

## Towards HIV Detection: Novel Poly(propylene imine) Dendrimer-Streptavidin Platform for Electrochemical DNA and gp120 Aptamer Biosensors

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The detection of sequence – specific DNA and HIV glycoprotein 120 (gp120) based on novel generation 4 poly(propylene imine) dendrimer/streptavidin platform is reported. The platform was prepared by the electrodeposition of the dendrimer on a carbon electrode followed by drop coating with streptavidin. The generation 4 poly(propylene imine) dendrimer/streptavidin platform was characterised using scanning electron microscopy, voltammetry and electrochemical impedance spectroscopy (EIS). Electrochemical measurements indicated that the platform was stable, conducting and exhibited reversible electrochemistry at a former potential of 140 mV in pH 7.5 phosphate buffer saline solution (PBS), while EIS interrogation using  $[\text{Fe}(\text{CN})_6^{3-/4-}]$  redox probe showed a marked reduction in charge transfer resistance ( $R_{ct}$ ) after modification. The biosensor was prepared by the immobilisation of single stranded 5'- biotinylated probe DNA and B40 aptamer on the modified electrode and then used to detect complementary DNA and gp120 respectively. The biosensor responses were evaluated with square wave voltammetry (SWV) and EIS in the presence of PBS and  $\text{Fe}(\text{CN})_6^{3-/4-}$  redox probe. A detection limit of  $3.44 \times 10^{-12}$  M (S/N = 3) and linearity  $r^2 = 0.9985$  were obtained for the DNA biosensor while the aptamer biosensor detected gp120 in the nanomolar concentration range. The DNA biosensor also showed excellent selectivity in the presence of non complementary and mismatch DNA strands.

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**Keywords:** Biosensor, Aptamer, HIV, gp120, poly (propylene imine) dendrimer, streptavidin

## 1. INTRODUCTION

In recent years, DNA and aptamer biosensors have attracted much attention due to their ability to specifically detect target molecules. Aptamers are synthetic oligonucleotides that bind to a specific target with high affinity and the ability to discriminate between closely related targets. Aptamers have been selected to bind to a wide range of different molecules such as organic dyes, amino acids, biological cofactors, antibodies, peptides, whole cells and proteins and have become important tools for diagnostics and therapeutics [1] which include treatment of cancer [2-3]. In the field of biosensing, aptamers are used as biorecognition element and are considered suitable alternatives to antibody and other bio-mimetic receptors [4].

Biosensors in general, have found wide applications in biomedical, biological warfare [5], environmental, food [6] and forensic fields [7]. There are now the possibilities of early disease detection even on the field using biosensors [8]. The unique affinity, specificity, selectivity, small size and flexibility of aptamers over antibodies highlight its potential for therapeutics and diagnostics [9]. An RNA aptamer, specific for trans-activator of transcription (Tat) of human immunodeficiency virus type 1 (HIV-1) has been reported using piezoelectric quartz crystals and diamond field effect transistor methods [10-11]. These reports suggest aptamer as an interesting approach for the development of biosensors for HIV-1 detection.

In the quest for HIV-1 prevention, a particular aptamer (B40) (our aptamer of interest) has been shown to bind to gp120 of R5 strains of HIV-1. B40 binds to the CCR5-binding site of HIV-1 surface envelope gp120 thereby potentially neutralising the infectivity of R5 strains [12]. A 77-nucleotide truncated form of aptamer B40 (B40t77) has also been shown to have neutralisation ability, which is believed to be due to the combination of steric inhibition of CCR5 binding to gp120 and the distant conformational changes upon binding that prevent the interaction of gp120 with host cells [13]. This neutralisation ability has also been shown recently to exist in a shortened synthetic derivative of the B40 aptamer, called UCLA1 [14]. The B40 aptamer and its derivatives have yielded promising results towards HIV-1 prevention. Although the B40 aptamer has promising HIV-1 prevention potential, to the best of our knowledge, it has not been used in the diagnostic sense i.e. it has not been used in the development of a biosensor. Since the protocols for DNA/RNA biosensor is well developed and can be easily found in literature, it is possible to develop an aptamer biosensor for gp120 and introduce a new paradigm for HIV sensing. Rather than the current indirect approach of testing, a direct approach of detecting the virus will be a plausible route. This direct approach will eliminate the so called window period. The aptamer – gp120 high affinities motivated us to have a diagnostic look at detecting HIV using electrochemical approach. Various techniques including optical, electrochemical and piezoelectric techniques have been used for sequence specific detection of DNA/RNA targets [15]. Electrochemical transduction has the advantage of simplicity and low cost [16] and this hold great promise for the rapid screening of genetic and infectious diseases [17]. Electrochemical DNA/Aptamer biosensors (also called genosensors) are developed by the immobilisation of DNA or aptamer biomolecule probe on a substrate (transducer) either by adsorption, cross linking, encapsulation or entrapment [17]. The choice of materials and chemistries of the immobilisation layer (or platform) is paramount to optimum biosensor performance. In this regard, the use of nanomaterials has gained

