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Gold–mercaptopropionic acid–polyethylenimine composite based DNA sensor for early detection of rheumatic heart disease†

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The first gold–mercaptopropionic acid–polyethylenimine composite based electrochemical DNA biosensor was fabricated for the early detection of *Streptococcus pyogenes* infection in humans causing rheumatic heart disease (heart valve damage). No biosensor is available for the detection of rheumatic heart disease (RHD). Therefore, the *mga* gene based sensor was developed by the covalent immobilization of a 5′-carboxyl modified single stranded DNA probe onto the gold composite electrode. The immobilized probe was hybridized with the genomic DNA (G-DNA) of *S. pyogenes* from throat swabs and the electrochemical response was measured by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance (EI). Covalent immobilization of the probe onto the gold composite and its hybridization with G-DNA was characterized by FTIR and SEM. The sensitivity of the sensor was 110.25 μA cm^{-2} ng^{-1} with DPV and the lower limit of detection was 10 pg per 6 μL. The sensor was validated with patient throat swab samples and results were compared with available methods. The sensor is highly specific to *S. pyogenes* and can prevent damage to heart valves by the early detection of the infection in only 30 min.

Introduction

Group A streptococci *Streptococcus pyogenes* infection in throat causes initially pharyngitis and if it is not treated in time, it may damage the mitral and aortic heart valves and finally lead to rheumatic heart disease (RHD) causing the death of the patient.[1–3] RHD is a global burden and it is more common in developing countries due to poor health care facilities.[4–6] The usual diagnostic methods are culture tests,[7] biochemical assays,[7,8] impedimetric sensors (antibodies against the bacteria),[9] PCR and genetic markers,[10–12] illumigene test kits[13] and fluorescent *in situ* hybridization (FISH).[14] All these methods are time consuming, less sensitive and specific, expensive and non-confirmatory on a single test. Even PCR and genetic marker based methods take more time for confirmation of the disease. Therefore, a DNA biosensor will be a better option for the early detection of rheumatic heart disease.[15] Presently, no biosensor is available for the early detection of RHD. Different types of biomaterials have been used for the development of electrochemical biosensors.[16,17] In the present investigation, a gold–polyethylenimine composite based DNA biosensor has been developed for early detection of the *S. pyogenes* infection from patients' throat swab samples.

Gold electrodes are very useful for the construction of electrochemical DNA sensors and nanosensors because of their chemical inertness and capability of working in a wide range of temperatures, pH and potential differences.[18–21] A thiol (SH) modified single stranded DNA (ssDNA) probe can be immobilized on a gold (Au) surface by the attachment of the SH group onto Au.[22] However, organic molecules having thiol groups (mercaptopropionic acid) can also be covalently attached to the gold surface and form S–Au bond.[14] Stability of the bond is very high, therefore, it can be used for designing electrochemical sensors.[23,24] The exposed carboxyl group (COOH) of mercaptopropionic acid (MPA) on the surface can be used to attach branched polyethylenimine (PEI) through covalent bond (amide bond) formation between the COOH group of mercaptopropionic acid and the amine (NH_2) group of polyethylenimine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide–*N*-hydroxysuccinimide (EDC–NHS) as the condensing reagent.[25,26] This reaction has a high conversion frequency, mild reaction conditions and has little effect on the bioactivity of the target molecules. Branched polyethylenimine has NH_2 groups in abundance. Hence, even after the covalent bond formation of the branched PEI with MPA, a number of the free NH_2 groups on the surface can be used for the covalent immobilization of carboxyl functionalized biomolecules (probe) for the development of the biosensor.

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The *mga* gene is the key regulator of virulence in Group A *Streptococcus* (GAS).[27] It is a large DNA binding protein with 500 amino acids and a molecular weight of 62 kD. It is a multiple gene regulator which activates the transcription of several genes like the antiphagocytic M protein (*emm*), C5a peptidase (*scpA*), M-like proteins and the serum opacity factor (*sof*). Although *mga* has various orthologs, it is conserved in GAS and hence can be used for the detection of the *S. pyogenes* infection.

