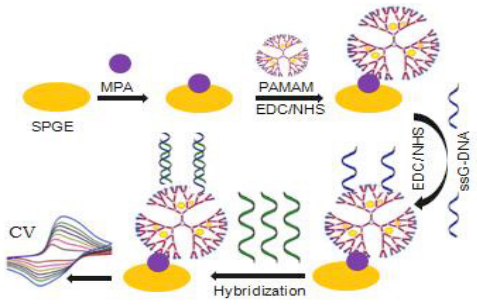
Isolation and hybridization of genomic DNA

The genomic DNA (G-DNA) was isolated from patient's throat swab samples as descried earlier [10, 16]. The purity of the DNA sample was determined by 260/280 ratio (~1.8 for pure DNA samples) using Nanodrop spectrophotometer. The different concentrations of patients G-DNA solutions (dsDNA) were denatured at 95°C for 5 min to make single stranded DNA (ssG-DNA) to hybridize with immobilized ssDNA probe on modified composite nanohybrid electrode (Au/MPA/PAMAM) for 10 min.

Construction of the sensor

A screen printed electrode with gold as working (0.126 cm^2) washed sufficiently with water and dried. After drying, 6 µL MPA (99%) was placed overnight onto the working surface of the electrode which binds with –SH groups to gold and form monolayer of MPA. An unbound MPA was removed thoroughly by several washing with Milli-Q water and dried at room temperature (25°C). The working electrode was further treated with (6 µL) equimolar mixture of 10 mM EDC and NHS (1:1, v/v) in Milli-Q water for 1h to activate the carboxyl groups on the surface and washed electrode with water and dried at room temperature. Further, 6 µL PAMAM (165 ng/µL in water) was placed on electrode and incubated for 2 h to form amide bond by binding few amino groups of PAMAM to carboxyl groups of MPA through EDC-NHS chemistry [10]. After incubation, the electrode was washed 3-4 times with water to remove unbound materials and dried at room temperature.

Further, a mixture of 3 µL (10 µM 5´carboxyl modified ssDNA probe) and 3 µL (10 mM EDC and NHS [1:1, v/v]) was placed on electrode for 3 h for formation of amide bond between the -COOH group of the probe and -NH_2 group of the PAMAM to make Au/MPA/PAMAM/ssDNA$_{probe}$ on working electrode [10]. The unbound probe and other chemicals were removed by several washing with Milli-Q water and further washed with TE (10 mM Tris, 1 mM EDTA) buffer, pH 8.0 and dried at room temperature. The ssG-DNA different concentrations (0.01-10 ng/6 µL) were made by denaturation at 95°C for 5 min in TE buffer, pH 8.0 which was hybridized onto the Au/MPA/PAMAM/ssDNA$_{probe}$ for 10 min at room temperature. After hybridization the electrode was washed with water and then 3-4 times with PBS (50 mM sodium phosphate buffer, 0.9% sodium chloride), pH 7.0 and dried as usual. Electrochemical measurements were taken by cyclic voltammetry (FRA2 µAutolab type iii, Metrohm, India) using redox indicator 50 µL methylene blue (5 mM MB in PBS, pH 7.0) [17].



Scheme 1: Fabrication of Au/MPA/PAMAM/ssDNA$_{probe}$ composite nanohybrid DNA sensor for amperometric detection of pathogen causing RHD. [SPGE: Screen printed gold electrode, MPA: Mercaptopropionic acid, PAMAM: Poly(amidoamine), EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, NHS: *N*-Hydroxysuccinimide, ssDNA: Single stranded DNA, ssG-DNA: Single stranded genomic DNA, CV: Cyclic voltammetry].

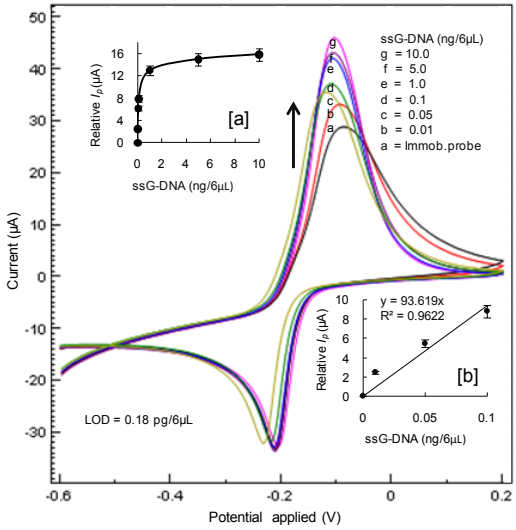


Figure 1: CV of (a) Au/MPA/PAMAM/ssDNA$_{probe}$ and (b-g) hybridization with 0.01, 0.05, 0.1, 1.0, 5.0 and 10 ng/6 µL ssG-DNA of *S. pyogenes* at 50 mVs^{-1} using 5 mM MB in 50 mM PBS, pH 7. The inset [a] shows hyperbolic curve between relative *Ip* (with respect to probe as zero) with increasing concentrations of hybridizing ssG-DNA of *S. pyogenes* and inset [b] shows linear curve for detection of LOD.

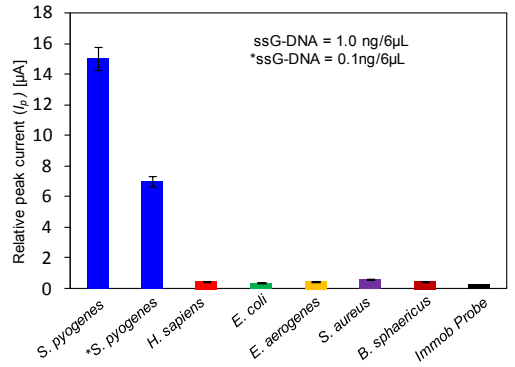


Figure 2: Specificity of the sensor for *S. pyogenes* and other possible pathogens. The relative I_p value of CV (with respect to immobilized probe as zero) after hybridization with 1.0 ng/6 µl with human and other pathogens besides *S. pyogenes* (1.0 ng and 0.1 ng/6 µl).

The fabrication of amperometric DNA sensor for detection of pathogen *S. pyogenes* is shown in Scheme 1.

Results and Discussion

Amperometric studies using cyclic voltammetry (CV)

The CV (Figure 1) of Au/MPA/PAMAM/ssDNA$_{probe}$ peak current (I_p) was higher (29.31 µA) than that of bare Au (not shown) due to the increased binding of redox indicator MB molecules to ssDNA probe. The I_p of Au/MPA/PAMAM/dsDNA (after hybridization) was higher than that of Au/MPA/PAMAM/ssDNA$_{probe}$ and it increases with the increase in concentrations of hybridizing with ssG-DNA of bacterial pathogen *S. pyogenes*. The increase in peak current was observed due to more binding of MB on dsDNA as compared to ssDNA resulting in more oxidation of MB molecules on the surface of electrode and hence increased in the current. The plot between the ssDNA concentrations and relative *Ip* values with respect to probe (zero) was hyperbolic (Figure 1 inset [a]). It was linear for 0-0.1 ng/6 µL ssG-DNA following