much attention in conditioning the electrodes for effective immobilisation and charge transfer as the case may be. Commonly used nanomaterials are gold nanoparticles, carbon nanotubes, quantum dots, metal oxide nanoparticles, metal nanoparticles, magnetic beads, polymer nanotubes or nanostructure, and most recently, dendrimers [18-19].

In this work, a novel platform consisting of dendrimer and streptavidin (a tetrameric protein) was fabricated on carbon electrode to develop a DNA biosensor and an RNA aptamer (B40) biosensor specific for HIV gp 120.

## 2. EXPERIMENTAL

### 2.1 Reagent

The synthetic oligonucleotides with the sequences below were purchased from Inqaba Biotechnical Industries (Pty) Ltd, South Africa.

5' biotin probe: 5'-GGT TGG TGT GGT TGG-3'

Complementary: 5'-CCA ACC ACA CCA ACC-3'

3-base mismatch: 5'-CCGCCAACACTAACG-3'

Non-complementary: 5' TCAATTA CTTCAAGG-3'.

B40 RNA aptamer (134 mer and 35 Kda), and gp120 were supplied by CSIR, Pretoria, South Africa.

Streptavidin and TE buffer were purchased from Sigma- Aldrich and Generation 4 (G4) PPI dendrimer were purchased from SyMO-Chem, Eindhoven, Netherlands. All other chemicals such as potassium chloride (KCl), ferri-ferro cyanide ( $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ), dipotassium hydrogen orthophosphate ( $\text{K}_2\text{HPO}_4$ ) and potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) were obtained from Sigma Aldrich. Ultrapure water with resistivity 18.2 M $\Omega$  was used to prepare buffer and redox active probe solutions. Stock solutions of DNA were prepared in TE buffer while the working solutions were prepared in phosphate buffer pH 7.2.

### 2.2 Instruments

All electrochemical measurements for Voltammetry (CV and SWV) and Electrochemical Impedance Spectroscopy (EIS) were carried out on a Zenium electrochemical workstation (Zahner, Germany). Thales software was used for experimental control and data collection. The traditional three electrode system that consisted of a Ag/AgCl (3 M Cl<sup>-</sup>) electrode as a reference electrode, a platinum wire as auxiliary or counter electrode and a 3 mm diameter glassy carbon electrode (GCE) as a working electrode were employed. Screen printed carbon electrode (Dropsens, Spain) was also used.

Scanning electron microscopy (SEM) images were captured using TESCAN performance in nanospace (VEGA3 TESCAN) Germany.

### 2.3 Platform Preparation

Prior to modification, a glassy carbon electrode (GCE) was carefully polished with 1.0, 0.3 and 0.05  $\mu\text{m}$  aluminium slurry respectively on a polishing pad and then washed ultrasonically in water and ethanol, respectively. The electrode was finally dried in air. After drying, the electrode was modified with 10 mM G4 PPI dendrimer by electrochemically depositing the dendrimer on the electrode surface. This was achieved by running 15 scans of cyclic voltammetry at a potential window of -200 mV to 1100 mV [20] at a scan rate of 50  $\text{mVs}^{-1}$ . This was followed by a gentle rinse in ultrapure water. The G4 PPI electrode was dried in air. The modified electrode was further modified with streptavidin by electrostatic attraction. 50  $\mu\text{L}$  of 100  $\mu\text{g mL}^{-1}$  streptavidin of pH 7.5 was drop coated on the modified electrode for 3 hr. This was also followed by a gentle washing step. The fabrication processes were followed by Voltammetry and Electrochemical Impedance Spectroscopy experiments in phosphate buffer pH 7.2 in the presence and absence of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox active probe.