Experimental

Materials

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), methylene blue (MB), mercaptopropionic acid (MPA) and polyethylenimine (PEI) were purchased from Sigma Aldrich, USA. Tris(hydroxymethyl) aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), ethanol, sodium chloride, potassium ferricyanide, sodium dihydrogen *ortho*-phosphate, di-sodium hydrogen *ortho*phosphate and other chemicals were obtained from Qualigens, India. Brain heart infusion broth was procured from Himedia, India. Genomic DNA isolation kit was obtained from Banglore Genei, India. 5′-Carboxyl modified ssDNA probe (5′-GCA-CAGCCAATTTCTAGCTTGTCG-3′) of *mga* gene was procured from Bio India Life Sciences, India. Screen printed gold electrode from Dropsens was modified at IGIB, Delhi, India.

DNA isolation from culture and throat swab

The genomic DNA was isolated from 18 h cultured *S. pyogenes* (strain M140, IMTECH Chandigarh) in brain heart infusion broth at the National Centre for Disease Control, Delhi using the phenol chloroform method (Banglore GeNei Kit).[11] The purity ($A_{260/280}$) and quantity (A_{260}) was measured using a nanodrop spectrophotometer.

The G-DNA was also isolated from throat swabs using 100 μL STE (50 mM Tris, 50 mM EDTA, 20% sucrose) buffer, pH 8 and heated at 95 °C for 5 min for bacterial cell lysis. After cell lysis, it was centrifuged at 5000 × *g* for 5 min and the supernatant was taken for the quantification of DNA by nanodrop. The DNA solution (dsDNA) after denaturation at 95 °C for 5 min (ssDNA) was directly used for hybridization with the immobilized probe.

Fabrication of the Au–MPA–PEI composite

A three electrode system based screen printed electrode with gold as the working and counter electrodes and silver as the reference electrode was modified for the fabrication of the gold composite based DNA sensor. The working Au surface was activated with 1 : 1 (v/v) mixture of 6 μL H_2SO_4 : H_2O_2 for 5 min. It was then washed 3–4 times with autoclaved distilled water and dehydrated with ethanol. MPA 6 μL was placed onto the surface of the working electrode for 24 h to form a monolayer and subsequently washed 3–4 times with autoclaved distilled water to remove unbound MPA and finally dried at 25 °C. The carboxylated electrode was treated with a mixture of 10 mM EDC and 10 mM NHS (1 : 1, v/v) in 50 mM PBS (phosphate buffer, pH 7 containing 0.9% sodium chloride) for 1 h to activate the COOH groups on the surface[26] and followed by reaction with PEI (6 μL) for 1 h. After the reaction, the electrode was washed 3–4 times with PBS, pH 7, to remove the excess of reagents and dried at 25 °C.

Immobilization of ssDNA probe

The COOH group on the ssDNA probe was activated by mixing 3 μL of 10 μM 5′-COOH modified ssDNA probe with 3 μL of equimolar mixture of EDC and NHS (10 mM of each) to make the final probe concentration 5 μM. Then, the reaction mixture was placed on the working electrode at 0.126 cm^2 surface area for 6 h to allow the formation of the amide bond between the COOH group of the probe and NH_2 group of the PEI to make Au–MPA–PEI–ssDNA electrode. The unbound probe was removed from the Au–MPA–PEI–ssDNA electrode by washing 3–4 times with PBS (50 mM sodium phosphate buffer, 0.9% sodium chloride), pH 7, and dried at 25 °C before electrochemical detection.

Hybridization with ssG-DNA

The G-DNA of *S. pyogenes* was denatured by heating at 95 °C for 5 min. The Au–MPA–PEI–ssDNA probe was hybridized with 0.5–25 ng per 6 μL ssG-DNA in TE (10 mM Tris, 1 mM EDTA) buffer, pH 8, for 10 min at 0.126 cm^2 surface area of the working electrode. After hybridization, the Au–MPA–PEI–dsDNA electrode surface was washed 3–4 times with TE buffer, pH 8 followed by PBS pH 7 and dried at 25 °C before electrochemical measurements. The fabricated sensor was used for the early detection of rheumatic heart disease electrochemically using methylene blue[28] as the redox indicator for CV and DPV, whereas, EI was measured using $K_3[Fe(CN)_6]$ in a potentiostat/galvanostat (FRA2 μAutolab type iii, Metrohm, India).

