (pH 7.40) to obtain a cell suspension used as a stock suspension with the final concentration of cells (1.0×10^6 cells mL^{-1}). The cell density was determined by using a haemocytometer, and this was performed prior to any experiments.

2.5. Fabrication of the biosensor

The anti-PSA antibody was pretreated, according to the reference [13], by using sodium periodate to oxidise the surface glycans of the anti-human PSA to reduce its binding to Ru1-labelled WGA. Briefly, 100 μL of 100 μg mL^{-1} anti-PSA antibody (dilution 1:10) was mixed with 50 μL of 20 mM sodium periodate, which was prepared using 150 mM NaCl and 100 mM sodium acetate (pH 5.50) and stirred for 1 h. Then, 50 μL of 1 mM MPBH was added above the mixture and stirred for 1 h, which was followed by the addition of 50 μL of 1 mM Cys-Gly dipeptide at 4 °C overnight. The prepared anti-PSA was used as a capture probe for the fabrication of the biosensor.

The GC electrode (2 mm dimmer) was polished with 0.3 μm alumina slurry and then ultrasonicated in water for 5 min. Then, 2.0 mg of GO was ultrasonically dispersed in 10 mL of water. In addition, 5 μL of the dispersed GO suspension was drop-coated onto the surface of the pretreated GC electrode and dried in air. The GO/GC electrode was washed with 10 mM PBS and activated in 1 mL of freshly prepared solution containing 2 g L^{-1} EDC and 5 g L^{-1} NHS for 30 min and then was washed with 10 mM PBS [9]. In addition, 10 μL of a 2.0 μg mL^{-1} pretreated anti-PSA antibody solution was drop-coated on the activated GO/GC electrode for 1 h at 25 °C. After rinsing with 10 mM PBS, the anti-PSA/GO/GC electrode was soaked in 100 μL of 1% BSA for 30 min to block the surface active sites of the GO/GC electrode. Finally, the ECL biosensor was washed with 10 mM PBS and stored at 4 °C in air before use.

2.6. ECL measurement

The fabricated biosensor was immersed in 100 μL of a fixed concentration of the target (PC-3 cells suspension or PSA) for an incubation time of 60 min at 37 °C and then rinsed with washing buffer. Then, the biosensor was incubated with 10 μL of 20 μM Ru1-WGA for 60 min and washed with washing buffer. The ECL measurement was performed in 2.0 mL of 10 mM PBS (pH 7.40) containing 0.10 M TPrA with a scan rate of 100 mV s^{-1} in the potential range from 0 to +1.5 V (versus Ag/AgCl, saturated KCl). The concentration of the target PC-3 cells was quantified by the increased ECL intensity (peak height).

3. Results and discussion

3.1. Characterisation of the probes

The synthesised signal probe (Ru1-WGA) was first characterised by UV–vis absorption spectrometry. As shown in Fig. 2A, a characteristic absorption peak of WGA appeared at 280 nm, whereas the characteristic absorption peaks of Ru1 were at 457 nm, which is assigned to the metal-to-ligand charge-transfer band, and at 287 nm, which is assigned to ligand-to-ligand charge transfer of π–π* transitions. The characteristic absorption peaks of the signal probe solution obtained from the dialysis bag were observed at 287 nm and 457 nm. This indicates that Ru1 was successfully WGA labelled [31].

The ECL behaviour of the synthesised signal probe was checked at the GC electrode. Fig. 2B shows the ECL intensity–potential profiles of Ru1 and the signal probe. The ECL peak of Ru1 appears at +1.10 V (I_{max} = 10917), whereas that of the signal probe appears at +1.11 V (I_{max} = 1491), indicating that the ECL behaviour of the probe is similar to the ECL behaviour of Ru1. The ECL intensity of Ru1 is 7.32 times higher than that of the signal probe at the same Ru1 content level. This is attributed to the fact that the ECL efficiency and the diffusion coefficient of the Ru1-labelled antibody is lower than that of Ru1 [12].

