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Electrochemical biosensing of mosquito-borne viral disease, dengue: A review

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ABSTRACT

Dengue virus is a mosquito-borne, single positive-stranded RNA virus that spread human being through infected female *Aedes* mosquito bite and causes dengue fever. The demand for early detection of this virus has increased to control the widespread of infectious diseases and protect humankind from its harmful effects. Recently, biosensors are found to the potential tool to detect and quantify the virus with fast detection, relatively cost-effective, high sensitivity and selectivity than the conventional diagnostic methods such as immunological and molecular techniques. Mostly, the biosensors employ electrochemical detection technique with transducers, owing to its easy construction, low-cost, ease of use, and portability. Here, we review the current trends and advancement in the electrochemical diagnosis of dengue virus and discussed various types of electrochemical biosensing techniques such as; amperometric, potentiometric, impedometric, and voltammetric sensing. Apart from these, we discussed the role of biorecognition molecules such as nucleic acid, antibodies, and lectins in electrochemical sensing of dengue virus. In addition, the review highlighted the benefits of the electrochemical approach in comparison with traditional diagnostic methods. We expect that these dengue virus diagnostic techniques will continue to evolve and grow in future, with exciting new possibilities stemming from advancement in the rational design of electrochemical biosensors.

1. Introduction

Newly emerging or re-emerging of the viral disease becomes a menace to the life of mankind. In particular, the vector-borne viral diseases pose the greatest threat to human society, which causes mild to severe infections and even premature death than any other disease process. These pandemic disease-causing viruses differ in their size, shape and behaviour, but only replicates inside the cells of living host (Labib et al., 2011). In the past 100 years, nearly 5000 species of viruses have been discovered from infected animals, plants, bacteria and archaea (Ramage and Cherry, 2015). The Ebola virus outbreak-2014 in West Africa, has been declared as historically largest tragedies with a total death of ~11,310 and the disease has been re-emerged again in Democratic Republic of the Congo with 1405 deaths and hundreds of infected cases as updated by World Health Organization (WHO) on June 13th, 2019 (Kaushik et al., 2016; WHO, 2015, 2019). In the beginning of 2016, WHO notified Zika virus, a mosquito-borne epidemic as a public health crisis around the world and it quickly spreads in Africa, Asia, America, and Europe (Chang et al., 2016; Li et al., 2017). Likewise, dengue is an endemic disease which causes major health problem in tropical and sub-tropical regions and considered to the most prevalent mosquito-borne viral disease affecting human population throughout the history (Back and Lundkvist, 2013; Fernandes et al., 2018). Annually, large numbers of dengue infections are reported, especially in monsoon season and period of high vector prevalence. The symptoms are often unobvious but leads to a wide range of clinical manifestations, from mild fever to fatal dengue shock syndrome (Chakravarti et al., 2012). Due to the absence of vaccines and specific treatments for dengue, immediate onset of epidemics could be extenuate using reliable and rapid diagnostic methods (Shu and Huang, 2004). In many cases, the dengue virus detection in human sera is laborious, cumbersome, and time-consuming to perform (Pejcic et al., 2006; Samuel and Tyagi, 2006). The major clinical diagnosis method involves virus isolation, serological test, detection of viral genomes and antigens (Darwish et al., 2015a; Kim et al., 2018; Kong et al., 2006; Parkash and Shueb, 2015b; Teles, 2011). Over the past decades, biosensors with analytical probes or biorecognition elements such as nucleic acid, immunoglobulins, antibodies, lectins, glycoproteins and viral

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particles used in the sensing system for rapid, sensitive and selective detection (Kirsch et al., 2013; Wong et al., 2014). A detailed survey of the literature showed that most of the biosensors used for dengue virus detection employ any one of the three transduction techniques, which includes optical, piezoelectric, and electrochemical. Recently, many electrochemical-based detection have been employed for the development of highly quantitative and qualitative biosensors for dengue virus (Abdulbari and Basheer, 2017; Felix and Angnes, 2018). Hence, this review concentrates and details the electrochemical detection methods of dengue virus with the biorecognition molecules.

2. Dengue: an overview

Dengue is the most common arthropod-borne viral disease, caused by the dengue virus which belongs to the family *flaviviridae* and genus *flavivirus*. It is further classified as 'arbovirus', hence it transmitted by an arthropod vector, in particular, the mosquitoes such as *Aedes aegypti* and a few extents in *A. albopictus* (Behnam et al., 2016). The dengue virus consists of four serotypes, DENV1, DENV2, DENV3 and DENV4 which have been emerged from the sylvatic strains in South-East Asia (Gubler, 2006; Parkash and Shueb, 2015a). Presently, all four serotypes are found worldwide especially in tropical and sub-tropical regions but now extensively spreading in higher latitudes (Darwish et al., 2015a). Generally, the dengue virus replicates inside the mosquito and infections can be transmitted quickly from one person to another by the bite of contaminated female *Aedes* mosquitoes (WHO, 2009). After incubation of 5–8 days, results with high dengue fever accompanied neurological disturbances and febrile seizures due to dehydration. The infections caused by the four serotypes may be asymptomatic to life treating lethal conditions such as dengue haemorrhagic fever and dengue shock syndrome. Mostly the symptoms of dengue are similar to other common febrile illnesses, so it is very difficult to diagnosis in their earlier stage. However, few laboratory diagnoses of dengue virus infections can be made by the detection of specific dengue viral antigen, antibodies, and genomic sequences. The most commonly used method includes dengue virus culture, nucleic acid amplification using real-time PCR, and serological test (Campuzano et al., 2017) (Fig. 1).

2.1. Electrochemical detection of dengue virus

Biosensor is a highly specific and sensitive diagnosis tool with fast detection in a small amount of sample. Generally, an analytical biosensor system works to detect and/or quantify the presence of specific compounds or biological analyte (Ronkainen et al., 2010; Teles, 2011). This analytical system consists of a transduction system with a biological recognition element and an electronic system with signal amplifiers, processors, and a display. In electrochemical sensors, the electrochemical interrogation includes current, impedance, and potential changes to transduce the biological recognition elements (Ahmad et al., 2018; Anusha et al., 2015b; Kausaite-Minkstimiene et al., 2018). In recent reports, more than half of the biosensors are used for the detection of human pathogens based on electrochemical transduction. Moreover, the biosensors are potential in virus sensing due to its real-time monitoring and rapid detection in the field (Kurtinaitiene et al., 2008). The early diagnosis with an adequate detection system is essential to minimize the proliferation of virus outbreak to the society. Thus, the biosensor technology has been flourished widely for the detection as well as the quantification of small molecules to nanosized viral particles. The biosensor with electrochemical approaches such as amperometric, potentiometric, voltammetric and impedimetric have been used to monitor virions (10–100 nm) or virus particles during virus outbreaks in epidemic areas. In recent years, there has been vast research on dengue virus detection using biosensor is trending especially in electrochemical biosensors (Table 1).

2.2. Biosensor electrodes

In a typical biosensor, the primary attention is devoted to the

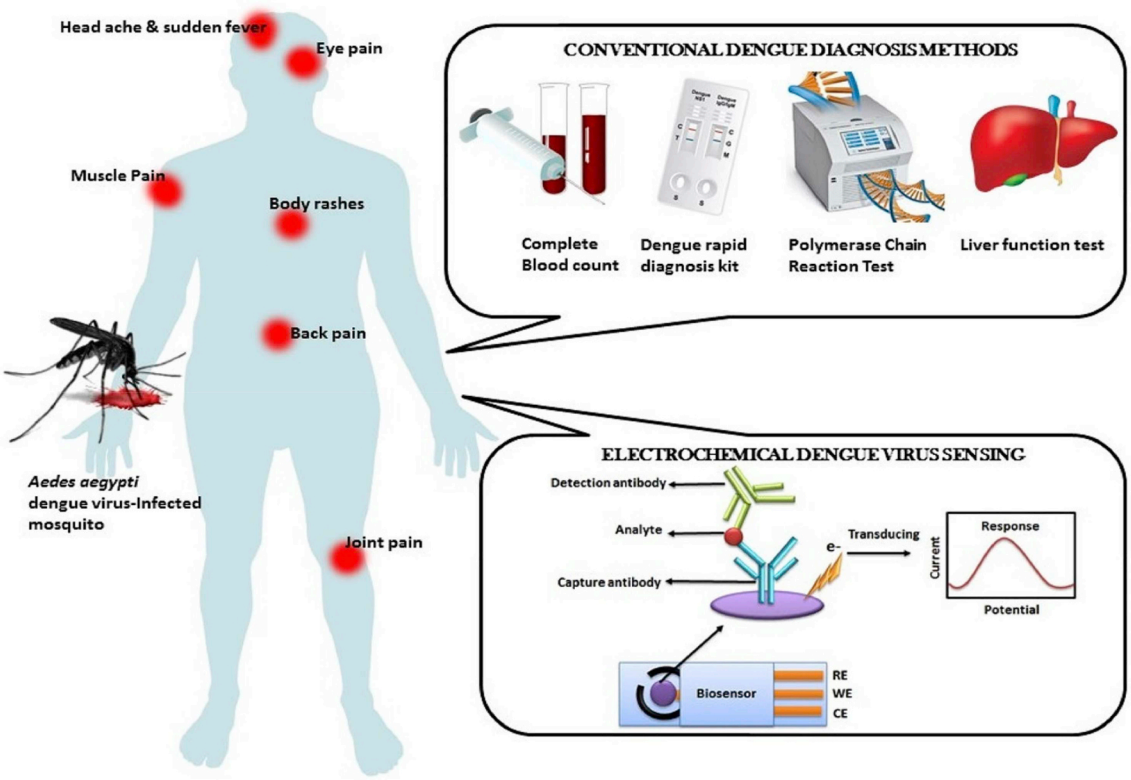

Fig. 1. Schematic representation of dengue virus infection, symptoms, conventional and electrochemical diagnosis methods.

Table 1
Recent developments in electrochemical dengue virus biosensors.