FTIR and SEM analysis

The Au–MPA–PEI, Au–MPA–PEI–ssDNA and Au–MPA–PEI–dsDNA electrodes were characterized using Fourier transform infrared spectroscopy (FTIR) at frequency 400–4000 cm^{-1}. (BX-59333, Perkin Elmer, USA). The FTIR spectrum of bare gold was taken as background and subtracted from the FTIR spectra of Au–MPA–PEI, immobilized probe Au–MPA–PEI–ssDNA and after hybridization Au–MPA–PEI–dsDNA electrode. Further, the spectra were carried out for baseline correction, normalization and the resulting spectra peaks at specific wavenumber (cm^{-1}) were analysed in % transmittance.

Scanning electron microscopy (SEM) was carried out for studying morphological changes on the surface of the electrode at different stages of immobilization on the working gold electrode to form composite electrodes with multiple probes attached through PEI and hybridized with a complementary strand of single stranded genomic DNA. The screen printed electrodes with different modifications were coated with gold (6 nm thickness) using sputter coater under vacuum. The gold coated electrodes were scanned under scanning electron microscopy (Carl Zeiss, Germany, Model No. EVO15) and different images of different modifications were characterized.

Results and discussion

The schematic fabrication of the Au–MPA–PEI composite electrode, immobilization of the ssDNA probe and hybridization with ssG-DNA of *S. pyogenes* to form Au–MPA–PEI–dsDNA and its electrochemical detection is shown in Scheme 1.

CV studies

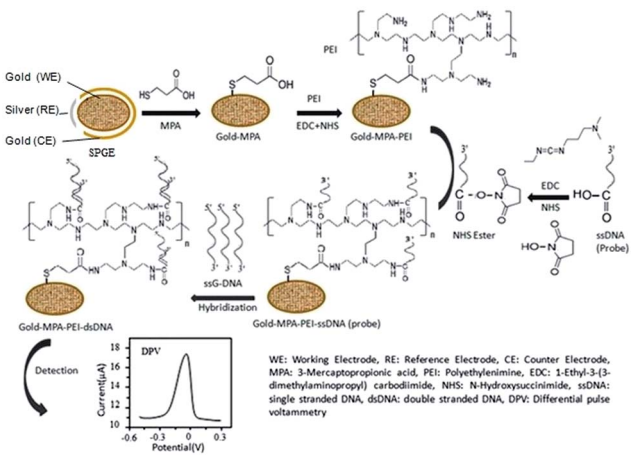
The cyclic voltammetry (CV) peak current (I_p) of Au–MPA–PEI–ssDNA (11.54 μA) (Fig. 1) was higher than that of bare gold (not shown in the figure). It may be due to the increased binding of MB molecules to the ssDNA probe and hence higher conductivity. CV of Au–MPA–PEI–ssDNA and Au–MPA–PEI–dsDNA is shown in Fig. 1. The I_p of Au–MPA–PEI–dsDNA was higher than that of Au–MPA–PEI–ssDNA and it increased with the increase in concentrations of hybridization of ssG-DNA of *S. pyogenes*. This may be due to the increased binding of MB on dsDNA as compared to ssDNA which leads to the oxidation of more MB molecules and hence leading to an increased current.[28] The plot between the concentrations of ssDNA used for hybridization and the relative I_p values with respect to the probe (zero) was hyperbolic (Fig. 1 inset).

It was linear for 0–1 ng per 6 μL ssG-DNA following the linear equation [I_p (μA) = 2.986 (μA ng^{-1}) × ssG-DNA (ng) + 0] and regression coefficient (R^2) 0.9109. The sensitivity (S) of the DNA sensor was calculated using the formula $S = m/A$ where, m is the slope of the linear equation and A is the area of the working gold composite electrode and it was found out to be 23.698 μA cm^{-2} ng^{-1}. The limit of detection (LOD) was calculated using the formula LOD = 3 (σ/S) where, σ is the standard deviation and S is the sensitivity.[29] It was calculated as 0.05 ng per 6 μL (Fig. S1†).

