International Journal of Biological Macromolecules 103 (2017) 355–359

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

DNA chip based sensor for amperometric detection of infectious pathogens

Swati Singh[a,b], Ankur Kaushal[a], Shashi Khare[c], Ashok Kumar[a,b,*]

[a] CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi, 110007, India
[b] Academy of Scientific and Innovative Research (AcSIR), New Delhi, 110025 India
[c] National Centre for Disease Control, Sham Nath Marg, Delhi, 110054, India

ARTICLE INFO

Article history:
Received 7 April 2017
Received in revised form 8 May 2017
Accepted 9 May 2017
Available online 11 May 2017

Keywords:
DNA chip
PAMAM dendrimer
Pathogens
Rheumatic heart disease
Streptococcus pyogenes

ABSTRACT

Several infectious pathogens are found in human whose detection is essential for rapid cure of diseases. The most commonly found pathogen in human is *Streptococcus pyogenes* which leads to a wide range of infections from mild pharyngitis to rheumatic heart disease. An ultrasensitive DNA chip based sensor was developed for quick identification of pathogen *S. pyogenes* from patient throat swab samples. The amperometric response was measured after hybridization of specific probe with single stranded genomic DNA (ssG-DNA) from the patient samples. The DNA chip was characterized by FTIR, SEM and validated with suspected patient real samples. The sensitivity of the DNA chip based sensor was found 951.34 ($\mu A/cm^2$)/ng DNA and lower limit of detection (LOD) was 130 fg/6 μL samples. The DNA chip based sensor is highly specific and takes only 30 min for identification of specific pathogen.

1. Introduction

Several pathogens cause infections to human but commonly known pathogen of Group A streptococcus (GAS) bacteria *S. pyogenes* causing pharyngitis at initial stages which if not treated may lead to the chronic valvular lesions and latter damage of mitral and aortic heart valves [1–4]. The autoimmune reactions are the hallmark of the pathogenesis of the disease [5–7]. The current methods of detection of *S. pyogenes* infection are culture test, biochemical assays, impedance, PCR, genetic markers, illumigene kit and fluorescent *in situ* hybridization (FISH) test and sensors [8,9]. These methods are time consuming, expensive, less sensitive and specific due to certain limitations. DNA chip based sensors are the modern diagnostic techniques which may be used to increase the sensitivity and specificity of the traditional diagnostic tests [10]. Our aim is to develop a rapid, accurate, sensitive and cost effective method for the detection of *S. pyogenes* [11–13]. Therefore, we designed *mga* (multiple gene activators) gene based carboxylated ssDNA probe (24 mer) which can be used as a specific probe for detection of the *S. pyogenes* [14,15]. The probe was chemically synthesized and immobilized on gold nanoparticles embedded modified electrode as DNA chip for hybridization with ssG-DNA of target pathogen *S. pyogenes*.

DNA chip consist of several small sized single stranded DNA probe (20–25 mer) attached on the surface of screen printed electrode which can bind to their complementary single stranded genomic DNA (ssG-DNA) and electrochemical changes are measured amperometrically using specific redox indicators for identification of pathogens [16–19]. Here, gold nanoparticles embedded carbon electrode was modified to develop DNA chip using cysteine and dendrimer for attachment of several specific DNA probes on small surface area for binding many target DNA. The thiol (-SH groups) of cysteine attached to gold (Au) at one side and free carboxyl group to the dendrimer through amide bond formation by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxy succinimide (EDC/NHS) chemistry [20–22]. Several left NH_2 groups of dendrimer (PAMAM) binds to the carboxylated single standed DNA probes for hybridization with target DNA. The schematic fabrication of the DNA chip based sensor is shown in Scheme 1.