Electrode	Biosensor Type	Biorecognition element	Analyte	Linear range	Limit of Detection	Reference
CNT-SPE	CAMP	Anti-NS1	NS1	0.04–2 μg mL^{-1}	12 ng mL^{-1}	Dias et al. (2013)
CS-CFE	AMP	Anti-DENV	DENV envelope	1.0–175 ng mL^{-1}	0.94 ng mL^{-1}	Cavalcanti et al. (2012b)
PAH/MWCNT	AMP	Anti-NS1	NS1	0.1–2.5 μg mL^{-1}	0.035 μg mL^{-1}	Silva et al. (2015)
CCTS/O_2/Si	AMP	Specific DNA probe	DNA-DENV2	100 f – 10 nM	17 nM	Odeh et al. (2017)
Au/CD-trode	DPV	Anti-NS1	NS1	1-100 ngmL^{-1}	0.33 ng mL^{-1}	Cavalcanti et al. (2012a)
Nanoporous alumina/Pt	DPV	Anti-DENV2	DENV2	1-10^3 pfu mL^{-1}	1 pfu mL^{-1}	Cheng et al. (2012)
Pencil GE	DPV	18-mer ss nucleic acid	DENV1-Specific Complementary oligonucleotides	1–40 nm	0.92 nm	Souza et al. (2011)
Nanoporous alumina/Pt wire	DPV	5′ DNA probe	DENV1(31-mer ssDNA)	10^{-12} -10^{-6} mL^{-1}	9.55 × 10^{-12} M	Rai et al. (2012)
ZnO/Pt–Pd	CV/DPV	DENV probe ssDNA	DENV target ssDNA	1 × 10^{-6} M- 100 × 10^{-6} M	4.3 × 10^{-6} M	Singhal et al. (2017)
Mn_2O_3 nanofiber	CV/DPV/IMPM	Dengue DNA	Dengue consensus primer	1 nM- 1 μM	120 × 10^{-21}M	Tripathy et al. (2017)
Thiophene-SPE/AuNPs/Protein A	CV	Anti-NS1	NS1	0.04–0.6 μg mL^{-1}	0.015 μg mL^{-1}	Silva et al. (2014)
PGE	DPV	DENV3 probe DNA	DENV3 complementary DNA	10–100 nM	3.09 nM	Oliveira et al. (2015)
GA/Cys/Au	POTM	Anti-NS1 IgY	NS1	1.1–10 μg mL^{-1}	0.09 μg mL^{-1}	Figueiredo et al. (2015)
Au	IMPM	Anti-NS1	NS1	0.01–1.00 μg mL^{-1}	30 ngmL^{-1}	Cecchetto et al. (2015)
ITO	IMPM	Anti-NS1	NS1	5–4000 ng mL^{-1}	0.035 μg mL^{-1}	Darwish et al. (2015b)
Nanoporous alumina/Pt	IMPM	Anti-DENV2 IgG	DENV2	1 to 900 pfu mL^{-1}	–	Nguyen et al. (2012)
Nanoporous alumina/Pt	IMPM	BSA and 2H2 antibodies	DENV2 and DENV3	1 to 900 pfu mL^{-1}	0.230 pfu mL^{-1} (DENV2) 0.710 pfu mL^{-1} (DENV3)	Peh and Li (2013)
Au/liposome	IMPM/CV	Con A lectin	DENV1, DENV2, DENV3,	–	–	Luna et al. (2014)
AuNPs/PVB	IMPM/CV	Con A lectin	Dengue fever and dengue haemorrhagic fever	–	–	Oliveira et al. (2009)
Fe_3O_4/PVB	IMPM/CV	CramoLL lectin	DENV1, DENV2, DENV3	–	–	Oliveira et al. (2011)
AuNPs/PANI/CramoLL	IMPM/CV	CramoLL lectin	DENV1, DENV2, DENV3			Avelino et al. (2014)
AuNPs/PANI/BmoLL	IMPM/CV	BmoLL lectin	DENV1, DENV2, DENV3			Andrade et al. (2011)
CNT/polypyrrole-NHS	IMPM/CV	Anti-NS1 (DENV2)	DENV2	10^{-13}-10^{-5} g mL^{-1}		Palomar et al. (2018)
Cys-MBA-AuNPs	IMPM/CV	Anti-DENV	DENV1, DENV2, DENV3, DENV4			Luna et al. (2015)
GO-PMC	IMPM/CV	Anti-DENV	DENV1, DENV2, DENV3, DENV4	1–2 10^3 pfu mL^{-1}	0.12 pfu mL^{-1}	Navakul et al. (2017)
AAO	IMPM/CV	DENV probe ssDNA	DENV complementary ssDNA	1 × 10^{-12} M- 1 × 10^{-6} M	2.7 × 10^{-12}M	Deng and Toh (2013)
SiO_2/APTES-GO	IMPM	Dengue Oligonucleotide sequences	Dengue DNA and RNA	–	1 fM	Jin et al. (2016)

***Abbreviations**: CNT-Carbon nanotubes, SPE-screen printed electrode, NS1-non-structural 1, AuNPs-Gold nanoparticles, GA-Gluteraldehyde, Cys-cysteamine, Au-gold, PAH-poly(allylamine), MWCNT-multi-walled carbon nanotubes, CD-compact disk, CS-chitosan, CFE-carbon fibre electrode, Pt-Platinum, GE-graphite, AuNPs-Gold nanoparticles, PVB-Polyvinyl butyral, NHS–N-hydroxysuccinimido 11-(pyrrol-1-yl) undecanoate, ZnO-Zinc oxide, Pd-palladium, MBA-4-mercaptobenzoic acid, Cys-cysteine, AAO-anodic aluminium oxide, CCTS-Cu_2CdSnS_4, O_2/Si-Oxygen-etched silicon substrate, PFU-Plaque forming units, Mn_2O_3-Manganese(III) oxide, SiO_2-Silicon dioxide, APTES-3-Aminopropyltriethoxysilane, GO-Graphene oxide, CAMP- Chronoamperometry, AMP- Amperometric, DPV- Differential Pulse Voltammetry, CV- Cyclic Voltammetry, POTM-Potentiometry, IMPM- Impedimetric.

selection of transducer surface in order to immobilize the biorecognition element for better detection of the analyte. Commonly, paper to the flexible surface with lightweight, biocompatible and inexpensive materials are utilized in various sensing platforms (Goode et al., 2015; Shah et al., 2015). In electrochemical biosensors, indium tin oxide (ITO) (Pruna et al., 2017) and fluorine-doped tin oxide (FTO) (Singhal et al., 2017) have been widely used as substrates due to its high transparency and good electrical conductivity. Similarly, opaque substrates such as gold, silver, platinum, stainless steel, graphite and glassy carbon are also used as electrodes due to elevated surface area and excellent electrical conductivity (Cheng et al., 2012; Deng and Toh, 2013; Janegitz et al., 2017; Oztekin et al., 2011; Peh and Li, 2013). For example, Oliveira et al. (2009) constructed a dengue biosensor in a gold electrode; where the concanavalin A was immobilized over the electrode using gold nanoparticles and polyvinyl butyral for the effective detection of dengue haemorrhagic fever and dengue fever from patient's sera. Likewise, screen-printed carbon nanotube (CNT) electrodes were covalently linked with anti-nonstructural protein 1 (NS1) antibodies by an ethylenediamine film process for NS1 protein detection, which resulted in high quantitative point-of-care testing with safe and disposable quantity (Dias et al., 2013). Similarly, screen-printed gold electrode (Sinawang et al. 2016, 2018) and thiophene-modified screen-printed electrode (Silva et al., 2014) were also used for the detection of dengue virus NS1 protein. Moreover, silica substrate (SiO_2) as well used for the fabrication of dengue virus detection because of its chemically inert nature and flat surface which prevents the reaction and easy removal of the buffer from the surface (Nuzaihan et al., 2016).

In addition, the biorecognition element or bio-receptors on the electrode are a crucial component to sense the biomolecules or pathogens. Generally, enzymes are used as biorecognition element in a catalytic biosensor, wherein affinity-based sensors employ antibodies, aptamers, nucleic acids or specific-engineered receptors. For example, the common glucose biosensor utilizes glucose oxide to analyse the glucose concentration in blood or urine samples (Anusha et al., 2015a; Krikstolaityte et al., 2014). However, in dengue virus biosensor, there are many lectin-based biosensors to detect dengue glycoprotein. Lectins are carbohydrate-binding proteins which are specific to carbohydrate structure and an optional to monoclonal antibody to detect dengue viral glycoprotein in serum; for example, *Concanavalin* A (ConA) lectin (Luna et al., 2014; Oliveira et al., 2009), *Cratyliamollis* (CramoLL) lectin (Avelino et al., 2014; Oliveira et al., 2011), *Bauhinia monandra* (BomLL) lectin (Andrade et al., 2011). In nucleic acid-based biorecognition, the direct interaction occurs by the complementary base pairing of a linear sequence and sense the analyte (Souza et al., 2011). The antibodies are produced in response to the antigen and which specifically binds to the antigen. This mechanism is used to identify particular antigens such as proteins or lipopolysaccharides at the surfaces of viruses and bacteria, or cell surface or blood sera or tissue samples (Kirsch et al., 2013). The interaction between the antibody at the transducer surface and antigen can be measured directly and indirectly (Figueiredo et al., 2015; Nguyen et al., 2012).

However, few types of biosensors are constructed by direct conjugation of the biorecognition element to the metallic electrode surface, but it often leads to the loss of biological activity. Hence, the biorecognition element is mostly immobilized in a thin tethering layer of polymers or self-assembled mono-layer which provides functional groups for chemical coupling (Goode et al., 2015). These elements are mainly immobilized into the transducers by adsorption, micro-encapsulation, entrapment, covalent attachment and crosslinking (Bazin et al., 2017). Altogether, the conjugation may be either non-covalent or covalent; but the non-covalent tethering has weak charge interactions and it can be easily dissociated when altering the pH or ionic strength of electrolyte, so covalent bonding of the biorecognition element to the transducer surface is more desirable (Zhang et al., 2017). Basso et al. (2018) detected all four serotypes of dengue virus through antigen-antibody binding interaction using gold nanoparticles as signalling antibody carrier.

2.3. Amperometric biosensor

Amperometric sensors continuously measure the current resulting from the oxidation and reduction of an electroactive species in a biochemical reaction, where the current is measured at a constant potential, so it is also referred to as amperometry. These sensors are more sensitive, rapid and inexpensive compared to other techniques. Meanwhile, amperometric biosensors become an essential tool in biomarker sensing, in which the electrode material and architecture plays a vital role in achieving accurate results. Recently, carbon nanomaterials in the form of nanotubes, nanowires, nanosheets etc., have been used as biosensor platforms (Pasinszki et al., 2017; Wang et al., 2009). An easily disposable chitosan-modified carbon fibre electrode for dengue virus envelope protein was reported, where the antibodies covalently immobilized on the chitosan matrix after activation with sodium periodate. The amperometric response of the competitive immunoassays was generated by hydrogen peroxide redox reaction with the peroxidase-conjugated to DENV and 2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) as mediator. The obtained results showed a linear range from 1.0 to 175 $ng\,mL^{-1}$ with the limit of detection $\sim 0.9\ ng\,mL^{-1}$ (Cavalcanti et al., 2012b). Another sensitive electrode was developed by immobilizing the anti-NS1 antibodies over poly (allylamine) and carboxylated CNTs. The amperometric response of immunosensor was based on the reaction between H_2O_2 and HRP conjugated to antibodies, it exhibited a linear range of 0.1–2.5 $\mu g\,mL^{-1}$ and limit of detection (LOD) of 0.035 $\mu g\,mL^{-1}$ (Silva et al., 2015).