DPV studies

The I_p value of the differential pulse voltammetry (DPV) of bare gold was similar as in the CV analysis. The I_p of Au–MPA–PEI–dsDNA was higher than that of Au–MPA–PEI–ssDNA and it increased with the increase in concentration of hybridization of *S. pyogenes* ssG-DNA (Fig. 2) in a similar pattern as in CV.

The inset plot between the concentrations of ssDNA used for hybridization and the relative I_p values with respect to the probe



Scheme 1 Schematic diagram for the immobilization of the carboxylated *mga* probe on the Au–MPA–PEI composite and its hybridization with ssG-DNA of *S. pyogenes*.

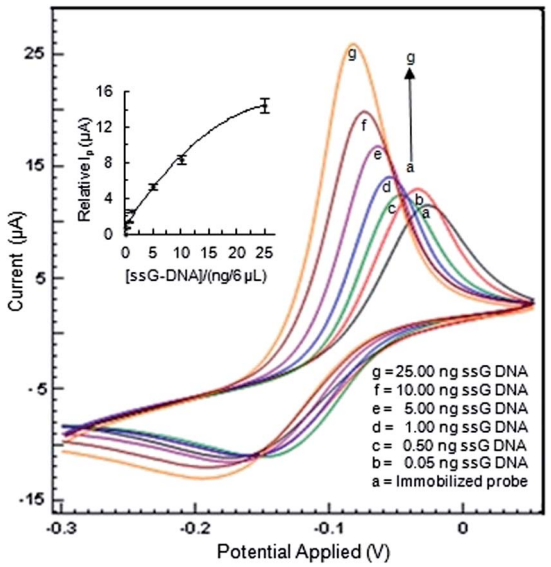


Fig. 1 Electrochemical response study using 1 mM MB in 50 mM PBS, pH 7. CV of Au–MPA–PEI–ssDNA (a) and (b–g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng per 6 μL ssG-DNA of *S. pyogenes* at 50 mV s^{-1}. The inset shows a hyperbolic curve between relative I_p (with respect to probe) and increasing concentrations of hybridizing ssG-DNA of *S. pyogenes*.

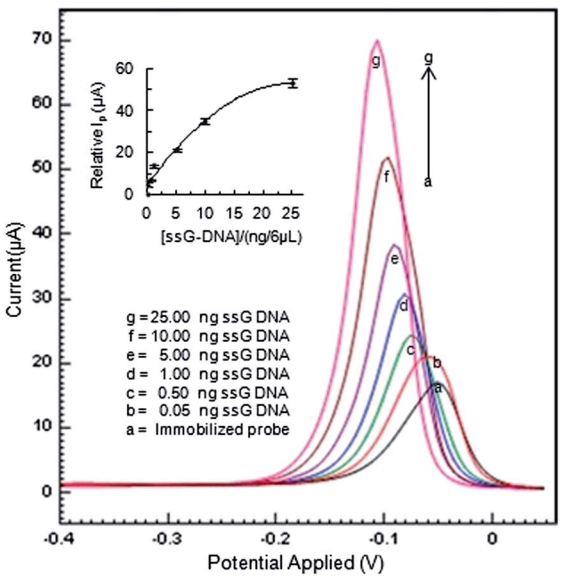


Fig. 2 DPV of (a) Au–MPA–PEI–ssDNA and (b–g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng per 6 μL ssG-DNA of *S. pyogenes* at an amplitude potential of 25 mV using 1 mM MB in 50 mM PBS, pH 7. The inset shows a hyperbolic curve between relative I_p (with respect to probe) and increasing concentrations of hybridizing ssG-DNA of *S. pyogenes*.

(zero) also shows linearity from 0–1 ng per 6 μL ssG-DNA following the linear equation [I_p (μA) = 13.892 (μA ng^{-1}) × ssG-DNA (ng) + 0] and regression coefficient (R^2) 0.8905. The sensitivity of the sensor was 110.25 μA cm^{-2} ng^{-1} and LOD was calculated as 0.01 ng per 6 μL due to a more sensitive DPV as compared to CV analysis (Fig. S2†).[29,30] Therefore, DPV can be used for the better detection of *S. pyogenes* from throat swab patient samples in 30 min with higher sensitivity and specificity than earlier reported methods.

