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Label-free electrochemical aptasensor constructed by layer-by-layer technology for sensitive and selective detection of cancer cells

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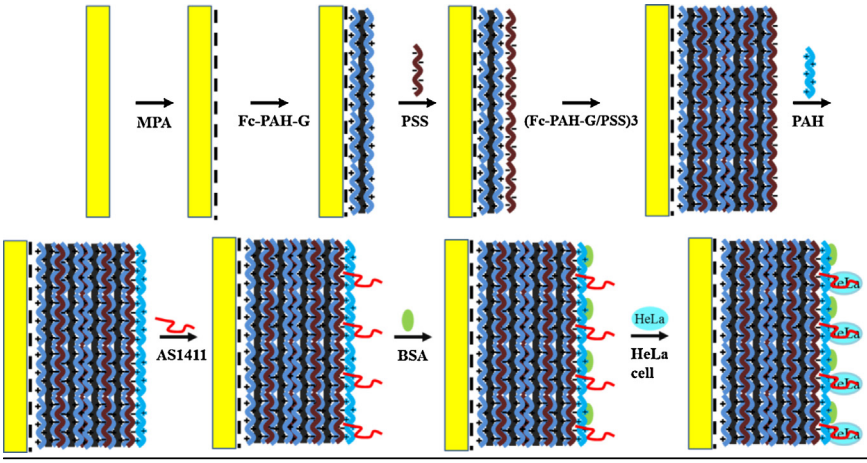
HIGHLIGHTS

- Fc-PAH was modified on the surface of graphene to prepare hybid nanocomposite (Fc-PAH-G).
- A cytosensor was constructed with Fc-PAH-G, PSS and aptamer AS1411 by LBL technology.
- The sensing interface introduced more redox probe and enhanced current signal on electrode.
- The sensor showed a detection range of 10–10^6 cells/mL with a detection limit of 10 cells/mL.

GRAPHICAL ABSTRACT



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ABSTRACT

Here, a cytosensor was constructed with ferrocene-appended poly(allylamine hydrochloride) (Fc-PAH) functionalized graphene (Fc-PAH-G), poly(sodium-*p*-styrenesulfonate) (PSS) and aptamer (AS1411) by layer-by-layer assembly technology. The hybrid nanocomposite Fc-PAH-G not only brings probes on the electrode and also promotes electron transfer between the probes and the substrate electrode. Meanwhile, LBL technology provides more effective probes to enhance amplified signal for improving the sensitivity of the detection. While AS1411 forming G-quardruplex structure and binding cancer cells, the current response of the sensing electrode decreased due to the insulating properties of cellular membrane. Differential pulse voltammetry (DPV) was performed to investigate the electrochemical detection of HeLa cells attributing to its sensitivity of the current signal change. The as-prepared aptasensor showed a high sensitivity and good stability, a widely detection range from 10 to 10^6 cells/mL with a detection limit as low as 10 cells/mL for the detection of cancer cells.

1. Introduction

Along with cancer becoming a leading cause death in the worldwide, an increasing interest has focused on early diagnosing and screening of cancer cells in recent years [1–3]. Early stage

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detection of cancer cells provides the great opportunities for the effective and low cost treatment. Therefore, highly sensitive, simple and rapid method for early detection of cancer cells is highly desired. Many techniques like fluorescent imaging, ultrasound and X-ray radiography have been applied on achieving early detection and monitoring of cancer cells [4,5]. However, electrochemical methods have attracted increasing interest due to its simplicity, low cost, high sensitivity and accuracy [6–8]. For example, Moscovici et al. reported a novel cell counting sensor via differential pulse voltammetry (DPV) to rapidly detect 125 prostate cancer cells containing non-target cells within 15 min [9].

Aptamer, artificial nucleotide sequences with stable structure and high selectivity and specificity to target molecules or cells, has been used as highly promising molecule probes among electrochemical methods. Thus, aptamer-based biosensor has been widely investigated in the early stage of cancer cells detection, allowing surface-immobilization in high density, rapid platform construction and short analysis time [10,11]. Aptamer-based cytosensor deserves further investigations, since the detection of cancer cells at the early stage is still a challenge to enable efficient treatment [12,13]. Kashefi-Kheyrabadi et al. investigated the detection of liver cancer cells based on the specific interaction of aptamer and cancer cells though electrochemical impedance spectroscopy (EIS) method [14]. Constructing platform for immobilization of aptamers on the electrode surface becomes a major concern. Graphene has attracted widely attraction in constructing electrochemical biosensor due to its unique physical structure and chemical properties (e.g., high surface area, excellent electrocatalysis and excellent electronic conductivity) [15,16]. Graphene-based hybrid film also has shown potential application in the field of electrochemical sensors, enhancing functionality of material and electrochemical properties of graphene [17–19]. Graphene used as matrix for other material opened up a new field of biosensor and provided promising platforms for a variety of biosensing applications.

