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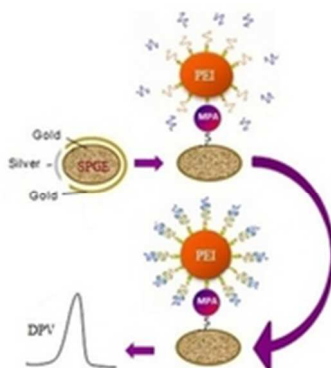
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ARTICLE TYPE

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

Swati Singh^{ab}, Ankur Kaushal^{ab}, Shashi Khare^c, Pradeep Kumar^{ab} and Ashok Kumar^{abd}

Abstract

A gold/mercaptopropionic acid/ polyethylenimine composite based first electrochemical DNA biosensor was fabricated for the early detection of *Streptococcus pyogenes* infection in human causing rheumatic heart disease (heart valve damage). No biosensor is available for detection of the rheumatic heart disease (RHD). Therefore, mga gene based sensor was developed by covalent immobilization of 5'-carboxyl modified single stranded DNA probe onto the gold composite electrode. The immobilized probe was hybridized with genomic DNA (G-DNA) of *S. pyogenes* from the throat swab and electrochemical response was measured by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance (EI). Covalent immobilization of 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 ($\mu\text{A}/\text{cm}^2$)/ng with DPV and lower limit of detection was 10 pg/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 save damage of heart valves by early detection of the infection only in 30 min.

Keywords

DNA sensor; gold composite; rheumatic heart disease; *Streptococcus pyogenes*

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 death of the patient¹⁻³. RHD is a global burden and it is more common in developing countries due to poor health care facilities⁴⁻⁶. The usual diagnostic methods are culture test⁷, biochemical assays^{7,8}, impedimetric sensor (antibody against bacteria)⁹, PCR and genetic markers¹⁰⁻¹², illumigene test kit¹³ 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, DNA biosensor will be a better option for the early detection of rheumatic heart disease¹⁵. Presently, no biosensor is available for early detection of RHD. Different types of biomaterials have been used for development of electrochemical biosensors^{16,17}. In the present investigation, gold-polyethylenimine composite based DNA biosensor has been developed for early detection of *S. pyogenes* infection from patient's 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 temperature, pH and potential differences¹⁸⁻²¹. Thiol (SH) modified single stranded DNA (ssDNA) probe can be immobilized on gold (Au) surface by the attachment of the SH group onto Au²². However, organic molecules having thiol groups (mercaptopropionic acid) which can also be covalently attached to the gold surface and form S-Au bond¹⁴. 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 COOH group of mercaptopropionic acid and amine (NH₂) group of polyethylenimine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) as condensing reagent^{25,26}. This reaction has high conversion frequency, mild reaction conditions and has little effect on the bioactivity of the target molecules. Branched polyethylenimine has NH₂ groups in abundance. Hence, even after covalent bond formation of the branched PEI with MPA, a number of the free NH₂ groups on the surface can be used for covalent immobilization of carboxyl functionalized biomolecules (probe) for the development of

1 biosensor.

2 *mga* gene is the key regulator of virulence in Group A
3 *Streptococcus* (GAS)²⁷. It is a large DNA binding protein with
4 500 amino acids and molecular weight of 62 kD. It is a multiple
5 gene regulator which activates the transcription of several genes
6 like antiphagocytic M protein (*emm*), C5a peptidase (*scpA*), M-
7 like proteins and serum opacity factor (*sof*). Although *mga* has
8 various orthologs, it is conserved in GAS and hence can be used
9 for detection of the *S. pyogenes* infection.

10 Experimental

11 Materials

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18 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-
19 hydroxysuccinimide (NHS), methylene blue (MB),
20 mercaptopropionic acid (MPA) and polyethylenimine (PEI) were
21 purchased from Sigma Aldrich, USA. Tris(hydroxymethyl)
22 aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA),
23 ethanol, sodium chloride, potassium ferricyanide, sodium di-
24 hydrogen ortho-phosphate, *di*-sodium hydrogen orthophosphate
25 and other chemicals were obtained from Qualigens, India. Brain
26 heart infusion broth was procured from Himedia, India. Genomic
27 DNA isolation kit was obtained from Bangalore Genei, India. 5'-
28 Carboxyl modified ssDNA probe (5'-GCACAGCCAATTTCT
29 AGCTTGTCG-3') of *mga* gene was procured from Bio India
30 Life Sciences, India. Screen printed gold electrode from Dropsens
31 was modified at IGIB, Delhi, India.