EI studies

The Nyquist plot for the electrochemical impedance (EI) studies of the gold composite biomaterial, Au–MPA–PEI–ssDNA and Au–MPA–PEI–dsDNA is shown in Fig. 3.

The diameter of the semicircle was equal to the charge transfer resistance (R_{ct}) at the electrode interface and the semicircle at the higher frequencies corresponds to the electron-transfer-limited process.[31,32] The Nyquist complex impedance obtained, best fit to Randles equivalent circuit of electrochemical interface where, R_s is the resistance of the electrolyte solution, C_{dl} is the double layer capacitance, R_{ct} is the charge transfer resistance and W_d is the Warburg diffusion element. C_{dl} is in series with R_s and parallel to R_{ct}.[33]

R_{ct} of bare gold was higher than that of the Au–MPA–PEI composite (not shown in the Fig. 3). This may be due to the presence of PEI in the composite which increased the conductivity and hence decreased the impedance. The R_{ct} value of Au–MPA–PEI–ssDNA was higher (13.56 Ω, curve a) than the Au–MPA–PEI composite electrode (not shown in the figure). This may be attributed to the negatively charged phosphate backbone of ssDNA which prevented the $[Fe(CN)_6]^{3-/4-}$ ions from reaching the electrode surface. The R_{ct} value of Au–MPA–PEI–dsDNA was higher than that of Au–MPA–PEI–ssDNA and it increased with increases in concentration of hybridization of ssG-DNA of *S. pyogenes*. This may be due to increases in the number of negatively charged phosphate groups on the electrode surface after hybridization which increases the surface thickness which in turn increase the R_{ct} value.[33] Electrochemical impedance also supports the CV and DPV of the sensor after hybridization with different concentrations of ssDNA with the probe for confirmation of the disease.

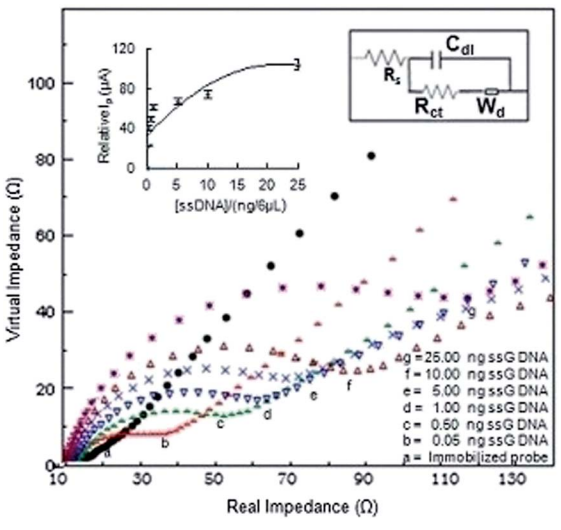


Fig. 3 EI spectra of (a) Au–MPA–PEI–ssDNA and (b–g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng per 6 μL of *S. pyogenes* ssG-DNA using the redox indicator 5 mM $K_3[Fe(CN)_6]$ in 50 mM PBS, pH 7. The inset shows a hyperbolic curve between R_{ct} (with respect to probe) and increasing concentrations of hybridizing ssG-DNA *S. pyogenes*.

FTIR spectra

The Fourier transform infrared (FTIR) spectra were taken for confirmation of composite gold electrode formation, immobilization of the probe and hybridization with the complementary ssG-DNA of *S. pyogenes* from throat swab samples (Fig. 4).