Chronoamperometry is the advanced form of amperometric technique, where a square-wave potential is applied to the working electrode and the steady current is measured as a function of time. In this case, alternations in the current arising from the expansion or reduction of the diffusion layer at the electrode surface. In a typical study, screen-printed electrodes were fabricated using carboxylated CNTs and carbon ink, where the anti-NS1 antibodies were covalently linked by an ethylenediamine (EDA) film strategy. The antigen was sandwiched with HRP-specific antibody and the amperometric responses were generated at -0.5 V vs Ag/AgCl by H_2O_2 reaction with horseradish peroxidase (HRP). The device resulted with an excellent detection limit (12 $ng\,mL^{-1}$) and sensitivity (85.50 $\mu A\,mM^{-1}\,cm^{-2}$) in a wide linear range (0.04–2 $\mu g\,mL^{-1}$) for dengue NS1 protein detection (Dias et al., 2013) (Fig. 2).

2.4. Voltammetric biosensor

Cyclic voltammetry (CV) is one of the commonly used electroanalytical methods, which is useful to obtain information about the redox potential and electrochemical rates of the analyte. In this technique, voltammetry measures the resulting current by varying potential to study the analyte. Based on the analysis, a partial cycle, one full cycle, or a series of cycles were carried out and used for the study of redox processes, monitoring reaction intermediates, and stability assessment of reaction products. Silva et al. (2014) developed thiophene-modified SPE using a sulfur-containing heterocyclic compound. In this, the thiophene was incorporated to the carbon ink and coated with gold nanoparticles to conjugate Protein A to form a nanostructured surface, in which the anti-NS1 antibodies were immobilized via the Fc portion. The CV analysis of dengue virus NS1 protein showed calibration curve with a linear response of 0.04–0.6 $\mu g\,mL^{-1}$ in a good linear correlation ($r = 0.991$, $p < 0.05$) and LOD (0.015 $\mu g\,mL^{-1}$NS1). Sinawang et al. (2016) developed the electrochemical lateral flow immunosensors combined with gold nanoparticles. Here, specific capture anti-NS1 protein was covalently immobilized over the screen-printed gold electrode (SPGE) by 11-mercaptoundecanoic acid and the secondary antibodies labelled with ferrocene were conjugated with gold nanoparticles. The specific immune-complex formation was recorded and showed a linear range from 1 to 25 $ng\,mL^{-1}$ with an ultrasensitive detection limit (0.5 $ng\,mL^{-1}$) towards dengue NS1 protein (Fig. 3a). Further, the same group developed an electrolateral flow

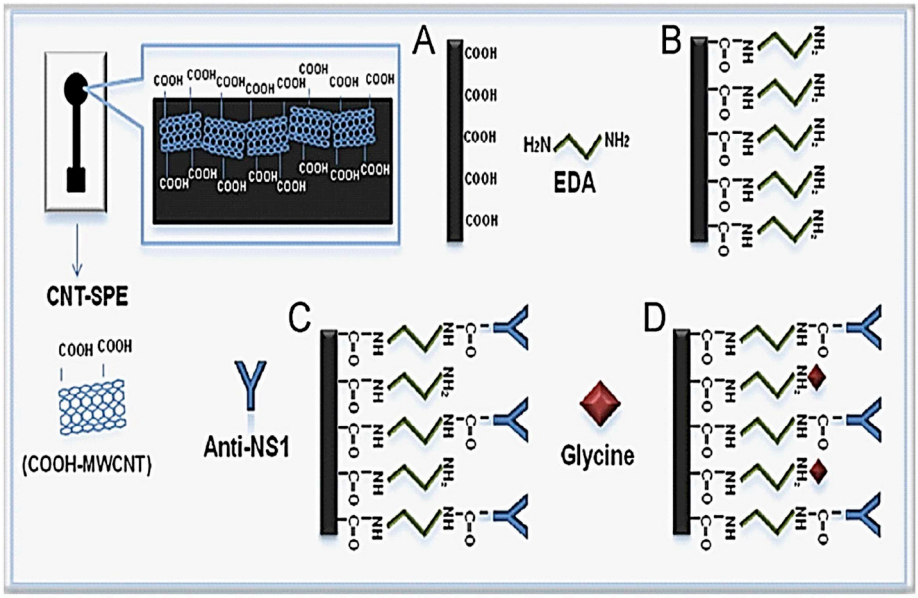

Fig. 2. Schematic illustration of an immunosensor tip to sense dengue virus NS1 protein. The sensor tip was a CNT-screen printed electrode (CNT-SPE); (A) bare CNT-SPE, (B) EDA film formation, (C) anti-NS1 immobilization, and (D) blocking with glycine. Herein, the EDA film with amine group was covalently bound with the anti-NS1. The other existing amine groups of the EDA film were utilized to form amine bonds with carboxyl groups of the CNT-SPE surface. Adopted from Dias et al. (2013). Copyright Elsevier.

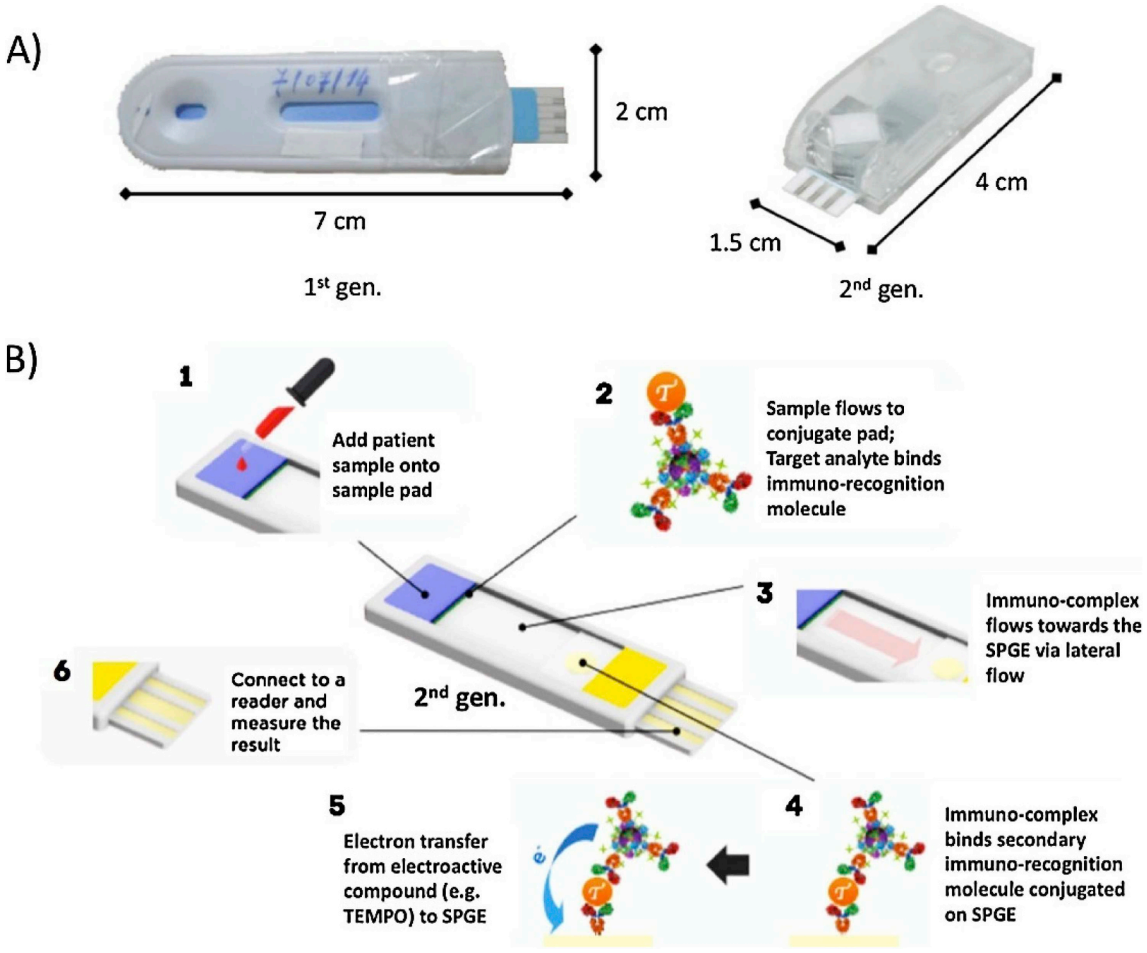

Fig. 3. A schematic representation of screen-printed gold electrodes (SPGE) based electrochemical lateral flow immunosensor for detection of dengue NS1 protein. Here, (A) 1st generation electrochemical lateral flow immunosensor prototype formulated with immunonanobeads (AuNPs-ab-Fc) and sandwich immunocomplex formation on SPGE with 7 cm long which is integrated with a cellulose paper strip including a lateral flow bed, a contact pad, a conjugate pad and a sample pad enclosed in a cassette (Sinawang et al., 2016) (B) 2nd generation electrochemical lateral flow immunosensor is 4 cm long miniaturized and more compact prototype with chemically modified PEG-stabilized and TEMPO-tagged AuNPs for point-of-care dengue NS1 protein detection in less than 30 min. Reproduced from Sinawang et al. (2018). Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

immunosensor (ELLI) with chemically modified PEG-stabilized and TEMPO-tagged AuNPs. This second-generation technology was based on the integration of SPGE into a shorter lateral flow setting, enabling the detection of target dengue NS1 protein in a convenient two-step process, self-contained assay, rapid (< 30 min) and quantitative (Sinawang et al., 2018) (Fig. 3b).

Differential pulse voltammetry (DPV) is another voltammetry technique, which measures the current immediately before each potential change and the current variation is plotted as a function of potential. Using DPV, a different approach to label-free electrochemical detection of DENV1 was made by Souza et al. (2011). They immobilized 18-mer single-stranded nucleic acid related to dengue virus gene-1 on activated pencil graphite. Then, the hybridization between the probe and its complementary oligonucleotides was analysed by guanine oxidation through DPV. The complementary oligonucleotides were quantified in a range between 1 and 40 nM with good linearity and LOD of 0.92 nM. Cheng et al. (2012) were the first to report an electrochemical biosensor for selective identification and quantification of DENV2 in the mosquito. From detail investigations on four different serotypes of dengue virus, they found DENV2 is one of the more prevalent and crucial types. Hence, the research aimed to construct a nanoporous alumina-modified platinum electrode with DENV2 monoclonal antibody (clone 3H5, isotype IgG), as biorecognition element for ultrasensitive detection of specific-dengue virus serotype (DENV2) by faradaic current response toward redox probe. They achieved LOD in plaque forming unit (pfu) of 1 pfu mL^{-1} with linear range 1 to 10^3 pfu mL^{-1} using DPV. Moreover, this biosensor is selective towards DENV2 with insignificant cross-reaction with non-specific viruses such as DENV3, West Nile virus and Chikungunya virus (Fig. 4). Another promising electrochemical immunosensor was developed on the gold film electrode obtained from a recordable compact disk for the early diagnosis of dengue haemorrhagic fever. Here, the anti-NS1 monoclonal antibodies were immobilized on the CD-trode via protein A and the analytical response was detected using DPV technique. The device showed a linear response from 1 to 100 ng mL^{-1} and LOD of 0.33 ng mL^{-1} with good reliability in serum samples (Cavalcanti et al., 2012a).

