The Combination of Molecularly Imprinted Polymer and Aptamer for Electrochemical Detection of Cancer Antigen 15-3

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***Abstract*—Cancer antigen 15-3 (CA 15-3) is a crucial biomarker for breast cancer monitoring. Tracking of CA 15-3 levels can aid in assessing treatment effectiveness and detecting disease recurrence. Herein, Molecularly Imprinted Polymer (MIP) and aptamer as recognition elements were developed to perform a sandwich assay for the selective electrochemical detection of CA 15-3. The sensitivity of Screen-Printed Carbon Electrode (SPCE) was improved by incorporating Multi-Walled Carbon Nanotubes (MWCNTs). Subsequently, MIP was fabricated layer on MWCNTs/SPCE for the initial selective capture of CA 15-3. The signal probe, a CA 15-3-specific aptamer functionalized with a polydopamine-cadmium complex (PDA-Cd-Apt), was then proposed for secondary selective binding to the captured CA 15-3. Additionally, PDA-Cd-Apt enabled signal generation for electrochemical measurement. Square Wave Voltammetry (SWV) was used to measure electrochemical response of cadmium ions, which was proportional to CA 15-3 concentration. The developed sandwich assay offered a detection range from 5 to 30 U/mL, with a detection limit of 4.44 U/mL. This sensor was suitable for CA 15-3 detection, offering cost-effectiveness, high sensitivity, simple fabrication, and ease of use.**

***Keywords*—cancer antigen 15-3, electrochemical sensor, molecularly imprinted polymer, polydopamine nanoparticles**

I. Introduction

Breast cancer is a condition in which tumors are formed due to the uncontrolled growth of abnormal breast cells. Approximately 2.3 million women around the world were diagnosed with breast cancer, and 670,000 patients died from the disease in 2022. Even in countries with a very high Human Development Index, 1 in 71 individuals is estimated to die from this disease [1]. Cancer antigen 15-3 (CA 15-3) is a carbohydrate-containing protein associated with MUC-1 and is widely used as a biomarker for breast cancer [2]. CA 15-3 serves as a crucial indicator of the severity and recurrence of breast cancer. Furthermore, this biomarker can provide valuable data for patient's recovery status [3]. Consequently, tracking CA 15-3 levels is highly useful for breast cancer monitoring.

Research publications from 2007 to 2023 indicate that Enzyme-Linked Immunosorbent Assay (ELISA) is a popular and effective method for the clinical detection of CA 15-3 [3]. Commercial ELISA kits, based on sandwich immunoassays from several manufacturers, are available for CA 15-3 quantification. However, performing ELISA in hospitals setting is costly and requires handlers with specialized skills [4]. Molecularly Imprinted Polymers (MIPs) are synthetic antibody mimics designed for selective binding to target molecules. In addition to their excellent affinity potential, MIPs offer easier fabrication and cost-effectiveness compared to natural antibodies. These advantages make MIPs a promising alternative to natural antibodies for CA 15-3 detection [5–7].

Aptamers have been widely employed in the field of biosensors due to their excellent properties, including high selective binding, ease of preparation, and cost-effectiveness [8]. Additionally, their simple modification potential allows aptamers to be applied in a variety of applications. Polydopamine (PDA) has garnered significant attention from researchers because of its biocompatibility, conductivity, and ability to polymerize under mild conditions. Since PDA is rich with amine, imine, and catechol groups, these functional groups offer favorable sites for anchoring with metal ions and also covalently modification with desired molecules [9, 10]. These characteristics make PDA-metal complexes suitable as electrochemical signaling molecules, while also allowing for selective binding when conjugated with target-specific aptamers.

This study presents a new, sensitive, and selective electrochemical biosensor for the quantitative determination of CA 15-3, using both MIP and aptamer as recognition elements for CA 15-3. The MIP was electrochemically polymerized on a Screen-Printed Carbon Electrode (SPCE) that was pre-coated with Multi-Walled Carbon Nanotubes (MWCNTs). PDA-metal nanoparticles, conjugated with a CA 15-3-specific aptamer, were introduced to generate an electrochemical signal and enhance selective binding to CA 15-3. In addition to the selective binding achieved by this MIP-aptamer sandwich assay, the sensor offers ease of operation, disposability, and cost-effectiveness for CA 15-3 detection.