32 DNA isolation from culture and throat swab

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35 The genomic DNA was isolated from 18h cultured *S. pyogenes*
36 (strain M140, IMTECH Chandigarh) in brain heart infusion broth
37 at National Centre for Disease Control, Delhi using phenol
38 chloroform method (Bangalore GeNei Kit)¹¹. The purity (A_{260}/A_{280})
39 and quantity (A_{260}) was measured using nanodrop
40 spectrophotometer.

41 The G-DNA was also isolated from throat swab using 100 μ L
42 STE (50 mM Tris, 50 mM EDTA, 20% Sucrose) buffer, pH 8 and
43 heated at 95°C for 5 min for bacterial cell lysis. After cell lysis, it
44 was centrifuged at 5,000xg for 5 min and supernatant was taken
45 for quantification of DNA by nanodrop. The DNA solution
46 (dsDNA) after denaturation at 95°C for 5 min (ssDNA) was
47 directly used for hybridization with immobilized probe.

48 Fabrication of Au/MPA/PEI composite

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51 A three electrode system based screen printed electrode with gold
52 as working and counter electrode and silver as reference electrode
53 was modified for the fabrication of the gold composite based
54 DNA sensor. The working Au surface was activated with 1:1
55 (v/v) mixture of 6 μ L H₂SO₄:H₂O₂ for 5 min. It was then washed
56 3-4 times with autoclaved distilled water and dehydrated with
57 ethanol. MPA 6 μ L was placed onto the surface of working

58 electrode for 24 h to form a monolayer and subsequently washed
59 3-4 times with autoclaved distilled water to remove unbound
60 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 surface²⁶
and followed by reaction with PEI (6 μ L) for 1 h. After reaction,
the electrode was washed 3-4 times with PBS, pH 7 to remove
the excess of reagents and dried at 25 °C.

61 Immobilization of ssDNA probe

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64 The COOH group on the ssDNA probe was activated by mixing 3
65 μ L of 10 μ M 5'-COOH modified ssDNA probe with 3 μ L of
equimolar mixture of EDC and NHS (10 mM of each) to make
66 the final probe concentration 5 μ M. Then, the reaction mixture
67 was placed on the working electrode at 0.126 cm² surface area for
68 6 h to allow formation of amide bond between the COOH group
of the probe and NH₂ group of the PEI to make Au/MPA/PEI-
ssDNA electrode. The unbound probe was removed from the
69 Au/MPA/PEI-ssDNA electrode by washing 3-4 times with PBS
70 (50 mM sodium phosphate buffer, 0.9% sodium chloride), pH 7
and dried at 25°C before electrochemical detection.

71 Hybridization with ssG-DNA

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74 The G-DNA of *S. pyogenes* was denatured by heating at 95 °C for
75 5 min. The Au/MPA/PEI-ssDNA probe was hybridized with 0.5-
25 ng/6 μ L ssG-DNA in TE (10 mM Tris, 1 mM EDTA) buffer,
pH 8 for 10 min at 0.126 cm² surface area of the working
76 electrode. After hybridization, the Au/MPA/PEI-dsDNA
77 electrode surface was washed 3-4 times with TE buffer, pH 8
followed by PBS pH 7 and dried at 25°C before electrochemical
78 measurements. The fabricated sensor was used for early
detection of rheumatic heart disease electrochemically using
79 methylene blue²⁸ as redox indicator for CV and DPV whereas, EI
was measured using K₃[Fe(CN)₆] in a potentiostat/galvanostat
(FRA2 μ Autolab type iii, Metrohm, India).