### 2.4 Biosensing Protocol

#### 2.4.1 Immobilization of probe DNA on the platform

By taking advantage of the affinity between biotin and streptavidin, 50  $\mu\text{L}$  of 2  $\mu\text{M}$  biotinylated probe DNA was immobilized on the glassy carbon modified electrode by drop coating for 1 hr at room temperature. This was then followed by gentle rinsing with ultra pure water and 10 mM phosphate buffer solution of pH 7.2 respectively to remove the unbound DNA probes. Thus, the biosensor (GCE/G4 PPI/Strep/ssDNA) was fabricated and ready for use. For the preparation of the aptamer biosensor, 47.5  $\mu\text{L}$  of 50 nM biotinylated aptamer B40 was heated at 95  $^{\circ}\text{C}$  for 3 min and cooled at room temperature for 10 min after which 2.5  $\mu\text{L}$  of 20x folding buffer was added. It was then cooled in ice for 5 min and drop coated on the GCE/G4 PPI/Strep platform for 30 min at room temperature.

#### 2.4.2 Hybridization with DNA or target detection

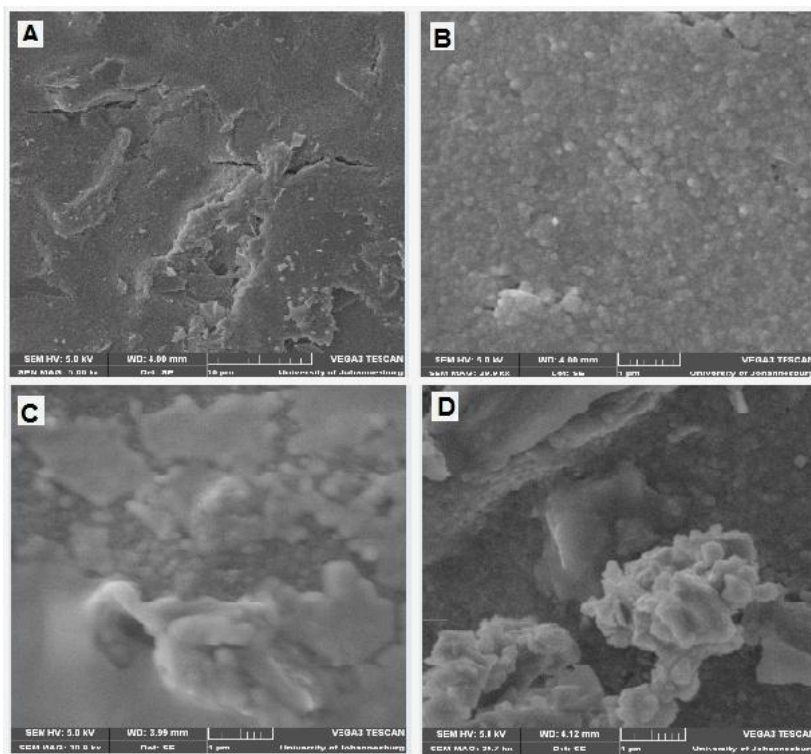
Target detection was performed by incubating Biotin-probe glassy carbon modified electrode with 50  $\mu\text{L}$  different concentrations (ranging from 0.1 to 16 nM) of target oligonucleotide (cDNA) in pH 7.2 10 mM phosphate buffer solution for 1 hr at 38  $^{\circ}\text{C}$ . This was followed by a gentle washing step in water and phosphate buffer solution respectively to remove any unbound molecules and then electrochemically characterized.

### 2.5 Electrochemical detection

Electrochemical Impedance Spectroscopy (EIS) and square wave voltammetric electrochemical measurement were conducted. Impedance measurements were recorded from 100 kHz to 100 mHz at sinusoidal voltage perturbation of 10 mV amplitude and bias potential of 0.25 mV. The fitting parameters were obtained by fitting with a Randle's equivalent circuit. Square wave voltammetric measurements were conducted at a window of -200 mV to 700 mV with amplitude 20 mV and frequency 25 Hz. The electrochemical experiments were carried out in an unstirred solution of 10 mM phosphate buffer solution (PB) of pH 7.2 and in 5 mM (1:1) mixture of  $\text{K}_3\text{Fe}(\text{CN})_6$  and  $\text{K}_4\text{Fe}(\text{CN})_6$  or  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox probe prepared in 10 mM phosphate buffer saline (PBS) solution.