II. Methodology

A. Preparation of MIP/MWCNTs Modified SPCE

MWCNTs were modified on SPCE by drop-casting. A 3 μL of aliquot well-dispersed MWCNTs (0.1 mg/mL in 40% ethanol) was dropped onto the SPCE and left to dry at Room Temperature (RT). Thereafter, MIP was electropolymerized on the MWCNTs-modified SPCE to create imprinting sites. The template CA 15-3 (10 U/mL) was mixed with pyrrole (5 mM) prepared in 0.1 M phosphate buffer at pH 7.4 containing 0.1 M KCl. Cyclic Voltammetry (CV) was performed from

−0.2 to 1.1 V at scan rate of 100 mV/s for 10 cycles to polymerize the film on top of MWCNTs-modified SPCE. After electrochemical polymerization, the sensor was placed in 0.1 M NaOH, and an additional 10 cycles were performed to remove CA 15-3, leaving imprinting cavities. The prepared sensor was designed as MIP/MWCNTs/SPCE. For the Non-Imprinted Polymer (NIP) sensor, the same procedure was followed, omitting CA 15-3.

B. Synthesis of Signal Probe

Self-oxidative polymerization was performed to synthesize PDA nanoparticles. Fifty milligrams of dopamine hydrochloride was dissolved in 100 mL of 10 mM Tris-HCl at pH 10.5. The solution was stirred at RT for 20 hours. The synthesized PDA nanoparticles were then collected by centrifugation at 15,000 rpm for 20 minutes, followed by washing twice with Tris-HCl and deionized (DI) water, respectively. The precipitated PDA nanoparticles were redispersed in 10 mL of DI water.

To absorb Cd^{2+} onto PDA nanoparticles, 1 mL of the synthesized PDA nanoparticles was mixed with 5 mL of 0.1 M $Cd(NO_3)_2{\cdot}4H_2O$ and stirred gently at RT overnight. The synthesized PDA-Cd was collected by centrifugation at 15,000 rpm for 5 mins, followed by washing five times with DI water. The resulting PDA-Cd was redispersed in 1 mL of DI water and stored at 4 ℃.

A 500 µL aliquot of the synthesized PDA-Cd was transferred to a new tube for functionalization with a CA 15-3-specific aptamer. The PDA-Cd nanoparticles were removed from DI water by centrifugation and transferred to 10 mM Tris-HCl at pH 10.5. Then, 500 µL of amine-modified CA 15-3-specific aptamer (1 µM) was added and incubated at Room Temperature (RT) with gentle stirring for 1 hour. Unbound aptamer was removed by centrifugation, followed by three washes with Tris-HCl at pH 10.5. The synthesized PDA-Cd-Apt was then redispersed in 1 mL of Tris-HCl at pH 7.4. To block non-specific binding on the PDA surface, the PDA-Cd-Apt was incubated with 0.1 M ethanolamine for 30 minutes. Excess ethanolamine was removed by centrifugation, followed by three washes with Tris-HCl at pH 7.4. Finally, the signal probe, PDA-Cd-Apt, was redispersed in 500 µL of Tris-HCl at pH 7.4 and stored at 4 ℃ until use.

C. Electrochemical Detection of CA 15-3

The fabricated MIP/MWCNTs/SPCE was incubated with 10 µL of CA 15-3 at various concentration for 1 hour. Unbound CA 15-3 was washed away with DI water, followed by a 5-minute wash with phosphate buffer containing 0.05% v/v TWEEN 20. Then, 10 µL of PDA-Cd-Apt was added and incubated for 1 hour. The sensor was washed with DI water to remove excess signal probe molecules. SWV was conducted in 0.1 M acetate buffer at pH 4.5. SWV parameters were set with a potential range from −1.2 to 0.0 V, an amplitude of 0.025 V, a step potential of 0.004 V, and a frequency of 25 Hz. Current responses were recorded after applying a deposition potential of −1.2 V for 120 seconds.