80 FTIR and SEM analysis

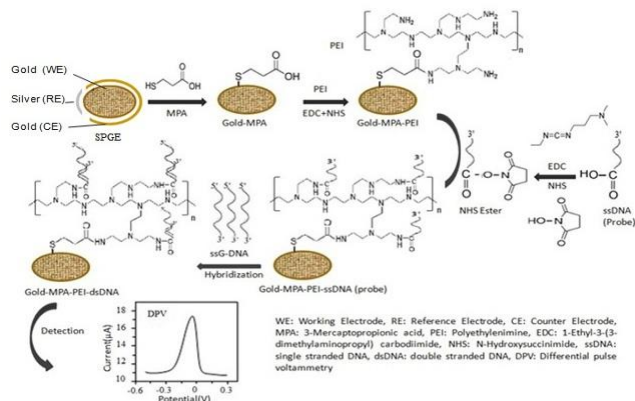
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83 The Au/MPA/PEI, Au/MPA/PEI-ssDNA and Au/MPA/ PEI-
dsDNA electrodes were characterized using Fourier transform
infrared spectroscopy (FTIR) at frequency 400-4000 cm⁻¹. (BX-
59333, Perkin Elmer, USA). The FTIR spectrum of bare gold was
84 taken as background and subtracted from the FTIR spectra of
Au/MPA/PEI, immobilized probe Au/MPA/PEI-ssDNA and after
85 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)
86 were analysed in % transmittance.

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

Results and discussion

The schematic fabrication of Au/MPA/PEI composite electrode,



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

immobilization of ss-DNA 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 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 increased binding of MB on dsDNA as compared to ssDNA which leads to oxidation of more MB molecules and hence leading to increased current²⁸. The plot between the concentrations of ssDNA used for hybridization and the relative I_p values with respect to probe (zero) was hyperbolic (Fig. 1 inset).

It was linear for 0-1 ng/6 μL ssG-DNA following the linear equation [I_p (μA) = 2.986 ($\mu\text{A}/\text{ng}$) x 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 ($\mu\text{A}/\text{cm}^2$)/ng. The limit of detection (LOD) was calculated using the formula $\text{LOD} = 3 (\sigma/S)$ where, σ is the standard deviation and S is the sensitivity²⁹. It was calculated as 0.05 ng/6 μL (Fig. S1).

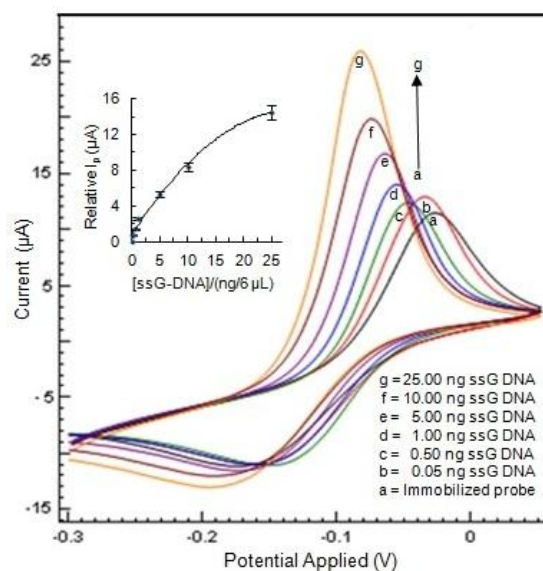


Fig. 1 Electrochemical response study using 1 mM MB in 50 mM PBS, pH 7. CV of Au/MPA/PEI-ssDNA (a) and (b-g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng/6 μL ssG-DNA of *S. pyogenes* at 50 mVs^{-1} . The inset shows hyperbolic curve between relative I_p (with respect to probe) and increasing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

DPV Studies

The I_p value of 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 similar pattern as in CV.