2.5. Impedimetric biosensor

In recent past, the electrochemical impedance spectroscopy (EIS) technique to detect analyte has gained popularity due to advantages over other electrochemical detection, where the biologically mediated redox reaction can be easily accessible to the analyte solution and in close proximity to the electrode surface. It determines the resistive and capacitive properties of materials upon perturbation of the system by a small amplitude sinusoidal AC excitation signal (~2–10 mV) and the wide range of spectrum can be obtained with respect to the applied frequencies. An impedimetric sensor was employed for the detection of dengue serotype 2 viruses by monitoring the impedance changes of the nanoporous alumina electrode towards the specific binding of DENV2 viral particles to its serotype 2-specific immunoglobulin G (IgG) antibody. The impedimetric response of the biosensor describes three distinct regions which match with the optimal equivalent circuit model; the electrolyte solution (R_s), the porous alumina channels (Q_1R_1) and the conductive electrode substrate layer (Q_2R_2). In particular, channel resistance R_1 and capacitance Q_1 change in response to the increase in DENV2 virus concentration and the Q_1 value doubles with the addition of DENV2. But the Q_1 remains unchanged for other non-specific viruses; West Nile and Chikungunya. Moreover, the linear relationship between R_1 and DENV2 concentration ranges from 1 to 900 pfu mL^{-1} according to the Langmuir-Freundlich isotherm model (Nguyen et al., 2012). Similarly, DENV2 and DENV3 were diagnosed by Peh and Li (2013) using the impedimetric technique. They fabricated nanoporous alumina membrane (60 µm thickness; 13 mm diameter) and coated both sides with a submicron layer of platinum, where the antibodies BSA and 2H2 were immobilized onto the surface of the membrane. The analysis was based on the change in pore resistance of the membrane and the results displayed a good correlation with the concentration of DENV2 and DENV3 viruses in pfu and show detection limit of 0.230 pfu mL^{-1} and 0.710 pfu mL^{-1} with the linear range between 1 and 900 pfu mL^{-1}. This thin piece of membrane sensor coupled with the simple electrochemical setup showed high sensitivity with a maximum detection time of 40 min. Darwish et al. (2015b) developed an electrochemical immunosensor for the direct detection of the dengue virus NS1 biomarker.

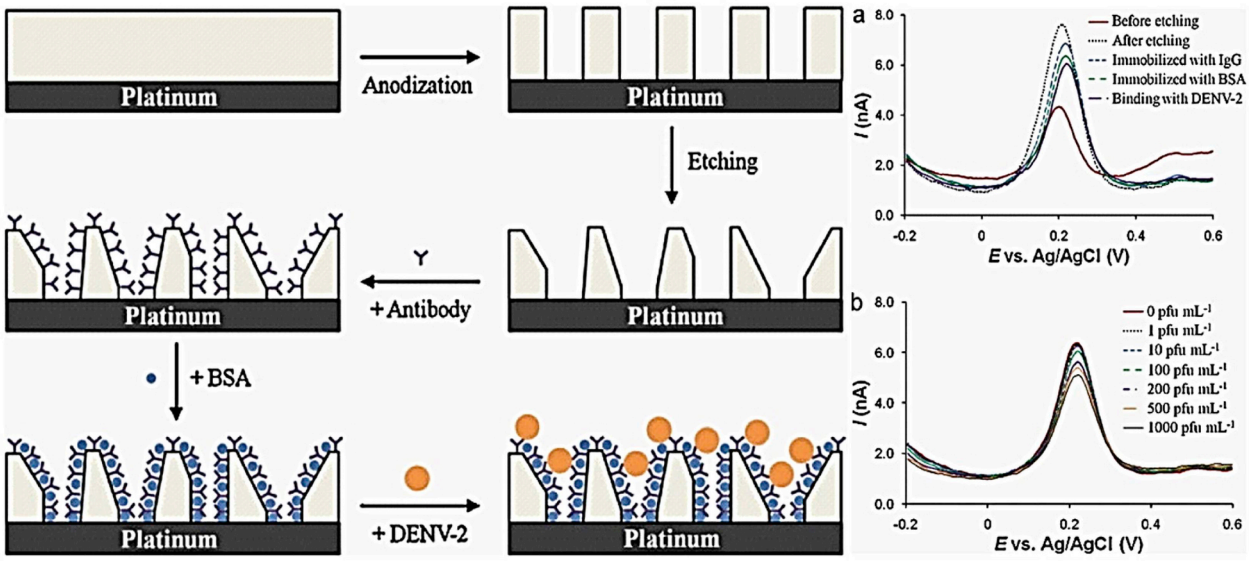

Fig. 4. An electrochemical membrane-based nanobiosensor with nanoporous alumina-modified platinum electrode for ultrasensitive detection of dengue virus. Anti-DENV2 monoclonal antibody such as clone 3H5 and isotype IgG is used as biorecognition element. Signal responses of electrode were derived from the DPV signals towards ferrocenemethanol (1.0 mM) in 0.1M phosphate buffered saline, (pH 7.4). (a) after each step of the biosensor construction procedure, DENV-2 concentration is 102 pfu mL^{-1}; and (b) after virus capture from solutions of different virus concentrations. Reprinted from Cheng et al. (2012). Copyright Elsevier.

This NS1 biosensor was fabricated in ITO electrode with a biosensing surface consisting of antifouling moieties and biorecognition molecules to enhance the specificity of analyte NS1 in human sera. The EIS exhibited a wide detection range (5–4000 ng mL^{-1}) with high reproducibility and good stability for 21 days at 4 °C. A different approach with DENV2 NS1 as specific and sensitive protein biomarker was used for the dengue fever diagnosis. The technique used polyvalent phage to identify unique affinity peptides that can bind NS1 protein and analysed the binding interactions using CV and EIS. The detection of NS1 proteins was monitored with the charge-transfer resistance of the sensor layer, where the R3#10 phage clones used in the study were much specific and high affinity for DENV2 NS1 proteins, as compared to wild type (Lim et al., 2018) (Fig. 5).

2.6. Potentiometric biosensor

Potentiometric biosensor measures the accumulation of a charge potential at the working electrode compared to the reference electrode in an electrochemical reaction. It works under equilibrium conditions and monitors the accumulation of charge at zero current created by selective binding in the surface of the electrode. The device has an electrochemical cell with two reference electrodes capable of measuring the potential across an ion-selective membrane that reacts with the charged ion of interest. For example, a potentiometric immunosensor was fabricated for the direct detection of NS1 from DENV2 virus using egg yolk immunoglobulin (IgY) as biorecognition element which was covalently immobilized over cysteamine modified disposable gold electrode with glutaraldehyde. The immunosensor exhibited a fast response, high sensitivity (3.2 ± 0.3 mV/μg mL^{-1}) and excellent LOD (0.09 μg mL^{-1}) with a linear range between 0.1 and 10 μg mL^{-1} for the direct diagnosis of dengue NS1 protein from real samples (Figueiredo et al., 2015).

3. Biorecognition elements for dengue virus sensor

3.1. Nucleic acid-based dengue biosensor

Nucleic acid such as DNA or RNA fragments are commonly used as biorecognition elements in dengue biosensor. It relies on either complementary base-pairing between the immobilized nucleic acid sequence and the analyte of interest. The nucleic acid used in biosensors could be either linear probe or stem-loop probe (Labib et al., 2016). A recent achievement in genosensor design with the utilization of nanotechnology provides a new direction to monitor the analyte. A genosensor commonly defined as a gene-based biosensor or DNA-based biosensor in which the DNA probes are immobilized in the sensor surface as a recognition element and measures the specific binding affinity. The binding formation or interaction occurs mainly between DNA-DNA, DNA-RNA, and protein-ligand molecules. For example, Singhal et al. (2017) demonstrated a genosensor based on ZnO/platinum-palladium modified ITO glass substrate; where the probe DNA which is common to all the four dengue serotypes were immobilized on the surface of the electrode for detection of consensus DNA sequence of dengue virus using methylene blue as an intercalating agent. Moreover, the probe DNA modified electrode served as a signal amplification platform for the detection of target hybridized DNA. The CV and DPV were used for the analysis of hybridization between the probe DNA and target hybridized DNA. The hybridization detected by reduction in current was generated by a interaction of anionic mediator that is methylene blue with free guanine (3′G) of ssDNA. The sensor showed a linear range between 1 and 100 μM with LOD of 4.3 μM and the limit of quantitation of 9.5×10^{-5} M. This genosensor facilitates the determination of all four dengue serotype infections (Fig. 6). Similarly, the silicon nanowire biosensor with novel molecular gate control was used for DNA detection of dengue virus. In this, the silicon nanowire was fabricated using nanolithography and the surface was functionalized by three-step procedure; such as surface modification, DNA immobilization and hybridization. These step-by-step process acts as a molecular gate control to establish the electrical detection for 27-mers base targets of dengue DNA oligomer. Here, the electrical detection is based on the changes in current, resistance and conductance of the biosensor due to accumulation of negative charges added by the immobilized probe DNA and hybridized target DNA. This DNA oligomer-based sensor demonstrated a wide detection range with LOD ~2.0 fM and sensitivity of 45.0 μ AM^{-1} (Nuzaihan et al., 2016).

In the past two decades, graphene is an attractive material in electrochemical biosensor and has been used in DNA sensing platform that enhances sensitivity, low LOD and long-term stability. Oliveira et al.