III. Results and Discussion

A. Characterization of PDA, PDA-Cd and PDA-Cd-Apt Nanoparticles

In this study, PDA nanoparticles were formed by self-oxidative polymerization under basis condition. The morphology of the synthesized PDA nanoparticles was investigated using SEM. Fig. 1 shows that the nanoparticles are spherical, uniformly sized, and well-dispersed. The average diameter observed from the SEM image is approximately 94.3 nm.

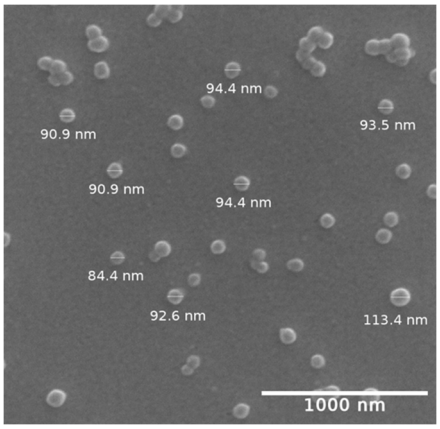

Fig. 1. SEM image of the synthesized PDA nanoparticles, showing details of size measurements.

Due to the abundant of amine, imine and catechol functional groups on PDA nanoparticles, these groups provide active sites for interactions with metal ions, enabling the formation of PDA-metal ion complexes [9–11]. SWV was performed to confirm the absorption of cadmium ions onto PDA nanoparticles. The synthesized PDA and PDA-Cd nanoparticles were mixed with acetate buffer at pH 4.5, and the electrochemical response of each nanoparticle type was compared, as demonstrated in Fig. 2. No oxidative peak was observed for PDA nanoparticles, but a well-defined characteristic peak for cadmium at −0.84 V was recorded from PDA-Cd nanoparticles. The SWV results confirm the successful adsorption of cadmium ions onto PDA nanoparticles.

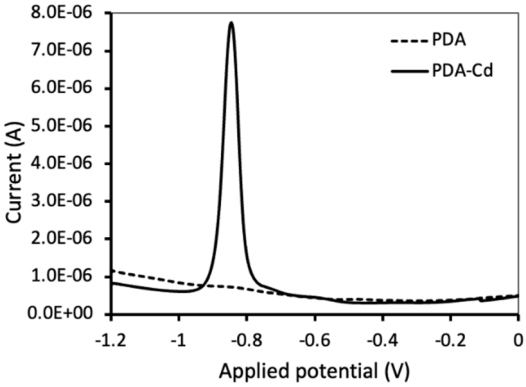

Fig. 2. Square wave voltammograms obtained from bare SPCE in the presence of PDA and PDA-Cd nanoparticles in acetate buffer pH 4.5.

DLS measurements were performed to evaluate the size of PDA, PDA-Cd and PDA-Cd-Apt nanoparticles. The hydrodynamic diameters of PDA, PDA-Cd, and PDA-Cd-Apt nanoparticles were 90.6 ± 2.4, 195.3 ± 12.0, and 211.1 ± 8.5 nm (Fig. 3), respectively, with polydispersity indices of 0.075, 0.379, and 0.430. The DLS-measured size of PDA nanoparticles closely matched the results obtained from the SEM image, further confirming the precise size of the PDA

nanoparticles. The hydrodynamic diameter of PDA-Cd increased, indicating successful cadmium ion adsorption. Given that PDA nanoparticles contain abundant functional groups such as catechol, imine, and amine, covalent immobilization is possible [12]. In this study, the amine group modified at the 5' end of the CA 15-3-specific aptamer was immobilized onto the PDA nanoparticles via Michael addition and Schiff base reactions [13, 14]. The DLS results revealed an increase in diameter for PDA-Cd-Apt compared to PDA-Cd, indicating the successful immobilization of the aptamer on PDA-Cd.

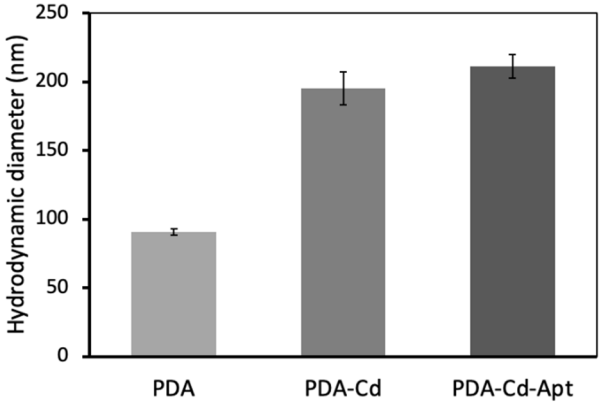

Fig. 3. DLS measurement of PDA, PDA-Cd and PDA-Cd-Apt nanoparticles.