The FTIR spectra in Fig. 4a shows the transmittance of the bare gold electrode.[34,35] Since the surface is completely made of bare gold, the transmittance is not specific to the functional groups. Fig. 4b shows the FTIR transmittance of Au–MPA–PEI electrode. The peaks visible at 546 and 662 cm^{-1} are specific for the gold–sulphur bond formed due to the attachment of mercaptopropionic acid on the gold surface.[36] The peak at 732 cm^{-1} is for the NH stretching and peaks at 3480, 3740 and 3872 cm^{-1} are for the C–H stretching. The peak at 1410 cm^{-1} is for the C–O–H and the peak at 1558 cm^{-1} is for the amide bond (CO–NH).[37] This peak confirms that PEI has attached to the MPA *via* the amide bond formation. The FTIR spectrum of Au–MPA–PEI–ssDNA shown in Fig. 4c exhibits peaks at 1044, 1182, 638 and 734 cm^{-1} corresponding to in-plane vibration of cytosine, C_7=N vibration of adenine, C_2=O stretch of thymine and C=O stretch of guanine, respectively, confirming the presence of four nucleotides of DNA.[29,30,38] The peaks of Au–MPA–PEI–dsDNA shown in Fig. 4d are similar to Au–MPA–PEI–ssDNA shown in Fig. 4c but with lower transmittance. It confirms the hybridization of ssG-DNA to the probe. The peaks at 1238 and 2983 cm^{-1} corresponding to the PO_2^- and C–H stretch, respectively

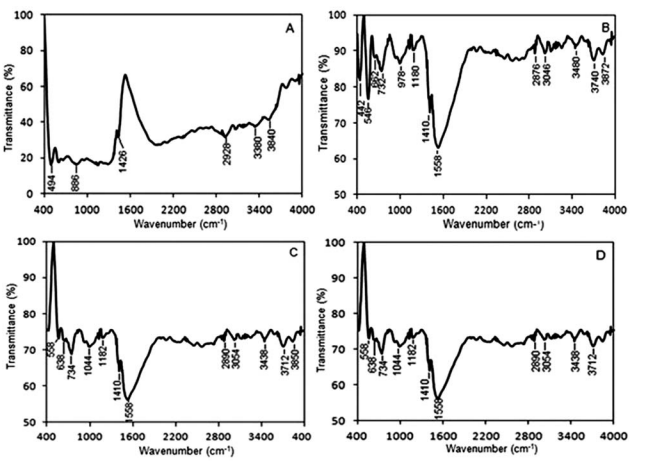


Fig. 4 FTIR transmission spectra of (A) bare Au, (B) Au–MPA–PEI, (C) Au–MPA–PEI–ssDNA and (D) Au–MPA–PEI–dsDNA at frequency 400–4000 cm^{-1}.

of deoxyribose backbone, increased in amplitude and became more prominent in case of Au–MPA–PEI–dsDNA.[29]

SEM analysis

The scanning electron microscopy (SEM) images of different stages of fabrication of the gold composite electrode are shown in Fig. 5.

The SEM image of the gold electrode with mercaptopropionic acid (MPA) in Fig. 5a exhibits a smooth and uniform structure.[39] Fig. 5b shows the image of Au–MPA–PEI and is different from the previous one showing changes in the surface morphology.[40] Fig. 5c shows Au–MPA–PEI–ssDNA and Fig. 5d shows Au–MPA–PEI–dsDNA.[41] The Au–MPA–PEI–dsDNA is denser in surface morphology than the Au–MPA–PEI–ssDNA confirms the hybridization of the ssG-DNA with the probe.[42]

Specificity, sensitivity and stability of the sensor

The specificity of the sensor with *S. pyogenes* and other possible pathogens (*E. aerogenes*, *E. coli*, *B. sphaericus*, *S. aureus and S. pyogenes*) is shown in Fig. 6. The DPV peak current (I_p) of the sensor after hybridization with 25 ng per 6 μL of ssG-DNA with other pathogens found in throat swabs were almost same as the immobilized ssDNA probe except with *S. pyogenes* which shows higher I_p even at a lower concentration (10 ng per 6 μL) of hybridization with ssG-DNA (Fig. 6 inset). The significant increase in I_p values was obtained only in the case of *S. pyogenes*, which confirms the specificity of the sensor only to *S. pyogenes*. The sensor is highly specific to the *mga* gene of *S. pyogenes* and has a low (0.01 ng per 6 μL) limit of detection. The lower LOD value may be due to the higher nucleotide guanine and cytosine (GC) content (50%) of the designed probe which binds strongly with the complementary ssG-DNA of *S. pyogenes*. The stability of the immobilized probe electrode was studied by measuring change in the DPV current every 30 days of storage at 4 °C. The sensor electrode was found to be stable for 6 months at 4 °C only with 10% loss in the initial I_p of the immobilized probe.