The synthesised signal probe was also checked by confocal laser scanning microscopy to assess whether it can bind with the target PC-3 cells. As shown in Fig. 3, the outlines of the cells clearly displayed orange fluorescence with a maximum emission wavelength of 620 nm, and the cell nucleus displayed blue fluorescence with a maximum emission wavelength of 461 nm from 4′,6-diamidino-2-phenylindole (DAPI), for the location of cell nucleus. The orange fluorescence from Ru1-WGA suggests that Ru1-WGA is attached to the receptors of WGA on the cell surfaces (Fig. 3a), whereas the weak orange fluorescence observed in the cytoplasm suggests that Ru1-WGA is trapped and transported in the vesicles. This observation of the colocalisations of WGA in a unique perinuclear region in the cells is in accordance with a previous report using QD-labelled WGA [32]. These findings are

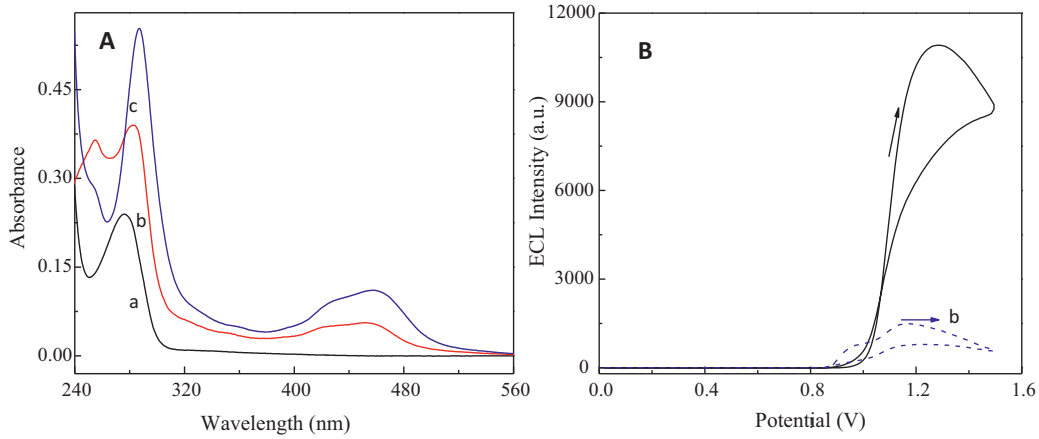

Fig. 2. (A) UV–vis absorption spectra obtained from (a) 2.7×10^{-5} M WGA, (b) 4.0×10^{-5} M Ru1, and (c) Ru1-WGA in 10 mM PBS (pH 7.40). Light-path length: 1.0 mm. (B) The ECL intensity–potential profiles of Ru1 (4.93 μM, a) and Ru1-WGA (1.23 μM, b) obtained with a GC electrode with a scan rate of 100 mV s^{-1} in 0.10 M TPrA.