A. Electrochemical Characterization of MIP and MWCNTs modified SPCE

CV was conducted to characterize the modification of MWCNTs and the MIP film on SPCE. Three stages of electrode modification including bare SPCE, MWCNTs/SPCE and MIP/MWCNTs/SPCE were evaluated using 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe. The electrochemical responses are shown in Fig. 4. Well-defined anodic and cathodic peaks of the redox probe were observed for all three electrode types. The highest current response was recorded on MWCNTs/SPCE, due to the excellent conductivity and high surface area of MWCNTs [15]. In contrast, the MIP/MWCNTs/SPCE showed a notable decrease in current response, as the polypyrrole film on the electrode surface inhibited the electron transfer of the redox probe, resulting in lower electrochemical responses. These results suggest successful modification of the SPCE with MWCNTs and the polypyrrole film.

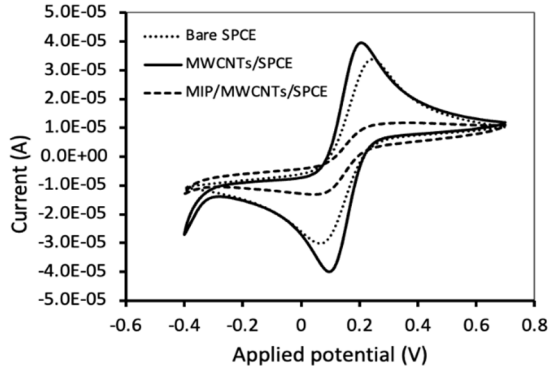

Fig. 4. Comparison of cyclic voltammograms for bare SPCE, MWCNTs/SPCE and MIP/MWCNTs/SPCE in 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.1 M KCl.

B. Preparation of MIP Film for CA 15-3 Detection

To study the binding affinity of the prepared MIP toward CA 15-3, three stages of MIP preparation consisting polymerization, elution and rebinding were characterized using SWV, as demonstrated in Fig. 5. The electrochemical responses were compared with NIP to confirm the successful preparation of CA 15-3 imprinted sites. All SWV measurements were conducted using the ideal redox probe, 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The polymerization step exhibited the lowest current response compared to the elution and rebinding steps. This was due to the blocking effect of the polymer film on the modified electrode. Furthermore, the lower current response of MIP/MWCNTs/SPCE compared to NIP/MWCNTs/SPCE confirmed the successful entrapment of the template molecule, CA 15-3, within the polymeric film [5]. Following electrochemical elution in 0.1 M NaOH for 10 cycles, the current response of MIP/MWCNTs/SPCE increased significantly. The template CA 15-3 was successfully removed, leaving imprinting cavities in the polymer film. These cavities allowed electrons to access the electrode surface. Additionally, only a slight increase in current response was observed on NIP/MWCNTs/SPCE after elution, further confirming the successful removal of the template protein from the polymer layer. The prepared MIP/MWCNTs/SPCE demonstrated detection performance for CA 15-3, as the current response decreased after rebinding with CA 15-3 at 30 U/mL. The binding between the imprinting cavities and the target analyte, CA 15-3, created a physical barrier to electron transfer, resulting in signal reduction. In contrast, barely any change in signal was observed on NIP/MWCNTs/SPCE after rebinding with CA 15-3, as no imprinting sites were present on this sensor. These results verify the performance of the prepared MIP for CA 15-3 determination [6].