## 3. RESULTS AND DISCUSSION

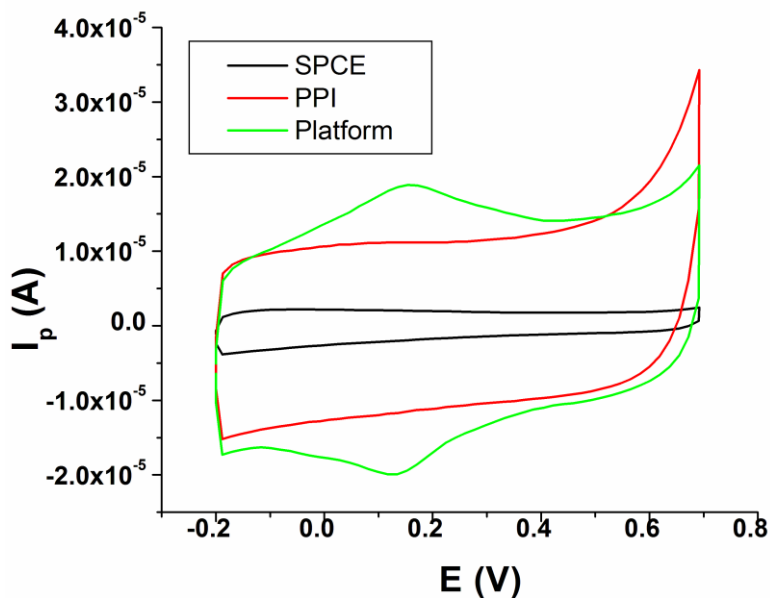
### 3.1 Morphology and Electrochemical Response of Platform



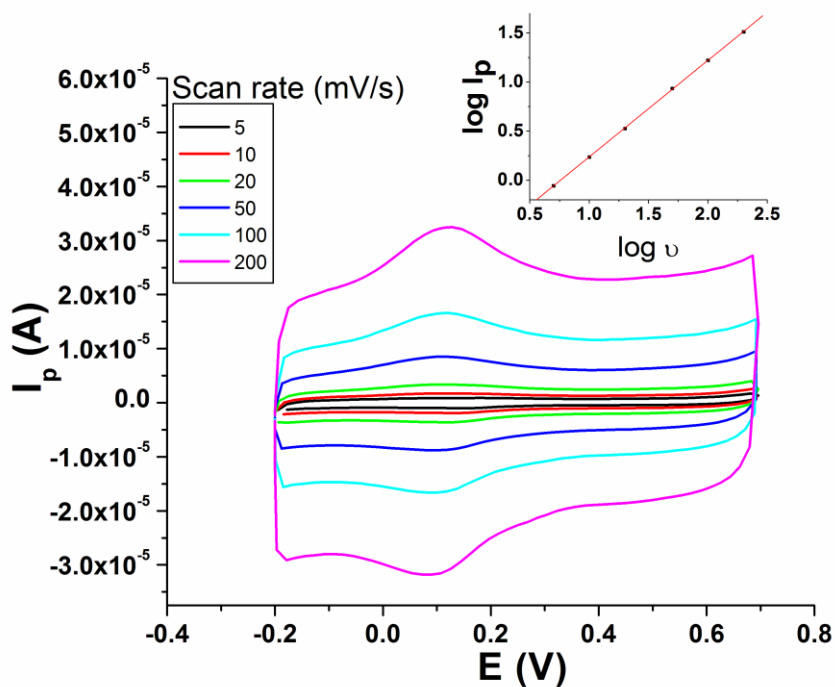
**Figure 1.** SEM (A) bare SPCE, (B) SPCE/PPI, (C) SPCE/PPI-Strep, (D) SPCE/Strep.

The preparation of the platform was microscopically monitored on a screen printed carbon electrode (SPCE) owing to the ease of picture capturing. Electrochemical characterizations were carried out on both SPCE (shown) and glassy carbon (GC) electrodes and similar results were obtained. The SEM image in Fig. 1B shows nano-globular deposits [21] of the dendrimer (as compared to the bare electrode) thus confirming the electrodeposition of poly (propylene-imine) dendrimer (PPI) on the surface of the electrode. The whitish clusters (Fig. 1D) show the immobilization of streptavidin

on bare electrode. Immobilization of the streptavidin on SPCE/PPI was confirmed by the attachment of this cluster to the PPI as shown in Fig. 1C. Streptavidin having an isoelectric point of 5 was made negative by preparing it in PBS of pH 7.5. The isoelectric point is the pH at which the net charge of a protein is zero. Below this point, proteins carry a net positive charge, and above it, a net negative charge.



**Figure 2.** Cyclic voltammogram showing the bare SPCE, G4 PPI modified SPCE and G4 PPI-Strep platform in pH 7.5 PBS at scan rate  $50 \text{ mV s}^{-1}$ .



**Figure 3.** Scan rate study of SPCE/G4 PPI-Strep platform in PBS. Inset: log-log plot of  $I_p$  vs scan rate to test for surface adsorbed specie of the platform.

