Nano-Au/cMWCNT Modified *speB* Gene Specific Amperometric Sensor for Rapidly Detecting *Streptococcus pyogenes* causing Rheumatic Heart Disease

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Abstract A specific 5' NH₂ labeled DNA probe of *speB* gene was immobilized onto the gold nanoparticles/carboxylated multi walled carbon nanotubes (Nano-Au/cMWCNT) screen printed electrode using EDC/NHS cross linking chemistry. This was followed by hybridization with 0.5–50 ng/6 µl of single stranded genomic DNA *Streptococcus pyogenes* infected patient throat swab samples. Electrochemical amperometric assay was deciphered by using cyclic voltammetry (CV) with methylene blue a redox indicator. The sensor had a sensitivity of 104.7 µA cm⁻² ng⁻¹ using CV with a R² of 0.907 and 0.01 ng/6 µl as the limit of detection (LOD). The modified electrode surface morphology was characterized using scanning electron microscopy. The stability of the electrode was seen at 4 °C for 180 days having 6% loss in the initial current. The sensor is *speB* gene specific and can detect the pathogen within 30 min.

Keywords Nano-Au/cMWCNT · *speB* sensor · Rheumatic heart disease · *Streptococcus pyogenes*

Introduction

Rheumatic heart disease (RHD) is a condition where human heart valves are damaged as a complication of rheumatic fever and pharyngitis [1]. Rheumatic fever is an inflammatory condition which occurs in children and adolescents. Main causative organism of RHD is a gram positive bacteria *Streptococcus pyogenes*. It causes infections of impetigo, scarlatina, pharyngitis and also leads to necrotizing fasciitis and sepsis diseases [2, 3]. Kalia et al. have several publications on genome based approach to identify specific biomarkers for identification of different strains of *Streptococcus* and many other bacterial pathogens [4–6]. One major protein streptococcal cysteine proteinase involve in the manifestation of infection- *SpeB*, streptococcal erythrogenic toxin B. It can degrades many host matrix and plasma proteins that activates or releases pro inflammatory molecules [7]. Most of the current tests rely on clinical methods, symptoms, history and location (geographical) of the patient which are not useful for therapy and prognosis of the disease. Recent advances in new diagnostic methods, causing improvements in the diagnosis of RHD. Several serological test based methods have emerged, which includes rapid antigen detection system, ELISA, RT-PCR, loop mediated isothermal amplification, luciferase immune-precipitation system and PCR [8–11]. All these assays are less sensitive, expensive, time-consuming and non-confirmatory on single test. However, use of nanoparticles and carbon nanotubes composite based nucleic acid sensors are becoming more popular over traditional diagnostic techniques [1, 12].

Present study reports immobilization of 5' NH₂ labeled probe of *speB* single stranded DNA (ssDNA) at the surface of Nano-Au/cMWCNT screen printed electrode for detection of RHD using ssG-DNA hybridization of patient swab

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Scheme 1 Schematic fabrication of sensor using immobilization of 5' NH₂ labeled ssDNA probe of *speB* gene on Nano-Au/cMWCNT and hybridization with ssG-DNA of *S. pyogenes*

samples. The amine labeled ssDNA probe (5'-GTAG-CAACACATCCTGTAGCTGCA-3') was chemically synthesized for identification of *S. pyogenes*. Screen-printed Nano-Au/cMWCNT electrodes were procured from DropSens, Spain. Electrochemical CV measurements were carried out using μ AutoLab potentiostat, Netherland with NOVA software. The patient samples were collected from Safdarjung Hospital, New Delhi. The swab was mixed with 100 μ L TE buffer (10 mM Tris and 1 mM EDTA), pH 8.0 and heated for 6 min at 94 °C for cell lysis. It was further centrifuged for 3 min at 5000 $\times$ g. The supernatant containing DNA was collected and quantified using Nanodrop spectrophotometer. The quantified DNA solution (dsDNA) was further heated at 94 °C for 5 min to make ssDNA and used directly for the hybridization with immobilized *speB* probe. Three electrode system [working (nano-Au/cMWCNT), counter (carbon) and reference (silver)] based screen printed electrode was used for the fabrication of *speB* gene based sensor. The electrode surface was washed thoroughly with autoclaved milli-Q water and dried properly. Nano-Au/cMWCNT was further treated with 6 μ L equimolar 1:1 ratio (v/v) of 10 mM EDC and 10 mM NHS (in milli-Q water) for 1 h to activate the carboxyl groups of MWCNT at the electrode surface for covalent binding with amino groups of the probe [1]. Besides, some amino groups of the probe also bind with Au nanoparticles which