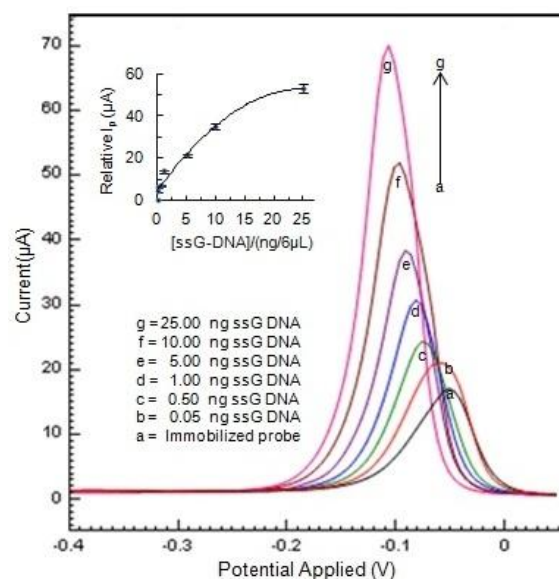


Fig. 2 DPV of (a) Au/MPA/PEI-ssDNA and (b-g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng/6 μL ssG-DNA of *S. pyogenes* at amplitude potential of 25 mV using 1 mM MB in 50 mM PBS, pH 7. The inset shows hyperbolic curve between relative I_p (with respect to probe) and increasing concentrations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

The inset plot between the concentrations of ssDNA used for hybridization and the relative I_p values with respect to probe (zero) also shows linearity from 0-1 ng/6 μ L ssG-DNA following the linear equation [I_p (μ A) = 13.892 (μ A/ng) \times ssG-DNA (ng) + 0] and regression coefficient (R^2) 0.8905. The sensitivity of the sensor was 110.25 (μ A/cm²)/ng and LOD was calculated as 0.01ng/6 μ L due to more sensitive DPV as compared to CV analysis (Fig. S2)^{29,30}. Therefore, DPV can be used for better detection of *S. pyogenes* from throat swab patient samples in 30 min with high sensitivity and specificity than earlier reported methods.

EI Studies

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

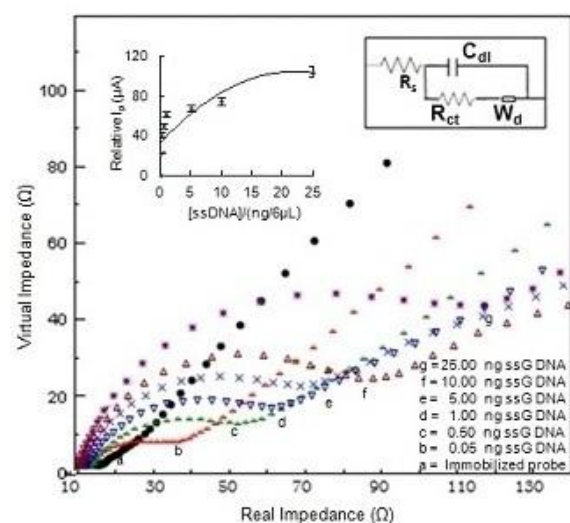


Fig. 3 EI spectra of (a) Au/MPA/PEI-ssDNA and (b-g) hybridization with 0.05, 0.5, 1.0, 5.0, 10 and 25 ng/6 μ L of *S. pyogenes* ssG-DNA using redox indicator 5 mM $K_3[Fe(CN)_6]$ in 50 mM PBS, pH 7. The inset shows hyperbolic curve between R_{ct} (with respect to probe) and increasing concentrations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

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} .

R_{ct} of bare gold was higher than that of 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 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 electrode surface.

The R_{ct} value of Au/MPA/PEI-dsDNA was higher than that of Au/MPA/PEI-ssDNA and it increased with increase in concentrations of hybridization of ssG-DNA of *S. pyogenes*. This may be due to increase 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} value³³. Electrochemical impedance also supports CV and DPV of the sensor after hybridization with different concentrations of ssDNA with probe for confirmation of the disease.

FTIR Spectra

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

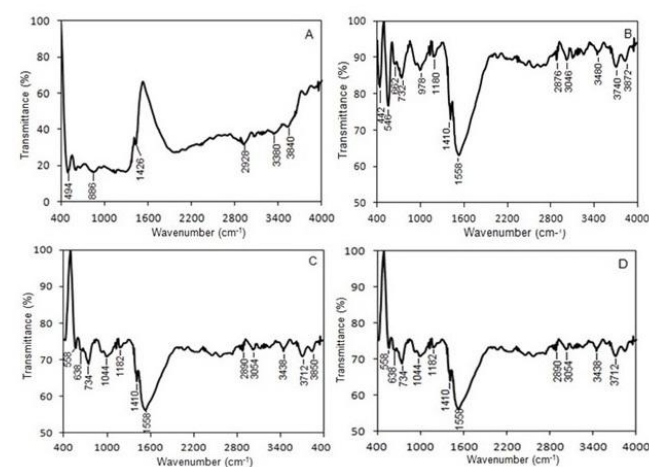


Fig. 4 FTIR transmission spectra of A) Bare Au, B) Au/MPA/PEI, C) Au/MPA/PEI-ssDNA and D) Au/MPA/PEI-dsDNA at frequency 400-4000 cm^{-1} .