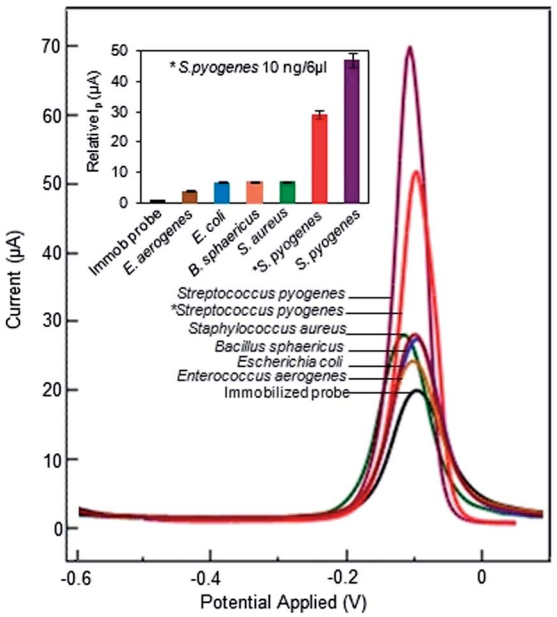


Fig. 6 Specificity of the gold composite DNA sensor with *S. pyogenes* and other possible pathogens found in the throat swabs of suspected RHD patients. The inset shows the average relative I_p value of DPV (with respect to the immobilized probe) after hybridization with ssG-DNA with other possible pathogens (25 ng per 6 μL) and *S. pyogenes* (10 and 25 ng per 6 μL).

The comparison of our method (gold composite sensor) with earlier reported methods for the detection of *S. pyogenes* causing rheumatic heart disease is summarized in Table 1. The gold–MPA–PEI composite sensor is better than earlier reported methods due to higher specificity, sensitivity, lower LOD and less detection time (30 min).

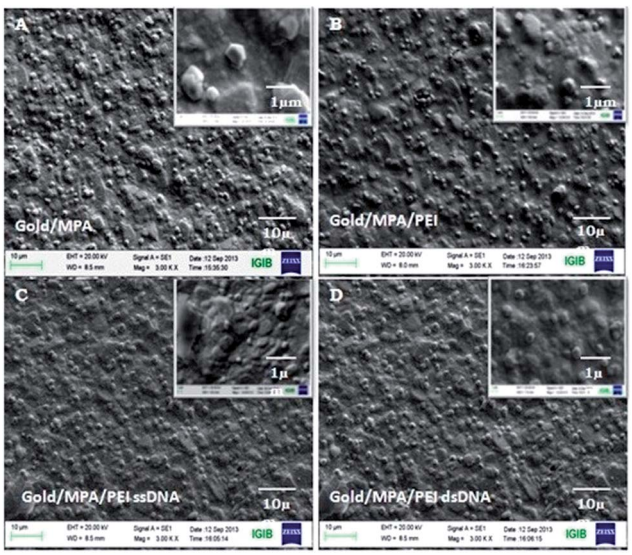


Fig. 5 SEM images of (A) Au–MPA, (B) Au–MPA–PEI, (C) Au–MPA–PEI-ssDNA and (D) Au–MPA–PEI–dsDNA composite electrodes.

Validation of sensor with patient throat swab samples

The sensor was validated with 20 throat swab samples of suspected patients using the DPV peak current (I_p) with respect to the probe as described above (Fig. 7).

The genomic DNA of patient samples was isolated and heated at 95 °C for 5 min to denature (ssG-DNA) and hybridized for 10 min with the immobilized probe. The change in I_p with respect to the probe, negative control (normal swab without *S. pyogenes*) and positive control (swab with *S. pyogenes* 10 ng per 6 μL) serves as a threshold for patient samples. In Fig. 7, the *y*-axis denotes the relative peak current in DPV with respect to the unhybridized probe (as control), negative control which do not have ssG-DNA in the sample to hybridize the probe (no increase in peak current), positive control with ssG-DNA of *S. pyogenes* (10 ng per 6 μL) was hybridized with probe and showed an increase in the average peak current with respect to the control (unhybridized probe). The patient bacterial ssG-DNA (10–12 ng per 6 μL) was hybridized with the probe and relative peak current was monitored using DPV.

