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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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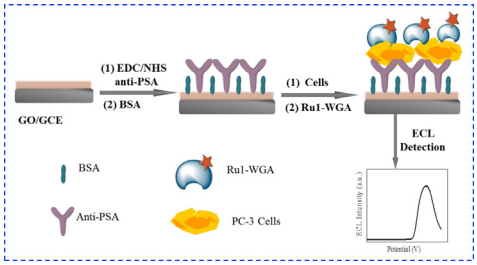
HIGHLIGHTS

- 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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