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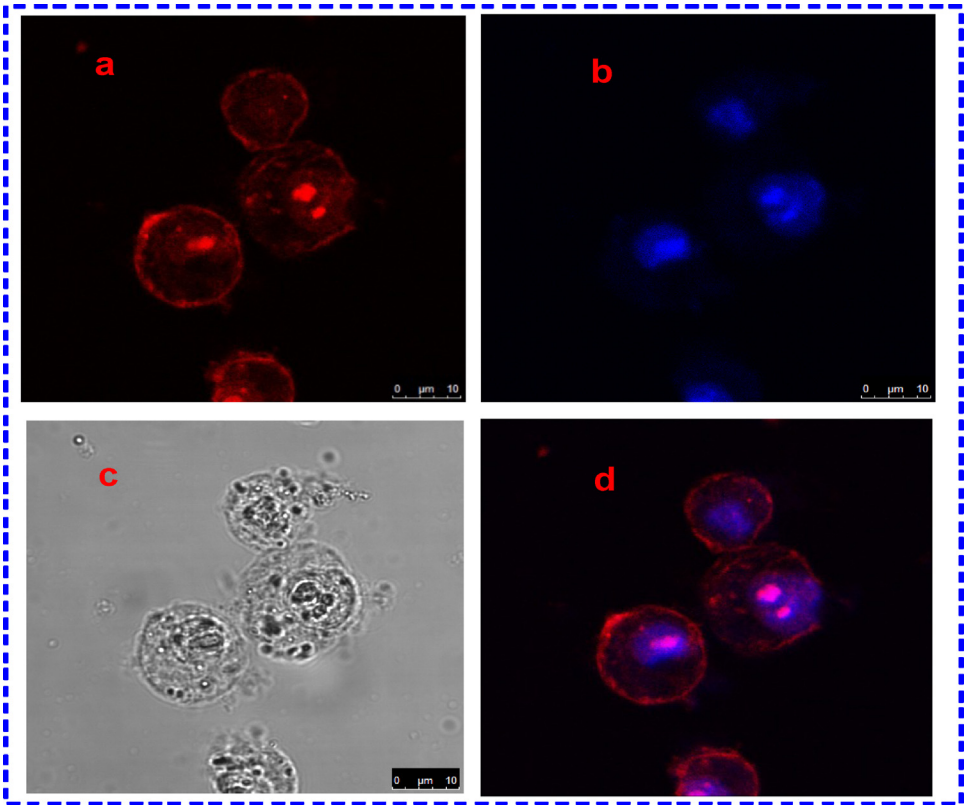

Fig. 3. Confocal fluorescence images of PC-3 cells incubated with the signal probe. (a) The cells ere stained with Ru1-WGA (orange); (b) the nucleus was stained with DAPI (blue); (c) the bright field of cells; (d) overlap of a, b and c. Ex = 458 nm, Em = 620 nm for Ru1-WGA. Ex = 358 nm, Em = 461 nm for DAPI. Live PC-3 cells were incubated with 20 μM signal probe at 37 °C, 5% CO_2 for 1 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

attributed to the fact the expression of sialic acid and *N*-acetylglucosamine on PC-3 cells surfaces [33,34] can be recognised by four carbohydrate-binding sites of one WGA. These indicate that the synthesised signal probe could bind the receptors on PC-3 cell surfaces.

Electrochemical impedance spectroscopy (EIS) has proven to be one of the most powerful tools for determining the characteristics of the interfacial properties in the biosensor fabrication process and binding process [18]. Nyquist plots of the GC electrode subjected to the step-by-step modification process, using $[Fe(CN)_6]^{4-/3-}$ as the redox indicator, were generated. As shown in Fig. 4 (inset), the Randles's equivalent circuit [35] was found to fit adequately the data obtained over the measured frequency range, which includes electron transfer resistance (R_{et}), electrode/electrolyte solution (R_s) and the Warburg impedance (Z_w). The two elements of the Randle's equivalent circuit, R_s and Z_w, represent the properties of the bulk solution and the diffusion of the redox probe, and they are not affected by the reaction occurring at the electrode surface. The other two elements, C_{dl} and R_{et}, depend on the dielectric and insulating features at the electrode/electrolyte interface, and there is always substantial change in R_{et} compared with C_{dl}. It is thus feasible to use R_{et} as the parameter to correlate the impedance response with different assembling steps [36]. The bare GC electrode exhibited a very small semicircle at high frequencies, and the electron transfer resistance (R_{et}) is 57 Ω. The GO-coated GC electrode resulted in an increase R_{et} from 57 Ω to 227 Ω, which is attributed to the fact that GO has a relative low conductivity [18]. In addition, GO has negative charges, such as hydroxyl, epoxyl, and carboxyl groups, which generate an electrostatic repulsive force to the negatively charged probe anions $[Fe(CN)_6]^{3-/4-}$ [15]. After the pretreated anti-PSA was covalently coupled with the carboxyl group of GO on the GC electrode, the R_{et} value greatly increased from 227 Ω to 1727 Ω. This indicates that the capture probe is covalently coupled onto the surface of the GO/GC electrode. Subsequently, after the functionalised electrode was blocked with BSA, the R_{et} greatly increased from 1727 Ω to 7208 Ω. After the biosensor incubating with