Electrochemical study confirmed that the PPI modified electrode did not show any noticeable electroactivity (Fig. 2). However, the platform (PPI-Strept modified electrode) was electroactive in 10 mM PBS solution as shown in Fig. 2. From the voltammogram, the following were obtained:  $E^{\circ'} = 140.3$  mV,  $\Delta E_p = 17.6$  mV and  $I_{pa}/I_{pc} \approx 1$ . For a reversible redox system, the  $\Delta E_p$  should be very small (though it also depends on the number of electrons involved) and the ratio of anodic to cathodic current should be unity. Thus the platform depicts a reversible system. To further probe the reversibility of the platform, a scan rate study was carried out (Fig. 3). The log-log plot (Fig. 3 inset) of peak current against scan rate obtained from the scan rate study gave a correlation coefficient,  $r^2 = 0.9999$  and a slope of  $\approx 1$ . These values are characteristic of a surface bound kinetics and reversibility [22]. Thus the PPI-Streptavidin interaction yielded a reversible electroactive conjugate. The conjugate film was stable as inferred from the peak potentials which were stable at different scan rate. The electrochemical reversibility and stability of the prepared platform are good potentials for biosensor developments with biotinylated probes.

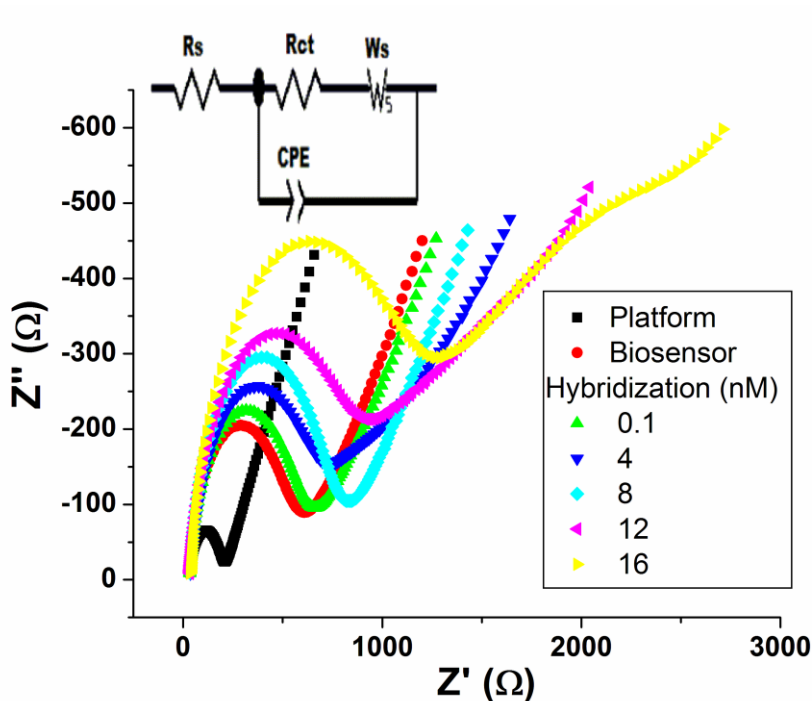
### 3.2 EIS Study of DNA Sensor

EIS technique, a method of measuring the impedance value of the electrode surface [15] was used for probing the electrode interface at each stage of the sensor fabrication. Its small amplitude perturbation signal makes it an excellent tool for obtaining highly resolved kinetic data related to thin films of electroactive surfaces [23]. This technique is used to determine the various properties of the electrode/solution interface including the solution resistance ( $R_s$ ), the charge transfer resistance ( $R_{ct}$ ), the capacity of the electric double layer ( $C_{dl}$ ) and the Warburg impedance ( $W_s$ ). Fig. 4 shows the Nyquist plots obtained from the platform (G4 PPI/Strept) to ssDNA immobilization and the hybridization with target DNA. The Randle's equivalent circuit (inset) was proposed to fit the experimental data obtained from the impedimetric measurement performed and Table 1 shows the experimental data obtained from the fit.

The capacitance of the EIS was fitted using the constant phase element (CPE). The representation of the double layer capacitance with constant phase element is required for better fitting of the experimental data due to electrode surface non-idealities [24].

From Table 1, the  $R_{ct}$  value (which corresponds to the semicircle in the Nyquist plot) increased after immobilization of the probe ssDNA and further increased after each hybridization step. This is believed to be caused by the charge repulsion between the anionic redox probe and the anionic skeleton of the DNA biomolecule, which increases in density after each hybridization step.