The FTIR spectra in Fig. 4A shows transmittance of the bare gold electrode^{34,35}. Since the surface is completely made of bare gold, the transmittance is not specific to 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 gold-sulphur bond formed due to attachment of mercaptopropionic acid on the gold surface³⁶. The peak at 732 cm^{-1} is for NH stretching and peaks at 3480, 3740 and 3872 cm^{-1} are for C-H stretching. The peak at 1410 cm^{-1} is for C-O-H and peak at 1558 cm^{-1} is for amide bond (CO-NH)³⁷. This peak confirms that PEI has attached with 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, $C=O$ 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 PO_2^- and C-H stretch, respectively of deoxyribose backbone, increased in amplitude and became more

prominent in case of Au/MPA/PEI-dsDNA²⁹.

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 gold electrode with mercaptopropionic acid (MPA) in Fig. 5A exhibits smooth and uniform structure³⁹. Fig. 5B shows the image of Au/MPA/PEI and is different from the previous one showing changes in the surface morphology⁴⁰. Fig. 5C shows Au/MPA/PEI-ssDNA and Fig. 5D shows Au/MPA/PEI-dsDNA⁴¹. 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 probe⁴².

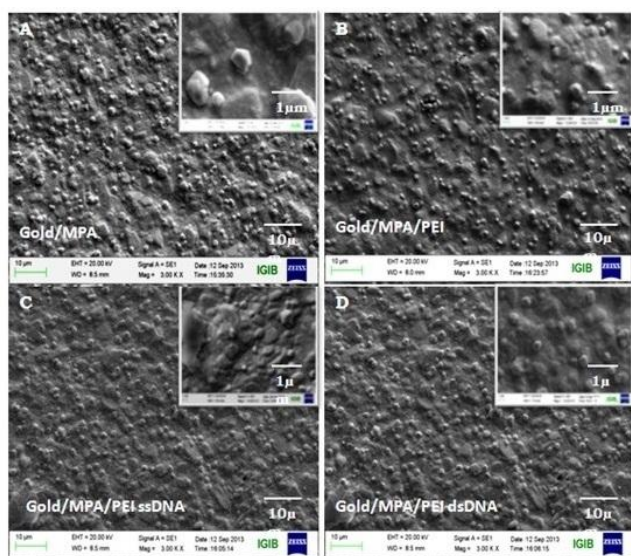


Fig. 5 SEM images of A) Au/MPA, B) Au/MPA/PEI, C) Au/MPA/PEI-ssDNA and D) Au/MPA/PEI-dsDNA composite electrodes.

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/6 μ L of ssG-DNA with other pathogens found in throat swab were almost same as the immobilized ssDNA probe except with *S. pyogenes* which shows higher I_p even at lower concentration (10 ng/6 μ L) 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 *mga* gene of *S. pyogenes* and has low (0.01ng/6 μ L) 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 DPV current at every 30 days on storage at 4 $^{\circ}$ C. The sensor electrode was found stable for 6 months at 4 $^{\circ}$ C only with 10 %

loss in initial I_p of the immobilized probe.