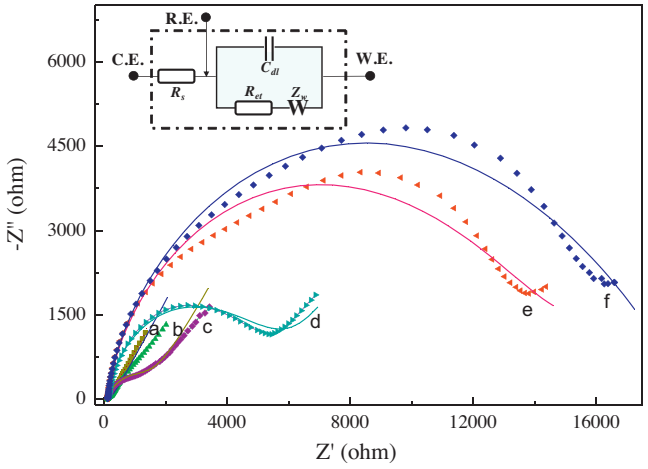

Fig. 4. Nyquist plots of the impedance spectra at different electrodes in each step of the fabrication of the biosensor and the determination of PC-3 cells in a solution of 10 mM PBS (pH 7.40) containing 5 mM $K_3[Fe(CN)_6]$–5 mM $K_4[Fe(CN)_6]$–0.1 M KCl. Curve a, bare GC electrode; curve b, GO/GC electrode; curve c, anti-PSA/GO/GC electrode; curve d, BSA/anti-PSA/GO/GC electrode; curve e and curve f, BSA/anti-PSA/GO/GC electrode after incubation with 1×10^3 cells mL^{-1} and 10 μM signal probe for 60 min, respectively. EIS measurement conditions: bias potential, 0.24 V; frequency, 100 kHz – 1.0 Hz; amplitude, 5.0 mV. The inset shows the equivalent circuit applied in the work.

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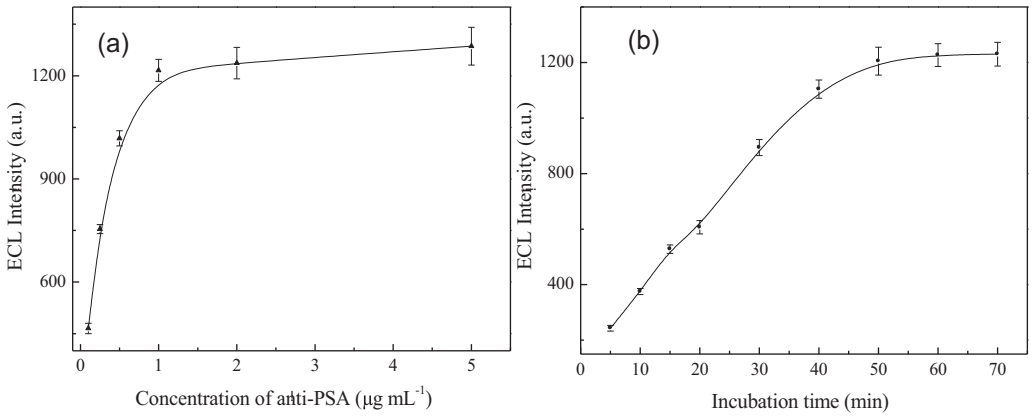

Fig. 5. (A) Dependence of the ECL intensity on the concentration of the capture probe. The biosensors were incubated with 3×10^3 cells mL^{-1} PC-3 cells for 60 min and then with 20 μM Ru1-WGA for 60 min. (B) Dependence of the ECL intensity on the binding time. The biosensors were incubated with 3×10^3 cells mL^{-1} PC-3 cells and then with 20 μM Ru1-WGA for 60 min. ECL measurements were performed with a scan rate of 100 mV s^{-1} in 0.10 M TPrA.