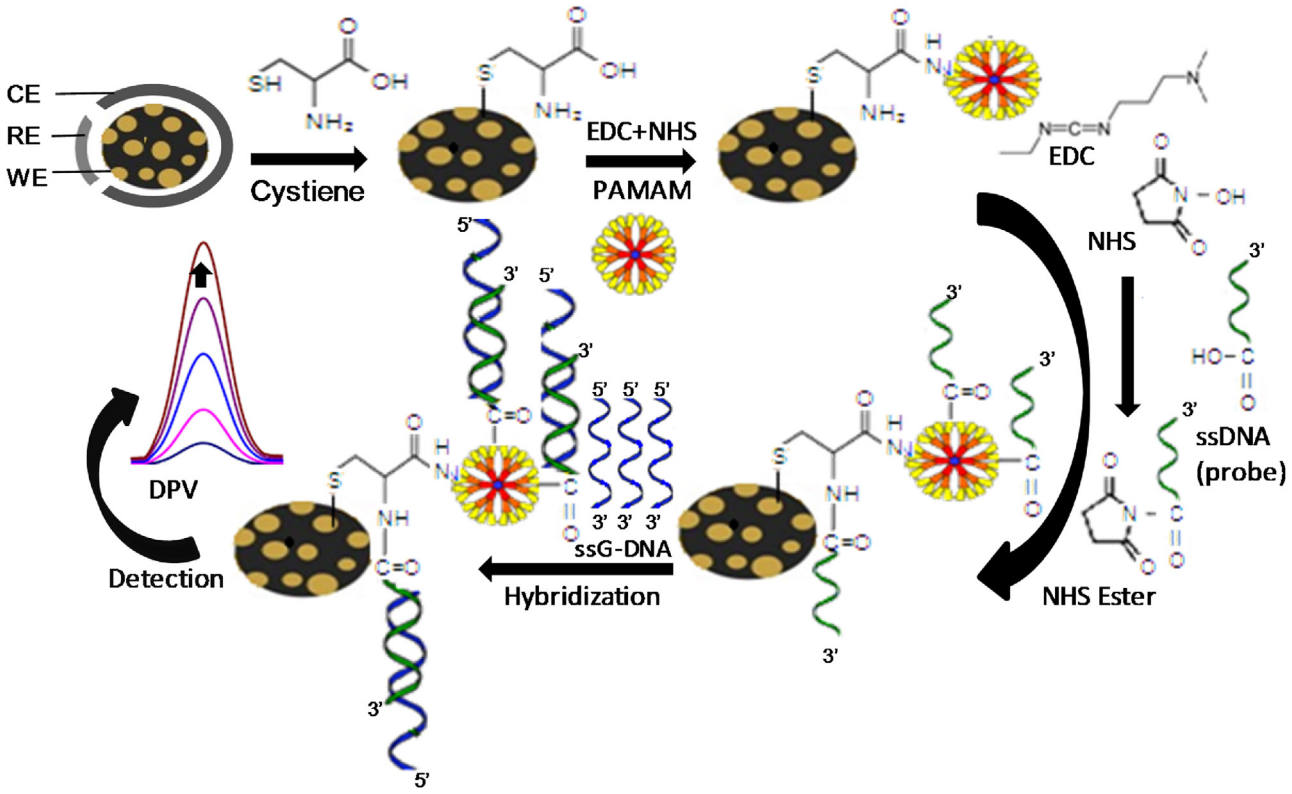
2. Experimental

2.1. Materials

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), methylene blue (MB), cystiene and

* Corresponding author at: CSIR-Institute of Genomics and Integrative Biology, Mall Road, Delhi, 110007, India.
E-mail address: ashokigib@rediffmail.com (A. Kumar).

http://dx.doi.org/10.1016/j.ijbiomac.2017.05.041
0141-8130/



Scheme 1. Fabrication of DNA chip sensor for amperometric detection of pathogens. CE: Counter electrode, RE: Reference electrode and WE: Working electrode of DNA Chip.

poly (amidoamine) dendrimer (PAMAM) (fourth generation, MW 14215.0) were obtained from Sigma-Aldrich, USA. Ethanol, hydrochloric acid, sodium chloride, sodium di-hydrogen *ortho*-phosphate, *di*-sodium hydrogen orthophosphate, Tris (hydroxymethyl) aminomethane (Tris), ethylenediamine-tetraacetic acid (EDTA) and other chemicals were received from Qualigens, India. 5′-Carboxyl modified ssDNA probe 24 mer (5′HOOC-GCACAGCCAATTTCTAGCTTGTCG-3′) of *mga* gene was synthesized from Bio India Life Sciences, India. Screen printed gold electrodes were purchased from Dropsens, Spain and modified for development of DNA chip based sensor at IGIB. India.

2.2. Isolation of DNA from samples

The genomic DNA (G-DNA) was isolated from patients' throat swab samples at National Center for Disease Control (NCDC) as described earlier [23]. The G-DNA solution (dsDNA) was denatured at 95 °C for 5 min to make ssDNA to hybridize (10 min) with immobilized probe on DNA chip sensor.

2.3. Development of the DNA chip sensor

A screen printed carbon electrode embedded with gold (Au) nanoparticles was used as working, carbon as counter and silver as reference electrode for the development of DNA chip sensor. Cysteine (6 μL, 5 mM) was placed on working electrode surface (0.126 cm^2) for overnight to attach with Au nanoparicles and unbound cysteine was removed by several washing with Milli Q water and dried at 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 1 h to activate the −COOH groups on the surface and followed by several washing with Milli Q water as described above. After washing, 6 μL PAMAM (165 ng/μL in water) was placed on working electrode for 2 h. The electrode was further washed 3–4 times with Milli Q water to remove the excess of the reagents and dried at room temperature (25 °C).

The carboxyl groups of the ssDNA probe (*mga* gene) was activated by mixing 3 μL of 10 μM 5′-carboxyl modified ssDNA probe with 3 μL of 10 mM mixture of EDC and NHS (1:1, v/v) to make the final concentration of probe 5 μM. The above reaction mixture was placed on the working electrode surface for 2 h to form amide bond between the —COOH groups of the probe and —NH_2 groups of the PAMAM. The unbound probe and other chemicals were removed by several washing with Milli Q water and further dried at 25 °C. The DNA chip (Au/Cys/PAMAM/ssDNA) sensor was hybridized with 0.001–10 ng/6 μL ssG-DNA in TE (10 mM Tris, 1 mM EDTA) buffer, pH 8 for 10 min at the working electrode of the DNA chip sensor. The chip was further washed with PBS (50 mM sodium phosphate buffer, pH 7 containing 0.9% NaCl, pH 7) and electrochemical amperometric measurements (FRA2 μAutolab type iii, Metrohm, India) were taken using 50 μL MB (1 mM in PBS, pH 7) as redox indicator [22].

2.4. Specificity test

The specificity test of the DNA chip sensor with *S. pyogenes* and other possible pathogens present in the mouth of humans such as *S. aureus*, *E. aerogenes* and *E. coli*, were procured from other labs of IGIB and cultured overnight in 250 mL LB (Luria- Bertani) media [24] and G-DNA was isolated using phenol-chloroform method [25] and quantified with nanodrop spectrophotometer. Human (*H. Sapiens*) DNA was also used for specificity test as it is present in the throat swab samples. The G-DNA of *H. Sapiens* was isolated from human blood sample using Quiagen DNA isolation kit (QI Aamp DNA Mini Kit) and was heated at 94 °C for 5 min to hybridize with immobilized probe on modified DNA chip sensor surface as described earlier. The change in peak current (I_p) with respect to probe (control) was measured as differential pulse voltammetry (DPV) with all pathogens