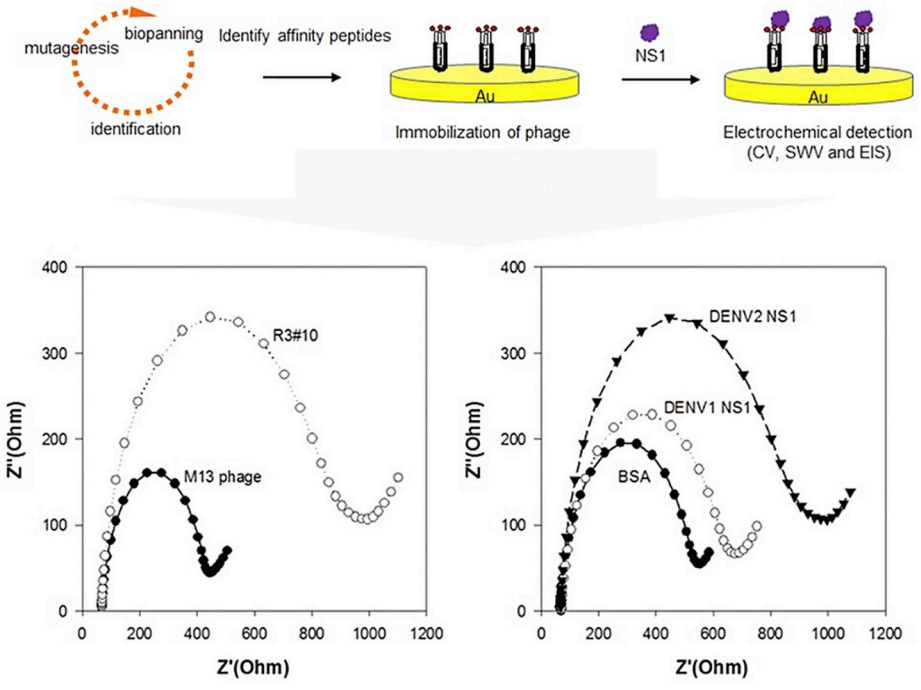

Fig. 5. A schematic illustration of electrochemical peptide sensor for direct detection of the biomarker NS1 during early stages of dengue virus infection. The selectivity test shows relative binding of M13 wild type phage and R3#10 phage to DENV2 NS1 protein, where the large increase in impedance was observed for R3#10 phage compared to M13 wild phage. A large impedance increase was observed upon addition of DENV2 NS1 and no dynamic change of impedance with DENV1 NS1 protein, while a small impedance changes with BSA (control) was found. Adopted from Lim et al. (2018). Copyright Elsevier.

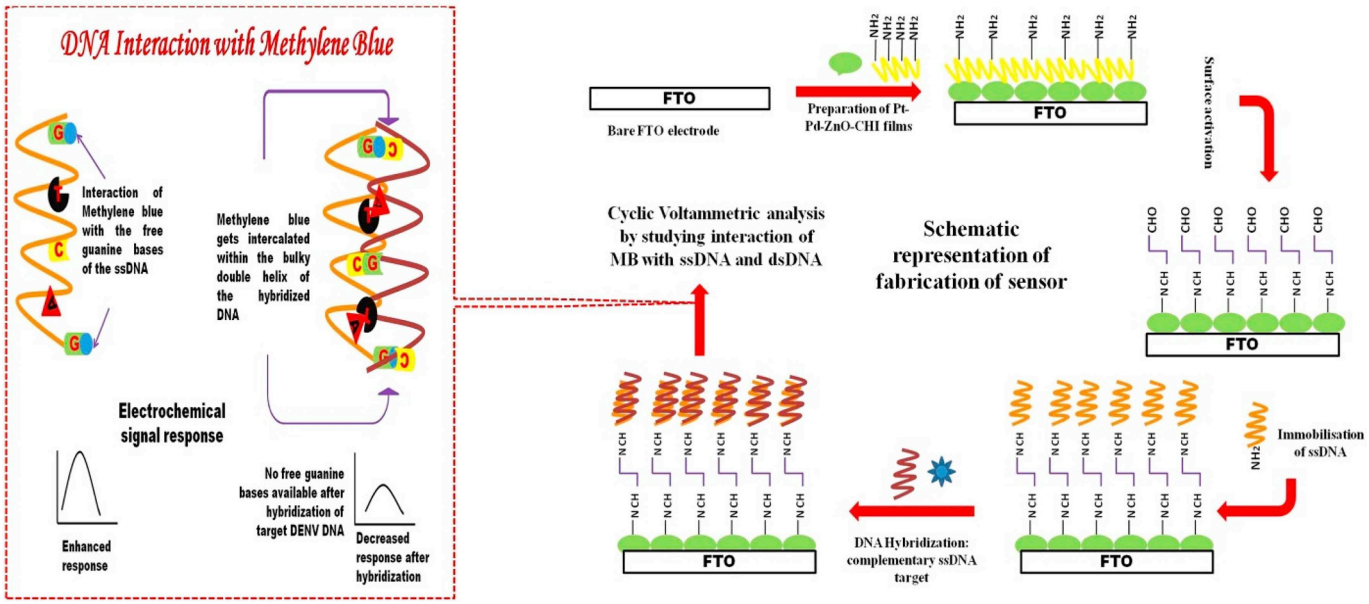

Fig. 6. The design and operation mechanism of the dengue virus genosensor; FTO glass plate was modified with ZnO/platinum-palladium and the probe DNA was immobilized over the electrode with an intercalating agent, methylene blue (MB). The hybridization between the ss probe DNA and ss target DNA forms double stranded DNA, at the same time MB gets intercalated within the bulky double helix of the hybridized DNA. The electrochemical response shows decrease in current due to intercalation of MB with double stranded DNA. Reprinted from Singhal et al. (2017) Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(2015) developed graphite-based DNA probe specific biosensor to sense DENV3 serotype. The 22-m sequence probe DNA was selected due to its highly conserved sequence and ability to detect envelope (E) gene, which is responsible for binding and fusion to host cell membrane, were immobilized onto the pencil-graphite electrode. The DPV analysed through hybridization reaction between the DENV3 DNA probe and the complementary DENV3 sequence; the peak current increase with an increase in the concentration of the target sequence (10 nM–500 nM) and exhibited the highest peak current of 135 ± 2.15 nA. The hybridization tests for selectivity was performed using complementary as well as non-complementary sequences revealed a highest significant difference in the voltammetric signal after hybridization (600 nA) when compared to the complementary DNA (135 nA), which was slightly lower than the probe-modified electrode (777 nA) having LOD of 3.09 nM. Moreover, the probe DNA-modified graphite electrode detected specific complementary sequences of the target DNA spiked in human serum. Another interesting DNA/RNA biosensor using graphene electrode was reported by Jin et al. (2016), where an oligonucleotide sequence was immobilized over 3-Aminopropyltriethoxysilane (APTES) functionalized graphene oxide on SiO_2. This genosensor exhibited enhanced dengue DNA and RNA detection with high sensitivity, superior selectivity and extremely low detection limit (1 fM).

Nanoporous anodized alumina with arrays of nanosized uniform pores has been widely used in biosensor owing to its numerous key factors such as ease fabrication, chemical stability, high surface area, and detection of biomolecules through interaction with active molecules. For example, nanoporous alumina and platinum wire electrode with 5′ aminated DNA probes were used for the electrochemical detection of dengue virus. As dengue virus genome is single-stranded RNA genome, therefore unique ss-31 mer DNA complementary sequences of DENV1 and DENV3 RNA genome were selected as the target analyte. The biosensor showed a linear range over 6 orders of magnitude with ultrasensitive LOD of $9.55\times\ 10^{-12}$ M for quantification of ss-31 mer DNA sequence (Rai et al., 2012). Similarly, an integrated membrane sensing platform based on an anodic aluminium oxide membrane with DNA probe sequence was also used for the detection of dengue virus DNA. The EIS was employed to monitor the impedance changes within the nanopores upon DNA binding; where the pore resistance (R_p) linearly increases in response towards the increase in the concentration of the target DNA in the range of 1×10^{-12} to 1×10^{-6} M with LOD of 2.7×10^{-12} M of 31-mer complementary analyte. This biosensor selectively differentiated the complementary sequence from target sequences with 21 base mismatch and single base mismatch (Deng and Toh, 2013).

Recently, the interdigitated electrodes are widely used as sensing capacitive structure since they are more preferred and highly effective in obtaining dielectric properties. The electrode is fabricated using photolithography, where the steps include photoresist coating, exposure and etching. In this pattern, the electric signals generated by the sensing material have to be detected by interdigitated electrodes. As a modification, Odeh et al. (2017) developed an integrated biosensor for DENV2 DNA detection in real time. In this approach, they synthesised Cu_2CdSnS_4 quaternary alloy nanostructures and deposited on an oxygen-etched silicon substrate (O_2/Si). Here, the quaternary alloy acts as a matrix for the immobilization of biorecognition element, dengue-specific DNA probe. Using amperometric analysis, the sensor successfully recognized the single-stranded DNA concentration from 100 fM to 10 nM with LOD of 17 nM. Moreover, the biosensor was found to be highly sensitive (24.2 μA $nM^{-1}cm^{-2}$), speedy response and low power consumption. In addition, the advancements in nanotechnology and improved understanding of nanosized biomolecular structures attained a position in biosensors due to their high-sensitivity and extremely low LOD. In particular, nanomaterial facilitated electrochemical detection of DNA hybridization has an exceptional impact on biomedical fields. For example, the zetamolar detection of dengue consensus primer by the electrochemical platform using electrospun semi-conducting manganese (III) oxide nanofibers for DNA hybridization detection showed an extremely low LOD 120×10^{-21} M with linear detection ranges between 1 nM and 1 μM (Tripathy et al., 2017) (Fig. 7).

3.2. Antibody-based dengue biosensor

Antibodies are the serum proteins produced by two types of blood cells, B-lymphocytes and plasma cells which empowered with the