increase the sensitivity of the sensor. The electrode surface was washed thoroughly with milli-Q water to remove unbound reagents and allowed to dry. The 6 μ L amine labeled DNA probe (10 μ M in TE buffer, pH 8) was immobilized onto screen printed working electrode (0.12 cm²) surface and kept for 2 h at 25 °C [1, 12]. The unattached ssDNA probe was removed by thoroughly washings with milli-Q water followed by TE buffer, pH 8.0 and dried as usual. The denatured genomic DNA (ssG-DNA) of different concentrations (0.5–50 ng/6 μ L in TE buffer (pH 8.0) was hybridized for 5 min with probe to make double stranded DNA (dsDNA) onto the electrode surface. After completion of hybridization, the electrode surface was washed thoroughly with milli-Q water and then 2–3 times with PBS buffer (sodium phosphate 50 mM containing 0.9% NaCl), pH 7.0 to remove the unbound ssG-DNA. Methylene blue (1.0 mM MB solution in PBS, pH 7.0) 50 μ L was used for oxidation–reduction reaction in CV measurement. The surface morphology of ssDNA/nano-Au/MWCNT and dsDNA/nano-Au/MWCNT electrodes was studied using SEM.

The fabrication of the *speB* sensor is shown in Scheme 1. The CV peak current (I_p) of immobilized ssDNA probe of *speB* gene was showing higher value than that of simple bare electrode (not shown in figure). It may be due to increase in binding of MB to *speB* ssDNA probe.

Fig. 1 CV of **a** ssDNA(probe)/nano-Au/MWCNT and **b–i** hybridization with 0.5, 1.0, 1.5, 3.0, 6.0, 12.5, 25 and 50 ng/6 μl of *S. pyogenes* ssG-DNA at 50 mVs^{-1} using 1 mM MB in 50 mM PBS, pH 7

Fig. 2 SEM micrograph of **a** nano-Au/cMWCNT **b** ssDNA/nano-Au/MWCNT and **c** dsDNA/nano-Au/MWCNT after hybridization with 50 ng/6 μl ssG-DNA of *S. pyogenes*

I_p of ssDNA (probe) was $38 \mu\text{A}$ (curve a) and after hybridization with genomic DNA oxidation peak current increases from 47–65 μA (curve b–i) for 0.5–50 ng ssG-DNA of *S. pyogenes*, respectively (Fig. 1). The peak current of oxidation reaction was found increasing along with increasing ssG-DNA concentrations due to the availability of extra guanine bases of unhybridized ssG-DNA that interacts with MB causing an increase in current. The ssDNA/nano-Au/MWCNT electrode contained limited unhybridized nitrogenous bases which interacted with MB

whereas, G-DNA made up of prolonged unhybridized single DNA sequence of unlimited nitrogenous bases causing an increase interaction with MB. Therefore, I_p increased in case of dsDNA/nano-Au/MWCNT composite electrode as compared to ssDNA/nano-Au/MWCNT electrode. The plot between the different concentrations of ssG-DNA and the relative I_p values with respect to the ssDNA probe was found hyperbolic (Fig. 1a). The sensitivity (S) of the *speB* sensor has been calculated by formula $S = m/A$, where ‘ m ’ represents the slope of the linear equation and

'A' is the surface area of the working nano-Au/cMWCNT electrode. The sensitivity of the sensor was found $104.7 \mu\text{A cm}^{-2} \text{ ng}^{-1}$. The LOD was determined using the formula $\text{LOD} = 3 (\sigma/S)$, where S is the sensitivity and σ is the standard deviation. The LOD was found $0.01 \text{ ng}/6 \mu\text{l}$ with R^2 (regression coefficient) of 0.907 (Fig. 1b).

SEM images of different surfaces modifications of nano-Au/cMWCNT electrodes are shown in Fig. 2. The nano-Au/cMWCNT electrode exhibits tubular crossed net like uniform, fibrous structure of cMWCNT and small rounded bubbles like structure shows the presence of gold nanoparticles as shown in Fig. 2a. Figure 2b shows the changed image of ssDNA/nano-Au/MWCNT with globular and tubular structure of DNA as compared to results in Fig. 2a. The globular and tubular morphology are appearing less dense due to small size of probe whereas, in Fig. 2c dsDNA/nano-Au/MWCNT shows the dense surface morphology (due to larger size of G-DNA) with globular structure confirming the hybridization of probe with ssG-DNA [13]. The present sensor showed high sensitivity, specificity (gene specific) and takes less time for detection of bacterial pathogen. The modified nanohybrid electrode was seen on storage at 4°C for 180 days having only 6% loss in original peak current (data not shown).

The peak current of the nanohybrid *speB* sensor with other pathogens was found almost the same as reported with the immobilized ssDNA probe of *speB* gene (no deviation in current) however, it was increased with *S. pyogenes*. Therefore, the specificity of the *speB* sensor was only with *S. pyogenes* (Supplementary material Fig. S1).

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Compliances with Ethical Standards

Conflict of interest Authors declare no conflict of interest.

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