A calibration plot of  $\Delta R_{ct}$  vs target DNA concentration was plotted from table 1. Where  $\Delta R_{ct}$  is the relative electron transfer resistance and it is defined as ( $\Delta R_{ct} = R_{ct} \text{ dsDNA} - R_{ct} \text{ ssDNA}$ ). The use of the relative electron transfer resistance in plotting the calibration curve is a form of normalization to cater for the slight changes of the surface properties of the electrode which may occur as a result of polishing and reuse [25]. The resistance to the flow of electrons increases linearly with target DNA concentration with a correlation coefficient/linearity of 0.9979.



**Figure 4.** Nyquist plot showing the immobilization and hybridization process of GC/PPI/Strept modified electrode in  $Fe(CN)_6^{3-/4-}$ .

**Table 1.** Fitting parameters obtained from the Randle’s equivalent fitting

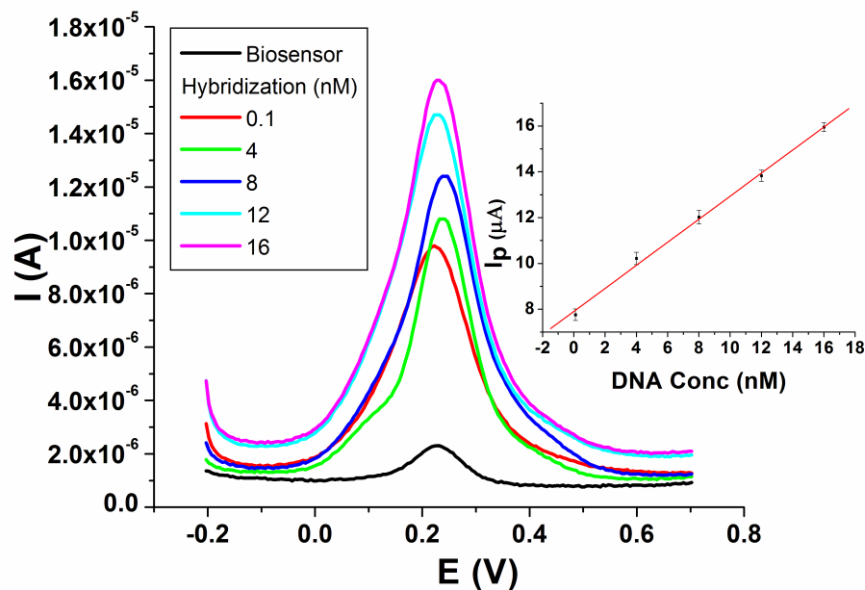
	$R_s$ (Ω)	$R_{ct}$ (Ω)	CPE (nF)	$W_s$ (DW)
Platform	36.46	170.44	295.9	497
Biosensor(ssDNA)	29.5	570.3	352.9	589.7
dsDNA (0.1 nM)	32.95	615.75	353.5	603.5
dsDNA (4 nM)	35.35	700.55	385.7	748.4
dsDNA (8 nM)	34.86	796.04	390.5	752.5
dsDNA (12 nM)	33.53	865.47	397.2	901.1
dsDNA (16 nM)	33.64	938.36	398.5	1142

### 3.3 Voltammetric Study of DNA Sensor

Fig. 5 shows the response of the biosensor to different concentration of target molecule in pH 7.2 phosphate buffer solution. It is interesting to note the increase in current as the concentration of the target DNA occurs. Most genosensors are based on impedance method (using a redox probe) owing to the poor conductivity and electroactivity of DNA. The voltammetric response in buffer observed in this work shows the suitability of the dendrimer-streptavidin conjugate as biosensor platform. In addition to the fact that double stranded DNA are more conducting than ssDNA, the configuration of this electrode must have created a favorable orientation of the probe which further facilitated electron transfer at the biosensor interface. Biosensor response in PBS rather than in ferrocyanide may be better because the former is closer to the physiological environment of the DNA probe. The calibration plot