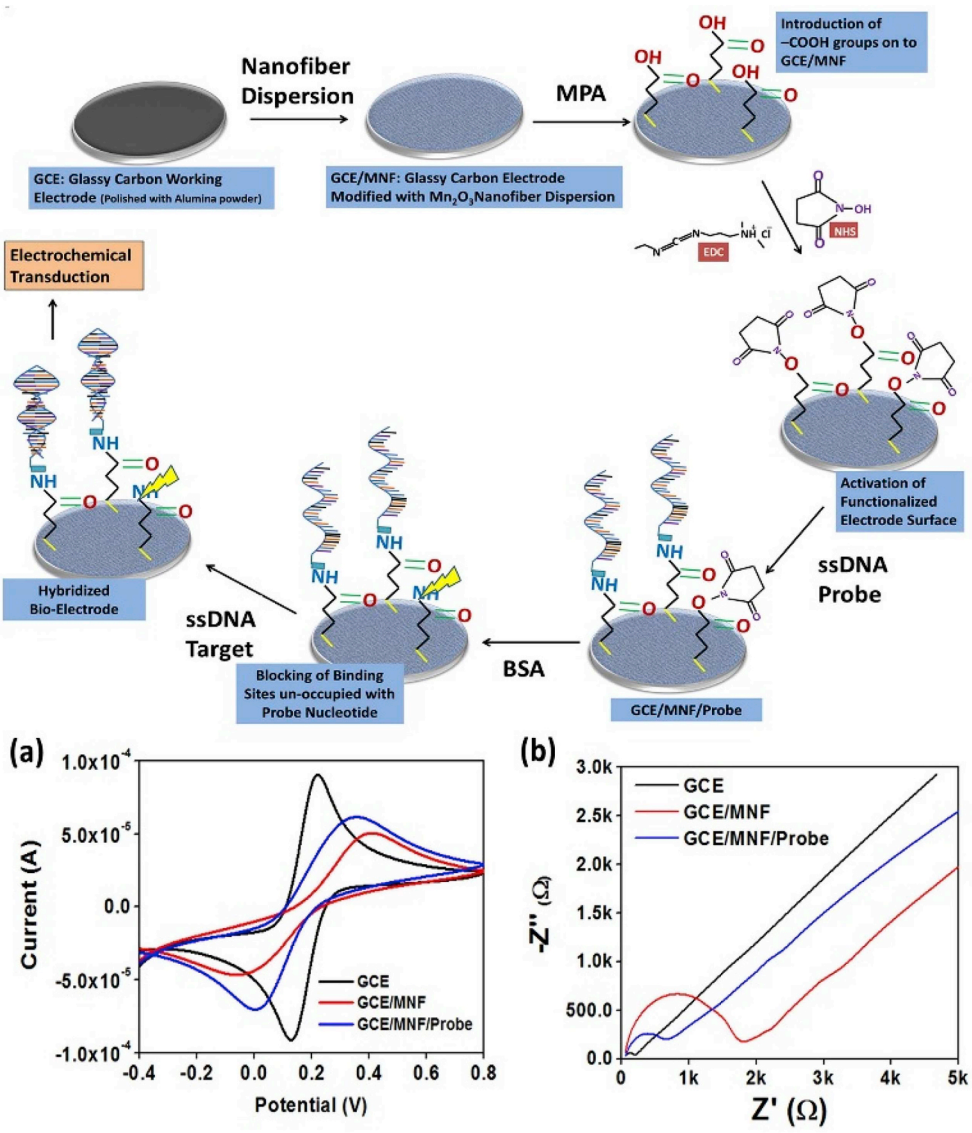

Fig. 7. Scheme of DNA-hybridization sensor, where the glassy-carbon electrode was modified with Mn_2O_2 nanofiber dispersion. This modified electrode in MPA which forms a self-assembled monolayer and it was subjected to NHS/EDC treatment in order to activate the carboxyl functional groups. Over the activated carboxylic group of the electrode, the ssDNA probe gets covalently immobilized by means of amine groups of the nucleotide. The bioelectrode was hybridized with complementary target DNA prior to electrochemical analysis. (a) CV analysis and (b) EIS studies of bare working electrode (GCE), nanofiber modified working electrode (GCE/MNF) and probe modified working electrode (GCE/MNF/Probe). Adopted from Tripathy et al. (2017) Copyright Elsevier.

capacity to recognize, by stereospecific association. It is produced in response to the foreign substance which is termed as the immunogen, because it evokes an immune response and in particular, an individual antibody will recognize only one substance known as an antigen. In immunoglobulins, whose selective binding strategies towards antigens are used in immunological analysis techniques. In electrochemical immunosensors, either antibodies or their complementary binding partners are used as biorecognition elements in combination with electrochemical transducers. To date, enormous immunoassay electrochemical systems are available due to their high specificity, extreme affinity and great sensitivity (Ronkainen et al., 2010). For example, IgG antibodies (Y-shaped glycoproteins of Mw ~ 150 kDa) are produced by a host in response to the presence of an antigen such as chemical compounds, proteins and particulate matter. It comprises of two antigen-binding fragments; heavy chain variable region (V_H) and the light chain variable region (V_L) which fold to provide a perfect fit for the specific antigen. Antibodies could be monoclonal or polyclonal; monoclonal antibodies are produced by a different immune cell and unique to the parent cell, whereas polyclonal antibodies are produced by different immune cells (Labib et al., 2016). These antibodies are immobilized onto the surface of the sensor either covalently or non-covalently; the antibodies interact with specific antigen/analyte to produce signals. Antibodies are very sensitive to environmental conditions; hence it has to be immobilized on a solid surface without affecting their biological binding efficiency (Grieshaber et al., 2008; Trilling et al., 2013). In most of the immunosensors, the common antibody immobilization methods are biotin-streptavidin linkages (Park et al., 2011) or conductive polymers (polypyrrole) by means of covalent bonding (Ramanavicius et al., 2014). Recently, Palomar et al. (2018) reported a dengue virus biosensor using dengue antibody, DENV2-NS1 glycoprotein. The biosensor electrode was fabricated utilizing CNT and the antibodies were immobilized by polypyrrole and N-hydroxysuccinimido 11-(pyrrol-1-yl) undecanoate film. The EIS and CV analysis tested in bovine blood plasma resulted in good linearity in a wide concentration range (10^{-13}- 10^{-5} g mL^{-1}) (Fig. 8).

As a different approach, Luna et al. (2015) used water-soluble 4-mercaptobenzoic acid (MBA) modified nanoparticles on self-assembled cysteine monolayers with DENV antibody as a sensing platform. Here, the anti-DENV antibodies were immobilized by covalent bonding between the amino group present in the amino acid residue of the biomolecules and the carboxylic group of the MBA. The CV analysis confirmed the ability to detect four dengue serotypes DENV1, DENV2, DENV3, and DENV4. Meanwhile, the performance of the biosensor was monitor through the changes in charge transfer resistance (ΔR_{CT}) and provided the quantitative information about the association of dengue virus and antibody. In this, the ΔR_{CT} (%) was calculated using the

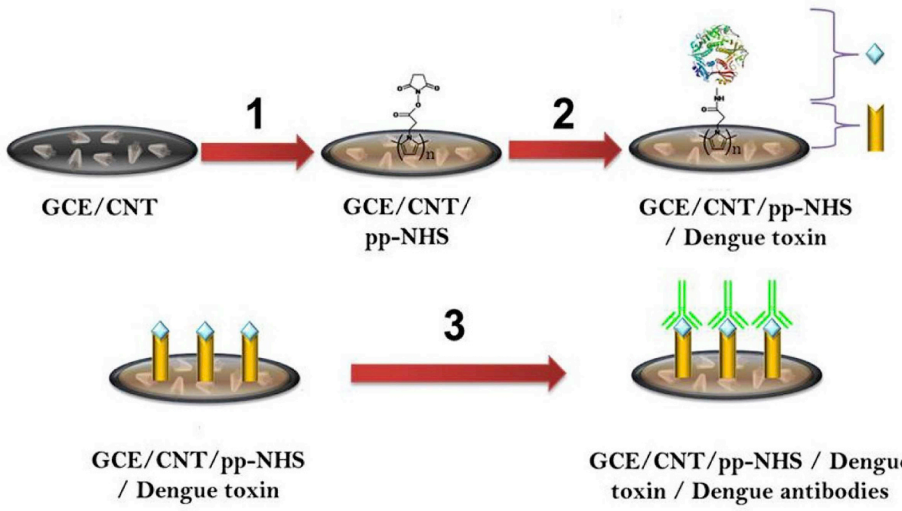

Fig. 8. Schematic representation of different fabrication steps of the dengue virus biosensor. (1) CNT coating via electrogeneration of polypyrrole-NHS (N-hydroxysuccinimido 11-(pyrrol-1-yl)undecanoate) film, (2) immobilization of the dengue toxin via peptide coupling between an amine function of the toxin and the NHS function of the polymer, (3) detection of specific dengue toxin antibodies. Reprinted from Palomar et al. (2018) Copyright Elsevier.

following equation (1),

$$\Delta R_{CT}\ (\%) = \frac{RCT(DENV) - RCT(Antibody)}{RCT(Antibody)} \quad (1)$$

where, R_{CT} (DENV) is the charge-transfer resistance of the sensor system after DENV recognition, and R_{CT} (Antibody) is the charge-transfer resistance of sensor system before exposure to different dengue. The results revealed a good correlation coefficient and showed better interaction between antigen and antibody for all DENV immunosensors (Fig. 9). Similarly, the polymer matrix composites/graphene oxide immunosensor for all dengue virus serotypes reported the linear dependence of R_{CT} verses virus concentrations ranged between 1 and 2×10^3 pfu mL^{-1} DENV with the LOD of 0.12 pfu mL^{-1} (Navakul et al., 2017) (Fig. 10).

In another study, an impedimetric label-free immunosensor based on anti- NS1 modified gold electrode was developed for the detection of viremia in dengue diagnosis. Here, anti-NS1 was immobilized in a mixed self-assembled monolayer consisting of 11-mercaptoundecanoic acid for covalent anti-NS1 attachment and 6-mercaptohexanol as a spacer. The impedance spectra recorded in the presence of $[Fe(CN)_6]^{3-/4-}$ showed linear range between 0.01μgmL^{-1} and 1.00 μgmL^{-1} with a sensitivity of 10.4% $decade^{-1}$ and 30 ngmL^{-1} LOD for NS1 diluted in a neat serum (Cecchetto et al., 2015) (Fig. 11). Santos et al. (2018) developed a label-free electrochemical dengue sensor with the dual maker for rapid sensitive quantification of NS1 and IgG. They used a self-assembled monolayer containing PEG moieties and a tethered redox thiol, both markers are detectable across clinically relevant levels within seconds with no impairment of analytical quality such as linearity, sensitivity, and variance (Fig. 12).

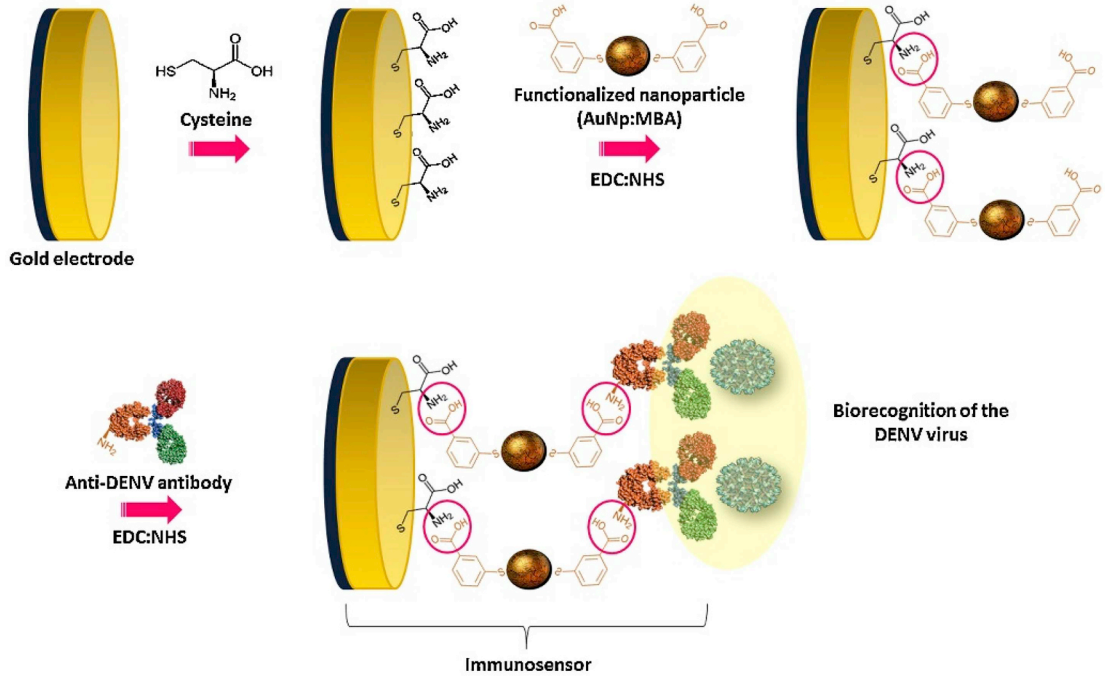

Fig. 9. A schematic representation of immunosensor based on gold nanoparticles and self-assembled monolayers of cysteine for all four types of dengue serotype detection using anti-DENV antibody as biorecognition element. Reprinted from Luna et al. (2015) Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