Table 1 Comparison of the gold–mercaptopropionic acid–polyethylenimine composite based DNA sensor with earlier reported methods for the detection of *S. pyogenes* causing rheumatic heart disease[a]

S. no.	Detection method	Target	LOD	Sensitivity (%)	Specificity (%)	Detection time	References
1	Bacterial culture test (microscopy, bacitracin, β-haemolysis, immunoassay)	Group A Streptococcus (GAS)	—	70–90	80–90	18–24 h	7
2	Impedimetric sensor (antibody against pathogen)	*S. pyogenes*	10^2 to 10^4 cells	—	—	3–6 h	9
3	Multiplex PCR	G-DNA	10 ng	—	—	10–12 h	10
4	*speB* genetic marker	G-DNA	100 ng dsDNA	90–96	80–90	80–120 min	11
5	*mga* genetic marker	G-DNA	100 ng dsDNA	90–95	80–90	80–120 min	12
6	Illumigene assay	Swab DNA	—	98	94.7	1–2 h	13
7	FISH	16s rRNA	—	88.90	97.8	3–6 h	14
8	Gold composite sensor CV	G-DNA	0.05 ng ssDNA	23.69 μA cm^{-2} ng^{-1}	100	30 min	Present
9	Gold composite sensor DPV	G-DNA	0.01 ng ssDNA	110.25 μA $cm^{-2}ng^{-1}$	100	30 min	Present

[a] CV, cyclic voltammetry; DPV, differential pulse voltammetry; FISH, fluorescent *in situ* hybridization; LOD, limit of detection.

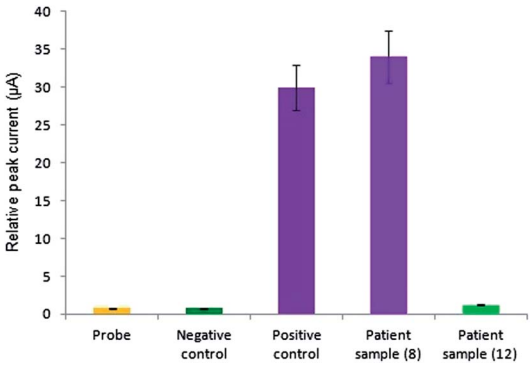


Fig. 7 Relative peak current value of DPV after hybridization with ssG-DNA from patient throat swab samples. Twenty throat swab samples were tested with gold composite DNA sensor using negative control (swab without *S. pyogenes*) and positive control (swab with *S. pyogenes*).

The average positive (8 samples) and negative peak currents (12 samples) were plotted with respect to the above mentioned controls. Negative patients do not have *S. pyogenes* (no complementary ssG-DNA) but may have other pathogens (non complementary DNA or even no DNA) causing no significant increase in peak current. The negative patient samples are negative because their peak current corresponds to the negative control whereas, the positive samples (with same ssG-DNA hybridizing concentration as in the positive control) peak current corresponds to the positive control (±10%) variation in average peak current. The usual methods of bacterial culture test[7] (18 h) and markers[12] (2 h) were also used for confirmation of the disease and results were found comparable with the sensor.

The probe of our sensor is based on the gene sequence of *S. pyogenes* which also showed 100% homology only with the genome of *S. pyogenes*, not with other pathogens or human on BLAST (basic local alignment search tools) suggest 100% specificity of the sensor only with *S. pyogenes*.

Conclusions

The gold composite based electrochemical DNA sensor is highly specific to the *mga* gene and can detect the early stage of infection of *S. pyogenes* (causing RHD) from throat swabs. The validation of the sensor was confirmed by CV, DPV and EI which showed a similar pattern of the results. The sensitivity of the gold composite based RHD sensor was 110 μA cm^{-2} ng^{-1} and the lower limit of detection was 10 pg ssG-DNA at 0.126 cm^2 surface area of the working electrode. This is the first report on a DNA sensor for the early detection of *S. pyogenes* causing rheumatic heart disease only in 30 min.

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