1×10^3 cells mL^{-1} PC-3 cells, R_{et} continually increase from 1727 Ω to 7208 Ω. This is attributed to the fact that a barrier of captured cells hampers the probes close to the surface of the electrode. This suggests that PC-3 cells can be captured onto anti-PSA/GO/GC electrodes. When PC-3 cells-bound biosensor was incubated with the signal probe, the R_{et} value increased from 7208 Ω to 9823 Ω. These indicate that the biosensor is successfully fabricated and could bind the target and the signal probe.

3.2. Optimisation of the capture probe concentration and the incubation times

Fig. 5A shows the dependence of the ECL intensity on the concentration of the capture probe. The ECL intensity sharply increases with the increase of the capture probe concentration from 0.1 to 1.0 μg mL^{-1}. This is attributed to the increase of the amount of pretreated anti-PSA covalently immobilised on the GO/GC electrode. Then, the ECL intensity reaches a plateau with the increase of the concentration of the capture probe from 1.0 to 5.0 μg mL^{-1}. This is attributed to the saturation of the capture probe on the GO/GC electrode. In addition, 2.0 μg mL^{-1} capture probe was used in the following experiments to save the capture probe and obtain a high sensitivity.

Fig. 5B shows the dependence of the ECL intensity on the incubation time for binding target PC-3 cells with the capture probe on the fabricated biosensor. The ECL intensity sharply increases with increasing incubation time from 5 min to 40 min. And then the intensity reaches a plateau after 50 min. This suggests that the binding reaction between PC-3 cells and the capture probe was complete within 50 min. Therefore, 60 min was chosen as the incubation time in the following experiments.

The incubation time between the bound PC-3 cells and the signal probe was also optimized. The results revealed that the incubation time of 60 min was sufficient to make the signal probe bind PC-3 cells on the surface of the biosensor. This incubation time coincides with that reported in our previous work [9]. Therefore, 60 min was chosen as the incubation time for PC-3 cells and the signal probe in the following experiments.

3.3. Analytical performance of the ECL biosensor for PC-3 cells

The quantitative behaviour of the ECL biosensor was assessed by measuring the dependence of the ECL intensity upon the concentration of PC-3 cells under the optimised conditions. Fig. 6A shows the ECL responses to different concentration of PC-3 cells in the range from 3×10^2 to 3×10^4 cells mL^{-1}. The inset of Fig. 6A shows that the ECL intensity is logarithmically proportion to the concentration of the cells in the range from 7×10^2 to 3×10^4 cells mL^{-1}. The linear regression equation was $I_{ECL} = 1275 \log C - 3082$ (C: cells mL^{-1}), with a correlation coefficient of 0.9917. The limit of detection (LOD) was calculated to be 2.6×10^2 cells mL^{-1} at 3σ, where σ was the standard deviation of the signals obtained in 7 parallel ECL measurements