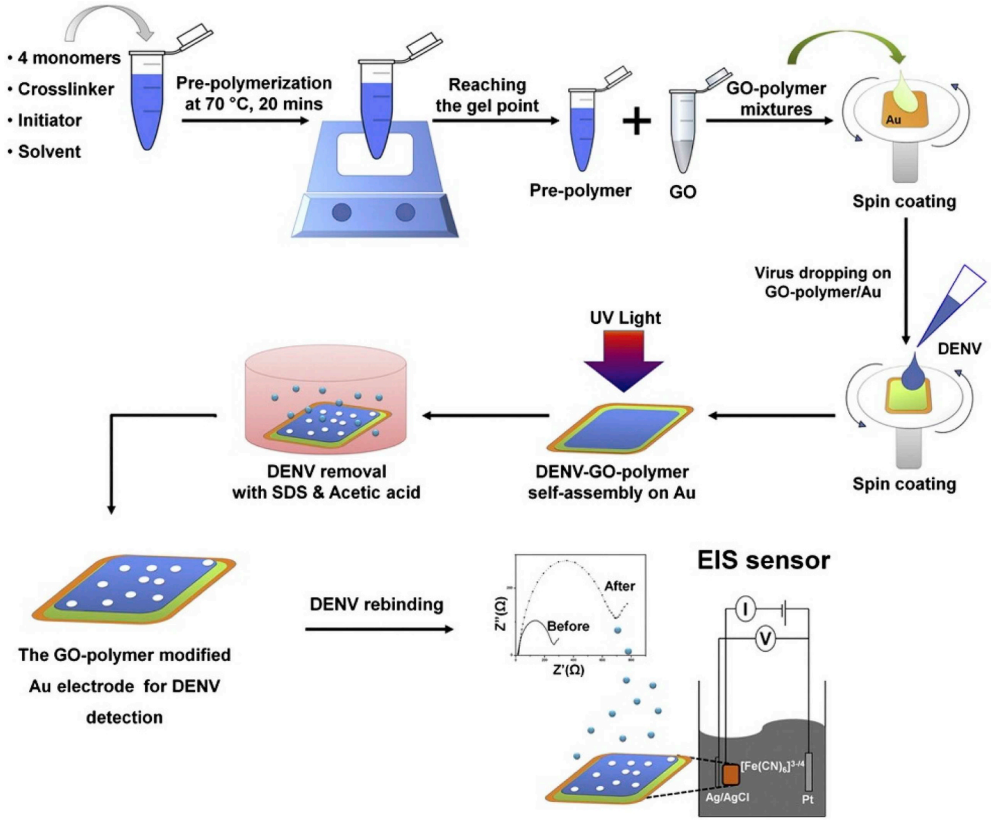

Fig. 10. A dengue virus biosensor developed on gold electrode coated with graphene oxide, where the graphene oxide was mixed with polymers and immediately the dengue virus was added before allowing a self-assembly process. Here, the EIS sensing can differentiate the dengue virus serotypes. Adopted from Navakul et al. (2017). Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

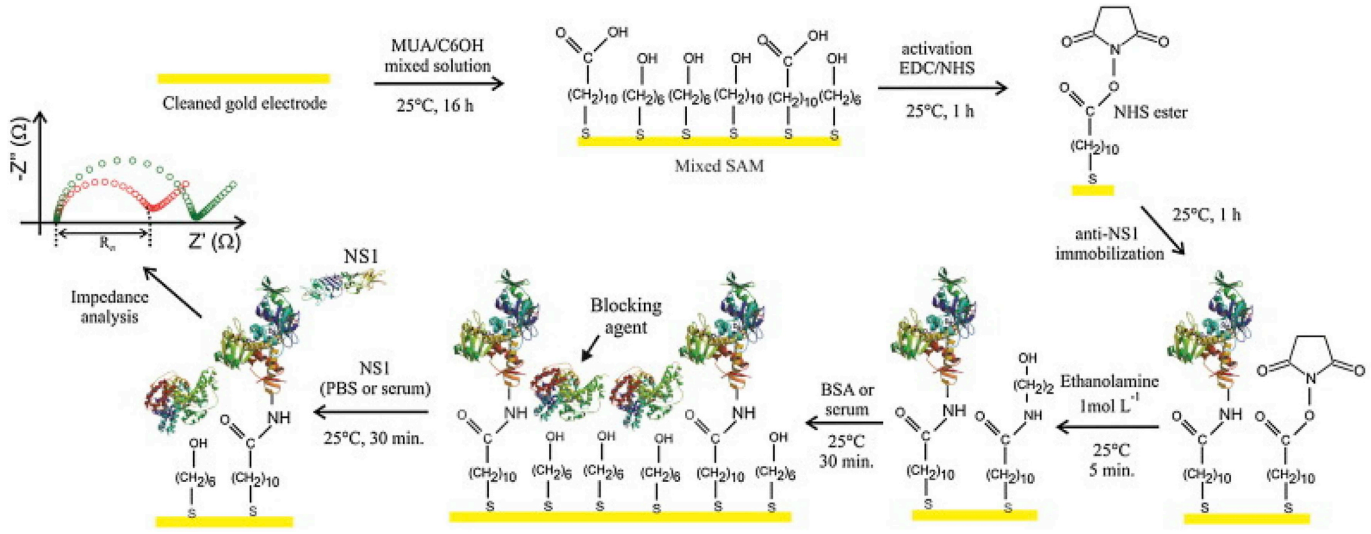

Fig. 11. Schematic representation of modified anti-NS1 gold electrode for dengue virus detection. The surface of gold was modified with thiol-SAM structures, in which 11-mercaptoundecanoic acid served as a receptor-supportive layer for anti-NS1 attachment, and 6-mercaptohexanol served as a spacer layer. Anti-NS1 immobilization was achieved through standard EDC/NHS bioconjugation, and the nonspecific sites were blocked with 0.1% BSA or neat serum. The faradaic impedance measurements were carried out using a redox probe in solution $[Fe(CN)_6]^{3-/4-}$. By the Nyquist plot, R_{CT} was measured from approximation of the diameter of the semi-circle of Z'. Reprinted from Cecchetto et al. (2015) Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Lectin-based dengue biosensor

Lectins are natural glycan-recognizing proteins found in viruses, bacteria, fungi, animals, and plants (Andre et al., 2015). Lectins are natural interacting partners of glycan; hence it is very essential to fractionate glycoproteins from other non-glycoproteins and essential for robust glycan measurements (Palecek et al., 2015). It is a large family of proteins not involved or nor active in the immune system, but has complex specificities that can recognize not only different monosaccharides within glycan chain, yet form different linkages between saccharide monomers or glycan branching (Belicky et al., 2016). Due to the specific characteristic features, lectins have been applied for the analysis and detection of glycoproteins, P-glycoprotein, carcinoembryonic antigen, α-fetoprotein, cancerous cells and viruses (Taniguchi and Kizuka, 2015). In recent years, lectins are used as biorecognition element in all types of biosensors; such as glucose biosensor (Zhang

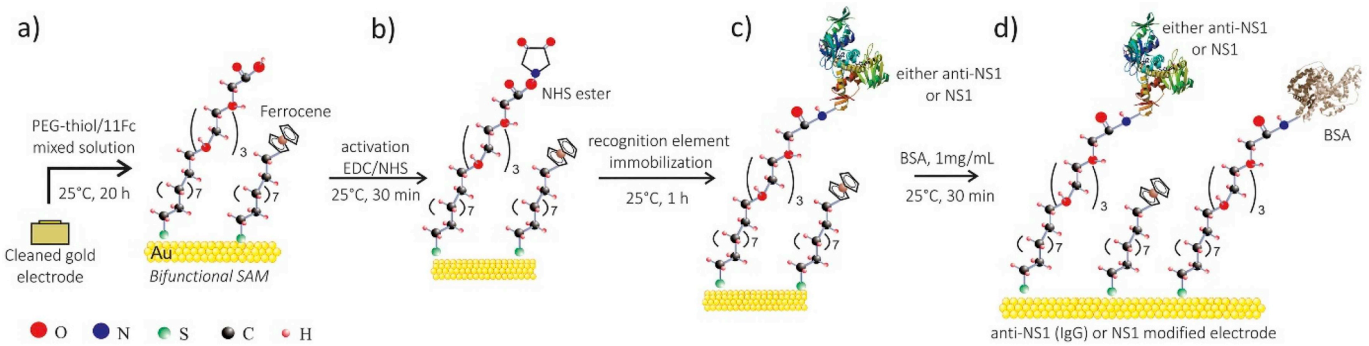

Fig. 12. A schematic illustration for step by step preparative depiction of the anti-NS1 and NS1 modified electrode surfaces. (a) Functionalization with a mixed solution containing 11Fc and PEG-thiol. (b) Activation of carboxylic groups prior to coupling with amino groups present in the recognition element as shown in (c) and (d) Blocking non-specific sites with BSA (1 mg/mL). Reprinted with permission from Santos et al. (2018). Copyright Elsevier.