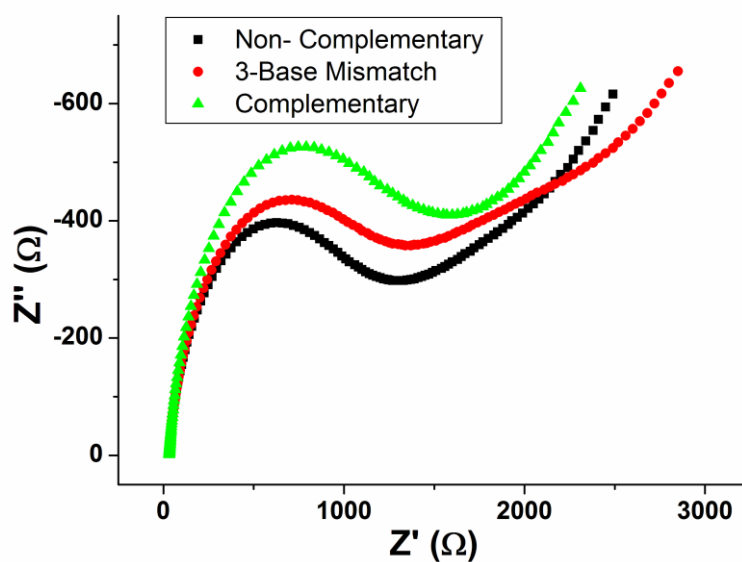


of current signal against ssDNA target concentration (Fig 5 inset) was linear with a correlation coefficient of 0.9985 and a detection limit of  $3.44 \times 10^{-12}$  ( $\frac{3\sigma}{slope}$ ). The ability to detect target in PBS is plausible because PBS provides a more natural environment for the oligonucleotide than ferrocyanide.



**Figure 5.** Square wave voltammetry of the immobilization and hybridization process in GC/G4 PPI/Strept modified electrode biosensor in pH 7.2 PB.

### 3.4 Sensitivity of the Sensor

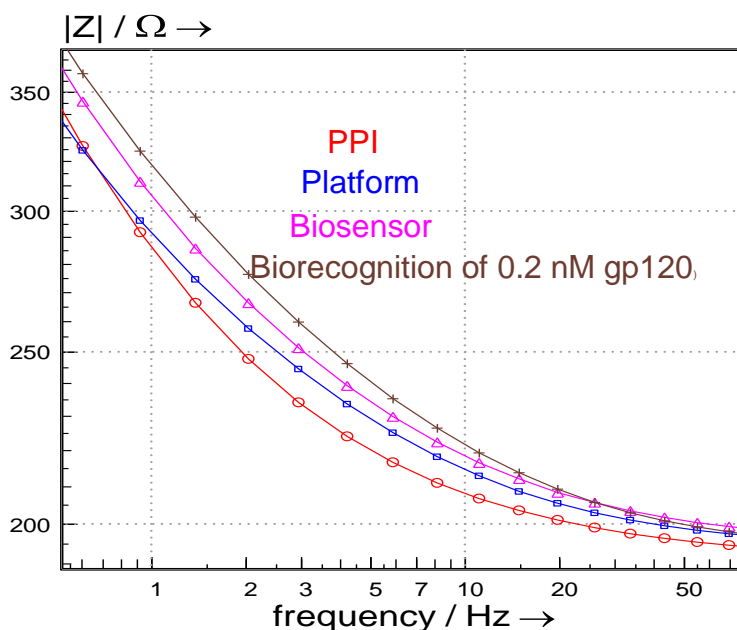


**Figure 6.** Nyquist plots of the impedance spectra of the DNA target hybridization in  $Fe(CN)_6^{3-/4-}$  showing selectivity of the sensor towards its complementary target.

The selectivity of the GC/G4 PPI/Strept/ssDNA biosensor was investigated using the same concentration of complementary target DNA sequence, 3-base mismatch sequence and the non-complementary respectively. Fig. 6 shows the electrochemical impedance spectroscopy result obtained in  $\text{Fe}(\text{CN})_6^{3-/4-}$  redox probe. The result reveals the selectivity of the biosensor for its complementary target sequence. The  $R_{ct}$  for the complementary target sequence is larger than the 3-base mismatch and the non-complementary. The trend in the decrease of  $R_{ct}$  impedance for 3 base mismatch and non complementary ssDNAs shows the extent of hybridization. This trend depicts the selectivity of the biosensor.

### 3.5 B40 aptamer biosensor for gp120 detection on dendrimer/streptavidin platform

A B40 aptamer biosensor was developed on a screen printed carbon electrode (SPCE) for the detection of HIV gp120 using the already proven dendrimer/streptavidin platform.