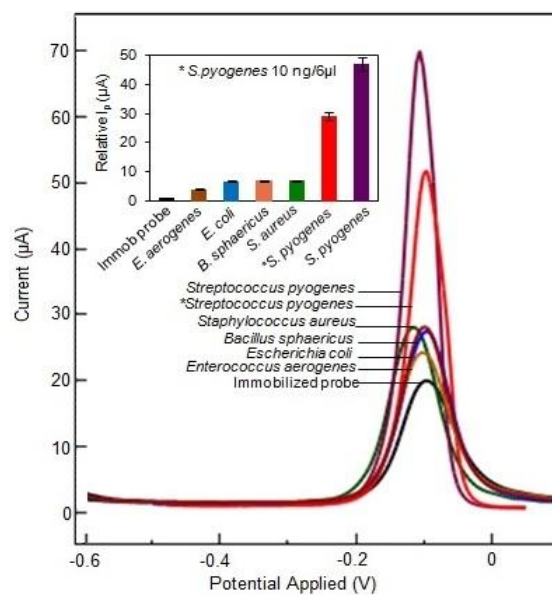


Fig. 6 Specificity of the gold composite DNA sensor with *S. pyogenes* and other possible pathogens found in the throat swab of suspected RHD patients. The inset shows the average relative I_p value of DPV (with respect to the immobilized probe) after hybridization with ssG-DNA with other possible pathogens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

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

Validation of sensor with patient throat swab samples

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

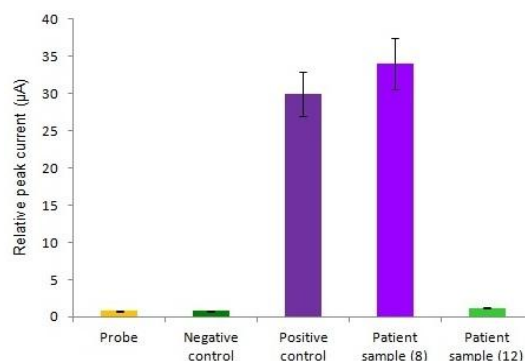


Fig. 7 Relative peak current value of DPV after hybridization with ssG-DNA from patient throat swab samples. Throat swab 20 samples were tested with gold composite DNA sensor using negative control (swab without *S. pyogenes*) and positive control (swab with *S. pyogenes*).

Table 1. Comparison of gold/mercaptopyronic acid/polyethylenimine composite based DNA sensor with earlier reported method for detection of *S. pyogenes* causing rheumatic heart disease

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	18-24h	7
2	Impedimetric sensor (Antibody against pathogen)	<i>S. pyogenes</i>	10^2 - 10^4 cells	-	-	3-6 h	9
3	Multiplex PCR	G-DNA	10 ng	-	-	10-12 h	10
4	<i>speB</i> genetic marker	G-DNA	100 ng dsDNA	90-96	80-90	80-120 min	11
5	<i>mga</i> 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 ($\mu\text{A}/\text{cm}^2$)/ng	100	30 min	Present
	Gold composite sensor DPV	G-DNA	0.01 ng ssDNA	110.25 ($\mu\text{A}/\text{cm}^2$)/ng	100	30 min	Present

CV Cyclic voltammetry; DPV Differential pulse voltammetry; FISH Fluorescent in situ hybridization; LOD Limit of detection

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

The average positive (8 samples) and negative peak current (12 samples) were plotted with respect to above mentioned controls. Negative patient 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 correspond to negative control whereas, positive samples (with same ssG-DNA hybridizing concentration as in positive control) peak current correspond to positive control (± 10 % variation in average peak current). The usual methods of bacterial culture test⁷ (18h) and markers¹² (2h) were also used for confirmation of the disease and results were found comparable with sensor.

The probe of our sensor is based on gene sequence of *S. pyogenes* which also showed 100% homology only with 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 *mga* gene and can detect in early stage of infection of *S. pyogenes* (causing RHD) from throat swab. The validation of the sensor was confirmed by CV, DPV and EI which showed similar pattern of the results. The sensitivity of the gold composite based RHD sensor was 110 ($\mu\text{A}/\text{cm}^2$)/ng and lower limit of detection was 10 pg ssG-DNA at 0.126 cm² surface area of the working electrode. This is the first report on DNA sensor for early detection of *S. pyogenes* causing rheumatic heart disease only in 30 min.

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Notes and references

- ^aCSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India
^bAcademy of Scientific and Innovative Research (AcSIR), New Delhi, India.
^cNational Centre for Disease Control, Sham Nath Marg, Delhi-110054, India
^dAddress for correspondence
 Prof. Ashok Kumar
^eCSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India
 Telephone:+91-11-27666156
 E-mail: ashokigib@rediffmail.com

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75 Graphical abstract

A novel first DNA sensor for detection of *S. pyogenes* pathogen causing rheumatic heart disease only in 30 min.