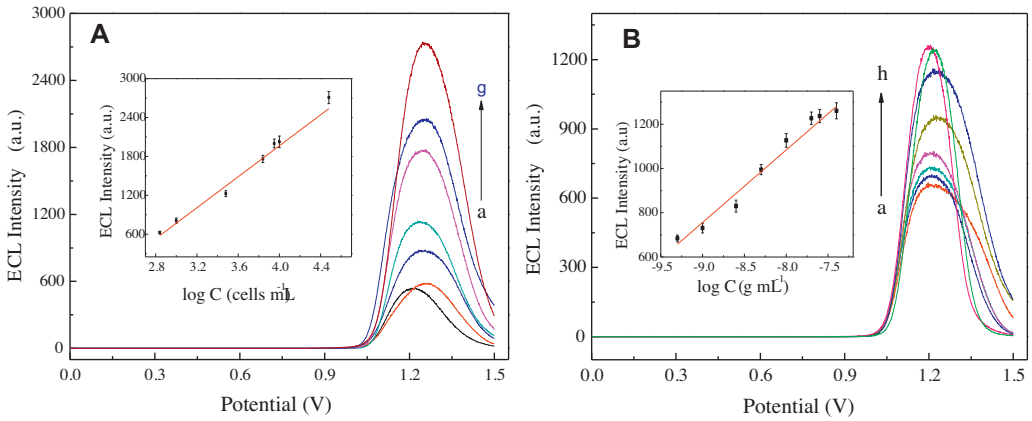

Fig. 6. (A) The ECL responses of the biosensors after incubation with PC-3 cells at concentrations of 7.0×10^2, 1.0×10^3, 3.0×10^3, 7.0×10^3, 9.0×10^3, 1.0×10^4, and 3.0×10^4 cells mL^{-1} (from a to g). Inset: calibration curve. (B) The ECL responses of the biosensors after incubation with PSA at concentrations of 0.5, 1.0, 2.6, 5.0, 10.0, 20.0, 26.0, and 40.0 ng mL^{-1} (from a to h). Inset: calibration curve. ECL measurements were performed with a scan rate of 100 mV s^{-1} in 0.10 M TPrA.

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Table 1
Comparison of the proposed method with some reported biosensors.

Detection technique	Recognition element	Target analytes	Detection limit (cells mL^{-1})	Refs.	Signal amplification strategy
ASV	*Con* A	CCRF-CEM cells	50	[10]	Quantum dots
EIS	*Con* A	Bel-7404 cells	2.34×10^2	[11]	No
EIS	Aptamer	Hela cells	1×10^3	[21]	Graphene
Fluorescence	Aptamer	CCRF-CEM cells	–	[19]	Silver nanocluster
ECL	Aptamer	MCF-7 cells	20	[23]	Gold nanoparticle
ECL	Antibody/lectin	PSA	1.35 ng mL^{-1}	[14]	No
ECL	Antibody	PSA	1.35 pg mL^{-1}	[40]	Magnetic enrichment
ECL	*Con* A	K562 cells	6×10^2	[12]	Gold-nanoparticle–silica nanoprobe
ECL	*Con* A	K562 cells	1.2×10^3	[38]	CdS quantum dots
ECL	Antibody/WGA	PC-3 cells	2.6×10^2	This work	No

EIS: electrochemical impedance spectroscopy; ASV: anodic stripping voltammetry.

using the blank solution [37]. A comparison of the LOD of the biosensor in our work with reported biosensors for the detection of cancer cells is listed in Table 1. The LOD obtained in this work is approximately four-fold lower than that obtained with the ECL biosensor using *Con* A [38] and the EIS aptasensor [21]. In addition, it is higher than of the reported ECL aptasensor incorporating gold nanoparticles amplification [23]. Moreover, the biosensor in our work displayed a relative standard deviation of 4.8% for five determinations at the cell concentrations of 3×10^3 cells mL^{-1} PC-3 cells, which indicates the good reproducibility of the biosensor. The storage stability of the fabricated biosensor was also examined. After stored at 4 °C in air for 1 week, the biosensor showed that the average ECL intensity was 94.6% of initial ECL intensity for 3×10^3 cells mL^{-1} PC-3 cells. Thus, the as-proposed biosensor exhibited good performance in the detection of cancer cells with broad detection range, low detection limit and good reproducibility and is promising for the detection of living cells.

3.4. Evolution of the selectivity of the biosensor

The selectivity of the biosensor fabricated in this work was checked through comparison of the ECL response to PC-3 cells, Ramos cells and K562 cells at a concentration of 1.0×10^4 cells mL^{-1}. The ECL intensity obtained was 2026 for PC-3 cells, whereas the value was 132 for Ramos cells and 120 for K562 cells. This suggests that the selectivity of the biosensor is quite satisfactory, as attributed to the specific interaction of the capture probe towards prostate membrane antigen on the surface of PC-3 cells [34].