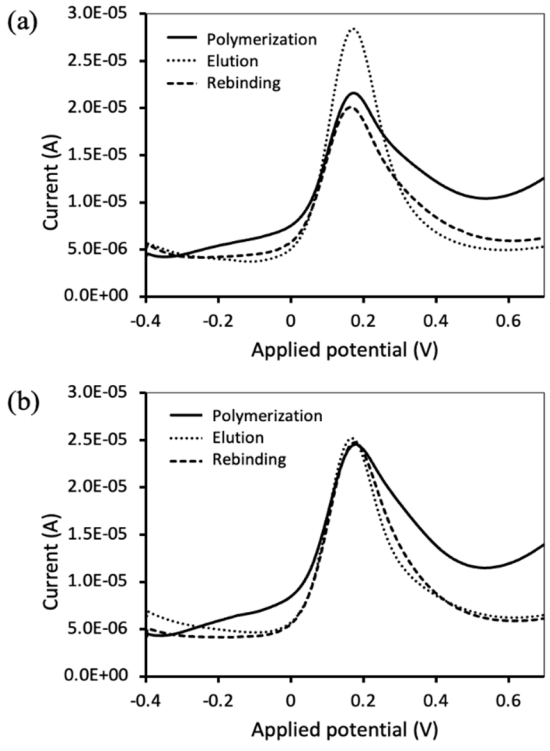

Fig. 5. Square wave voltammograms obtaining from MIP/MWCNTs/SPCE (a) and NIP/MWCNTs/SPCE (b) at different step of MIP preparation.

C. Performance of the Developed Sensor for CA 15-3 Determination

The electrochemical performance of the prepared biosensor was evaluated using SWV. The developed MIP/MWCNTs/SPCE was incubated with CA 15-3 at concentrations of 5, 10, 15, 20, and 30 U/mL. After washing away the unbound molecules, the signal probe, PDA-Cd-Apt, was added. As the signal probe contains CA 15-3-specific aptamer, it can selectively bind to the captured CA 15-3 on the sensor to form a sandwich assay. The square wave voltammogram for each CA 15-3 concentration was recorded in acetate buffer (pH 4.5). The current response of cadmium ions from the signal probe was measured and plotted as proportional to the CA 15-3 concentration, as shown in Fig. 6. The linear detection range was observed for CA 15-3 concentrations from 5 to 30 U/mL, with the linear equation I (μA) = 0.0496 [CA 15-3] (U/mL) + 3.8201, R^2 = 0.9931. The limit of detection (LOD) was calculated to be 4.44 U/mL. This LOD is lower than the clinical cut-off value of 30 U/mL for assessing breast cancer progression and recurrence [16, 17]. Therefore, the proposed sensor is reliable for CA 15-3 determination.

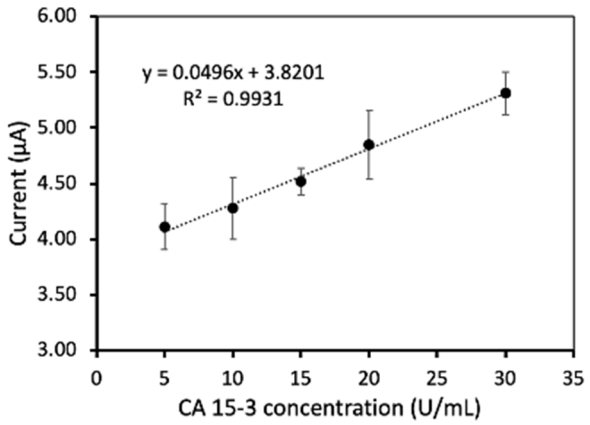

Fig. 6. The plot of current response versus CA 15-3 concentration on MIP/MWCNTs/SPCE.

IV. Conclusion

In the present study, a sandwich assay was proposed for the measurement of the CA 15-3 biomarker. SPCE was modified with MWCNTs to improve sensitivity and further functionalized with MIP for selective binding to CA 15-3. Additionally, the selective binding capability of the aptamer in the signal probe, PDA-Cd-Apt, enhanced the sensor's performance for CA 15-3 detection. Beyond demonstrating good performance for CA 15-3 determination, the sensor fabrication process is easy, and the materials used in this approach are cost-effective. Therefore, the sensor presented here holds potential for further development as a device for breast cancer monitoring.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

C.A.: writing—original draft, methodology, investigation, validation, visualization. P.K.: methodology, supervision. R.P.P.: writing—review & editing, conceptualization, supervision, methodology. All authors had approved the final version.

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