Here, a new label free aptasensor was reported for detecting cancer cell constructed though layer by layer assembly technique (LBL) with Fc-PAH-G, PSS and aptamer AS1411. In our work, the aptamer AS1411, could form stable G-quardruplex structure while binding cancer cells at certain environment [20]. Ferrocene-appended poly(allylamine hydrochloride) (Fc-PAH), positive charged reversible redox-active probe, was decorated on the surface of graphene through electrostatic interactions. Here, graphene was employed as the matrix for Fc-PAH to bring probes and promote electron transfer on the electrode to enhance the amplified signal. The nanocomposite provided ferrocene as signal indicator, avoiding the influence of the solution phase redox probes to cancer cells immobilized on the electrode surface during the potential scan [21]. Also, the nanocomposite showed high analytical performs attributing to taking advantages of both electroconductibility of graphene and free label of Fc-PAH. PSS, a negative polymer, was employed as the linker of Fc-PAH-G though LBL self-assemble techniques. LBL technology provided more effective probes to enhance amplified signal for improving the sensitivity of the detection. AS1411 was immobilized on the outmost layer of electrode through electrostatic interactions, reducing cost and saving time for developing the aptasensor platform. After the cancer cells were bonded with aptamer, the electron transfer was hindered on the surface of cytosensor because of the insulating property of the cell membranes. Electrochemical measurement was used to detect the current response which was related to the number of cancer cells adsorbed on the electrode surface [22]. The cytosensor exhibited good stability, selectivity and high sensitivity for the detection of Hela cells.

2. Material and method

2.1. Materials

Poly(allylamine hydrochloride) (PAH) and poly(sodium-p-styrene-sulfonate) (PSS) was both obtained from Aldrich. Ferrocene-carboxaldehyde was purchased from J&K Chemical Ltd. Fc-PAH was synthesized according to the previous procedure [23]. Graphite was purchased from Alfa Aesar. Ammonia solution (25–28 wt%) and hydrazine solution (50 wt%) were purchased from Beijing Chemical Reagent factory (Beijing, China). Acridine Orange (AO) was obtained from Dingguo Co., China. 3-Mercaptopropionic (MPA) was bought from Alfa Aesar Company. AS1411 aptamer sequence (5′-TTGGTGGTGGTGGTTGTGGTGGTGGTGG-3′) was synthesized by Shanghai Songong Biotechnology Co., Ltd. (Shanghai,

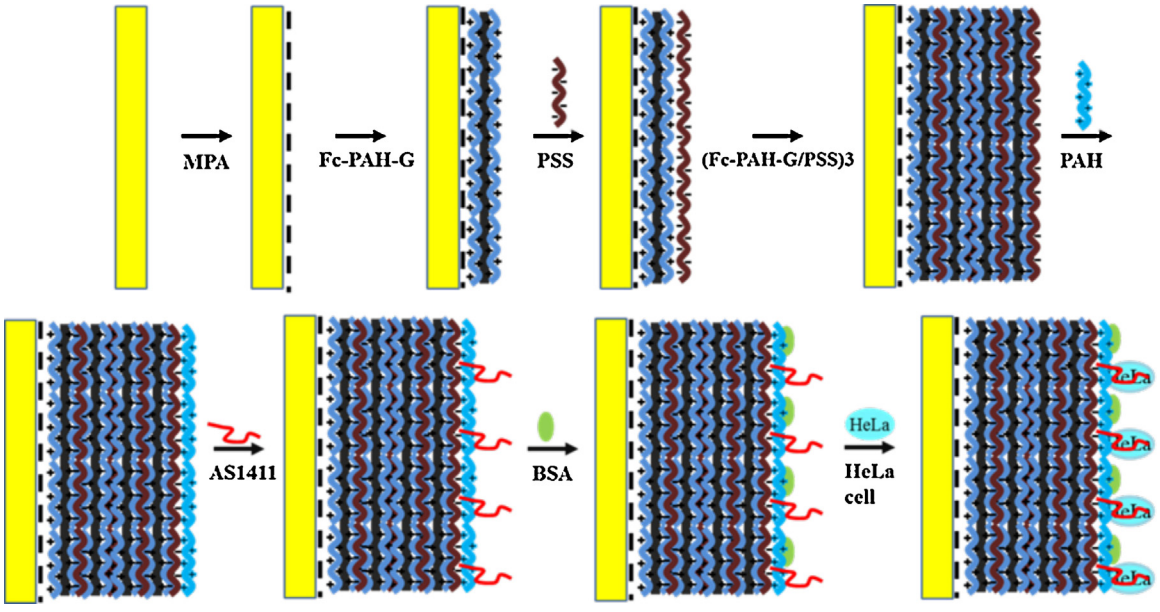

Fig. 1. Schematic diagram of the construction process of the biosensor by LBL technology for detection of HeLa cells.

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