To demonstrate the high selectivity of the biosensor in the proposed approach, a comparison with the biosensor using the one type probe approach was performed. To reach this, the biosensors were also fabricated using WGA as a capture probe following the protocol described in Section 2.5, and checked following the protocol described in Section 2.6. The results revealed that the ECL intensity obtained at the biosensors fabricated using WGA as a capture probe was 1801, 892 and 1690 for PC-3 cells, Ramos cells and K562 cells, respectively. Compared with the biosensor fabricated using anti-PSA as a capture probe, the biosensor using WGA displayed a low selectivity for the tested cells. This is attributed to the fact that the cancer cell surfaces are always covered with a dense and complex layer of glycoconjugates, which can be bound by WGA. WGA has four carbohydrate-binding sites that can recognise sialic acid and *N*-acetylglucosamine (GlcNAc), which is a fragment of the *N*-glycans on the cell surface that share a common pentasaccharide core structure consisting of Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc. Accordingly, WGA can bind PC-3 cells, K562 cells and Ramos cells because of the universal binding capacity of WGA for GlcNAc on the surface of the cancer cells [32,34]. Therefore, a high selectivity when using the proposed approach is validated.

3.5. Analytical performance of the ECL biosensor for PSA

To validate the obtained results, PSA was used to challenge the ECL biosensor, which is a serum biomarker used for the screening of prostate cancer [13]. The quantitative behaviour of the ECL biosensor was also assessed by testing the concentration of PSA under the optimised conditions discussed above. Fig. 6B shows that ECL intensity is logarithmically proportion to the concentration of PSA in the range from 0.5 to 40 ng mL^{-1}. The linear regression equation is $I_{ECL} = 343 \log C + 3834$ (C: g mL^{-1}) with a correlation coefficient of 0.9973. The LOD was calculated to be 0.1 ng mL^{-1} PSA at 3σ, where σ was the standard deviation of the signals obtained in 7 parallel ECL measurements using the blank solution [37]. This LOD is slightly lower than the 0.76 ng mL^{-1} PSA obtained using an electrochemical immunosensor [39]. It is higher than the 1 pg mL^{-1} obtained from an ECL immunosensor with a magnetic enrichment process [40]. This indicates that the biosensor fabricated displays high sensitivity for the detection of PSA.

The selectivity of the biosensor was also checked through the comparison of the ECL response to PSA, BSA, α-fetoprotein (AFP), carcino-embryonic antigen (CEA) and thrombin at a concentration of 10 ng mL^{-1}. The ECL intensity was 1149, 134, 129, 137 and 118 for PSA, BSA, AFP, CEA and thrombin, respectively. These results indicate that the designed biosensor also displayed satisfactory selectivity for the tested proteins. It should be mentioned that the ECL biosensor could be applied in both the existing PC-3 cells and PSA in clinical prostate serum samples [41]. In this case, PSA can be separated from cancer cells by centrifugation [42]. Thus, the ECL biosensor could potentially detect PSA and cancer cells individually.

4. Conclusions

An ECL biosensor for the detection of prostate cancer cells was designed using prostate specific antibody as a capture probe and ruthenium complex-labelled *wheat germ agglutinin* lectin as a signal probe. The ECL biosensor displayed a detection limit of 2.6×10^2 cells mL^{-1} for PC-3 cells and 0.1 ng mL^{-1} for PSA individually. The synthesised Ru1-WGA can be trapped and transported in the cell vesicles, as observed by confocal laser scanning microscopy. The relatively higher selectivity of the biosensor using the antibody and the lectin as probes was confirmed by comparison with that only using the lectin as probe. The strategy developed in this study is a promising approach and could be extended to the design of ECL biosensors for highly sensitive and selective detection of other cancer-related cells or cancer biomarkers.

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