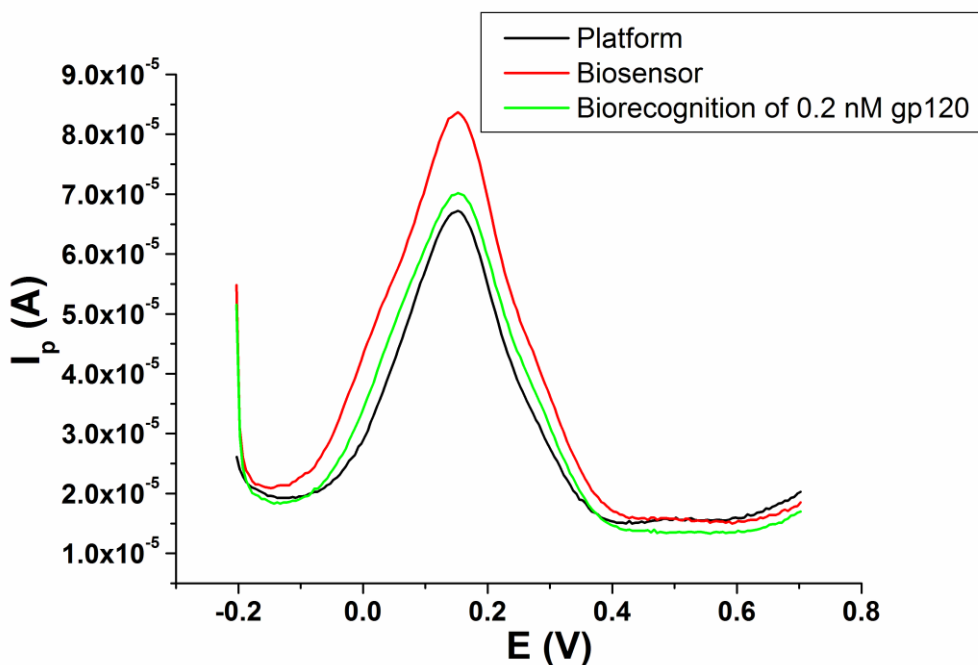


**Figure 7.** Impedance plots of biosensor fabrication and response to gp120 target in  $[\text{Fe}(\text{CN})_6]^{-3/4}$  redox probe.

The impedance changes in Fig. 7 in the presence of the redox probe confirm the biosensor fabrication and the biorecognition processes. The lowest impedance (PPI) can be attributed to the attraction between the cationic dendrimer nanomaterial and the anionic redox probe thereby increasing the rate of diffusion of the redox probe to the electrode surface (electron transfer enhanced). Electrostatic attraction of streptavidin on the PPI surface induced a small increase in impedance which was invoked by the diffusion inhibition of the redox probe to the electrode surface bearing in mind the anionic state of streptavidin at pH 7.5. The biotinylated biomolecule was linked to the G4 PPI-Strep

platform by biotin-streptavidin affinity and this was confirmed by a further increase in impedance due to the repulsion between the anionic backbone of the biomolecule and the anionic redox probe. Biorecognition of the gp120 target by the immobilized gp120 was further confirmed by the rise in impedance owing to the increase in the non-conducting gp120 species at the electrode-electrolyte interface.

Fig. 8 shows that gp120 could be determined using voltammetry. A marked reduction in current was observed after the biorecognition event between the B40 aptamer and gp120. This is expected because gp120 is bulky and not electroactive. A gp120 concentration of 0.2 nM was conveniently detected. There was no significant shift in the anodic peak potentials in the square wave voltammeter. This observation depicts a diffusion controlled system and good platform stability [26]. A blank measurement did not give any noticeable change in the square wave current. We are still in the process of optimizing this HIV biosensor performance. The challenge encountered in the work is the folding and unfolding nature of the B40 RNA aptamer which poses a challenge in calibration. While research is ongoing on this challenge, we propose that a DNA aptamer for gp120 may be a better bioreceptor for HIV biosensor development because of DNA's stability and stable structural configuration.



**Figure 8.** Square wave voltammetric response of the Biosensor in PBS

#### 4. CONCLUSION

This work has exploited the counter charges of dendrimer and streptavidin at the same pH to prepare a novel poly (propylene imine) dendrimer-streptavidin platform for DNA/aptamer immobilization in biosensor design. This platform lends itself to the immobilization of a myriad of

other biotinylated bioreceptors owing to the strong interaction between biotin and streptavidin. The potential of this platform is demonstrated by the ability to detect complementary DNA at a picomolar concentration by both impedimetric and voltammetric transduction.

On the other hand, this work has attempted, through the use of an aptamer, to detect HIV directly through the binding between a B40 aptamer and HIV-1 gp120. This approach has the possibility of paving way to a new paradigm in HIV diagnostics because we have shown that aptamers generated for the purpose of HIV prevention or therapy can be applied *in vitro* as biosensors. In the quest for HIV prevention and therapy, we hope more aptamers (especially DNA aptamers with shorter base lengths) will be selected in the near future so as to widen the scope of HIV biosensing.

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