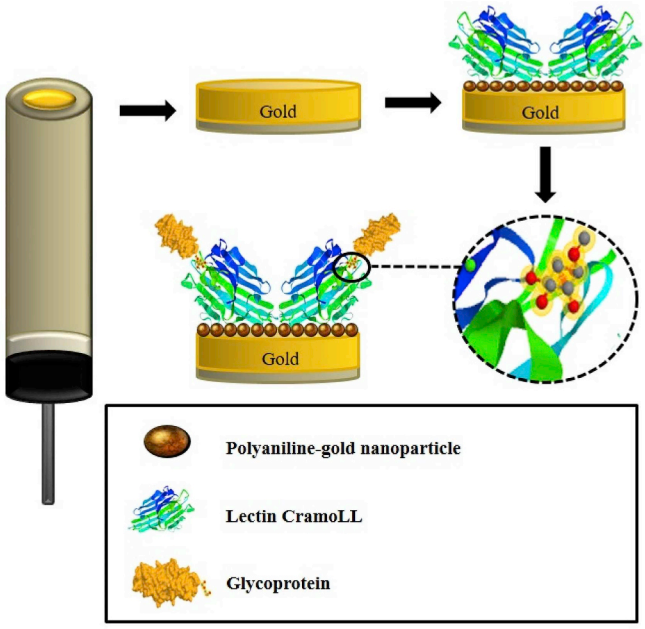

Fig. 13. Schematic model a lectin-based biosensor fabricated over gold electrode. Here, the bare gold electrode was polished with alumina and coated with CramoLL solution containing AuNPs-PANI composites. Adopted with permission from Avelino et al. (2014). Copyright Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al., 2013), bacterial sensor (Wang and Anzai, 2015), virus sensors (Hong et al., 2015; Hushegyi et al., 2016), cytosensors (Hu et al., 2013; Xue et al., 2010) etc. Among various types of lectins, concanavalin A (Con A) is one of the most utilized and studied lectins, which is commonly extracted from jack bean seeds (*Canavalia ensiformis*). This has a molecular weight of 110 kDa with an overall size of 6.7 nm × 11.3 nm × 12.2 nm and it exists as a tetramer with a higher degree of organization at pH 7.4 (Ballerstadt et al., 2006). In a dengue virus biosensor, Con A lectin was immobilized on the gold electrode using polyvinyl butyral and investigated the sensing response by CV and EIS in a phosphate buffer solution containing 10 mM of $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture as a redox probe. Here, the variation in R_{CT} was used to distinguish the sensor response for the different sera from patients infected by dengue fever and dengue haemorrhagic fever. In addition, the evaluation of 3D impedance spectra showed the best response for all different sera samples (Oliveira et al., 2009). On the other hand, biosystem based on Con A lectin and lipid membranes was developed to recognize glycoproteins from the serum of patients with DENV1, DENV2, and DENV3. This biosystem showed the redox probe reactions with a wide linear response to different concentration sera of DENV1, DENV2, and DENV3, respectively. Among these three serotypes, a higher impedimetric response was observed to glycoproteins present in the DENV3 serotype (Luna et al., 2014).

CramoLL is another type of lectin which is isolated from *Cratylia mollis* (Family: *Leguminosae*) seeds. Oliveira et al. (2011) described a biosensor system to analyse the interactions between CramoLL lectin and fetuin for the detection of glycoprotein from the serum of patients contaminated with dengue serotypes, DENV1, DENV2, and DENV3. The CramoLL lectin was immobilized on a gold electrode modified with Fe_3O_4 nanoparticles and polyvinyl butyral as an occlusive layer. The Fe_3O_4-CramoLL-PVB biosystem exhibited a wide linear response to different concentration sera of dengue serotypes with a higher response to glycoproteins present in DENV2 serotype. Similarly, Avelino et al. (2014) reported a biosensor developed by immobilizing CramoLL on gold nanoparticles/polyaniline electrode for the detection of dengue serotypes, DENV1, DENV2, and DENV3. The analytic performance of the interaction between AuNps-PANI hybrid composites and CramoLL was determined using the impedimetric sensor and it is efficient to differentiate the glycoproteins of dengue serotypes (Fig. 13). A similar study was carried out by Andrade et al. (2011) using another type of lectin, BmoLL, which is isolated from *Bauhinia monandra*. A sensitive and selective biosensor was developed with the immobilization of BmoLL over gold nanoparticles-polyaniline hybrid composite. Here, the hybrid composite was applied over bare gold electrode surface by chemical adsorption and the negatively charged BmoLL was electrostatically adsorbed to the positively charged nanocomposite-modified surface. The AuNPs-PANI-BmoLL was able to detect all the three dengue virus serotypes especially DENV2 with a high degree of specificity, reproducibility, and selectivity (Fig. 14). Thus, the investigation based on naturally occurring lectins as biorecognition element in dengue virus biosensors is promising and significant to attribute the different patterns of glycoproteins in the sera infected by dengue virus.

4. Advantages of electrochemical dengue biosensor

The diagnosis of the dengue serotypes is utmost important to know the evolutionary behaviour of virus and infections. The commercially available diagnosis is mainly based on the specific antibody to dengue, viral RNA detection, and the soluble dengue viral antigen (Rathakrishnan and Sekaran, 2013). The presence of dengue infection in blood samples are analysed using anti-dengue IgM, where in case of early phase (within 5 days of illness) of dengue; RT-PCR is used to test dengue viral RNA and serotyped by type-specific multiplex PCR (Chakravarti et al., 2012). In most cases, the diagnosis for dengue infection is established by culture of the virus, polymerase-chain-reaction or by means of serological assays. An acute patient serum with a sufficient amount of virus collected before fever onset in a short period is required for the successful isolation and culture of the virus. Moreover,

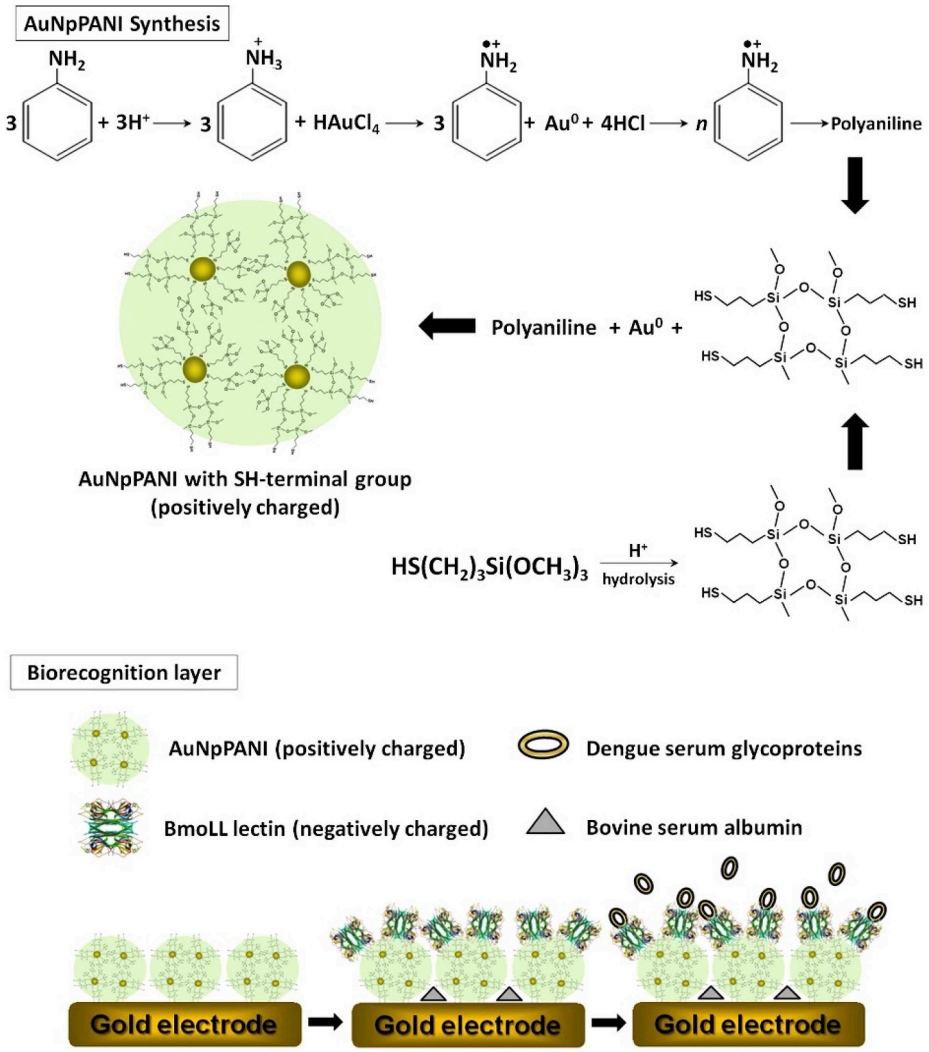

Fig. 14. Schematic diagram representing the mechanism for the reaction of aniline with chloroauric acid trihydrate and the step-by-step details of AuNpPANI-BmoLL-BSA-DEN biosensor system. Reprinted with permission from Andrade et al. (2011). Copyright Elsevier.

the bio-safety level 3 laboratory with expensive laboratory equipment, chemicals and professional are necessary to carry out these techniques, so it is both time and labour intensive (Peeling et al., 2010). The analysis using RT-PCR protocols show problems with a false-negative result because of the variation in the dengue virus serotypes. In some cases, the PCR inhibitors such as antibiotics and haemoglobin will result in loss of sensitivity due to their direct conjugation with DNA and DNA polymerases. The PCR can only be used to detect dengue infections during the early phase of their infections, and it is not efficient after 5–7 days (Anez et al., 2008). In serological detection, the antibodies such as IgM, IgG and NS1 are diagnosed from serum and other body fluids. False-positive results in the serology test using IgG and IgM have been mainly attributed to cross-reactivity with co-circulating antibodies from other flaviviruses antibodies as a result of prior infection or vaccination. On other hands, the ELISA method is rapid and cheaper, but it is time-consuming and requires the analysis of paired sera from the acute and convalescent phase to confirm a positive dengue infection (Ortega et al., 2017). On the whole, the existing conventional methods are expensive, laborious, require long sample preparation protocol and need sophisticated laboratory with bulky and costly equipment. Meanwhile, the electrochemical biosensors are inexpensive, easy to use, minimal sample preparation procedure and utilize the fully automated and portable system.

5. Conclusion and future outlooks

In the past two decades, the biosensing methodologies have achieved huge development for pathogenic microorganism detection that offers promising candidates for future disease diagnostics. This article provides a general overview of electrochemical detection of dengue virus, which is being developed intensively day-by-day. A detailed outlook on electrochemical sensing technology, fabrications, design and the basic principles of transduction were presented in this review. Furthermore, few attempts were made to reflect the types of electrochemical sensing techniques used for dengue diagnosis with examples. In addition, the importance of biorecognition elements in dengue biosensor and different types of biological molecules used in the sensor were described.

Although some progress has been made in dengue electrochemical biosensing, further advances are still needed that should improve the performance of the biosensors. At this point, the bottleneck of a biosensor is the lack of fundamental understanding of the biorecognition molecule, which is important for high-throughput biosensing applications. The simultaneous detection of dengue serotypes requires less or even no-cross reaction between the ligand and target biomolecules. Hence, more studies should be consecrated to understand; how a nanoscale biorecognition element binds, how it changes when immobilized, how it can be manipulated to occur perfect orientation to

execute the biorecognition event, how it remains viable and stable in ambient conditions for long period and how it attains better selection interaction of biorecognition molecule and target analyte biomolecule. Also, significant as well as innovative sampling strategies are required to simplify the process during electrochemical detections. Furthermore, incorporating nanomaterials into the dengue sensors highlights the necessity to assess the nanoscale structures with eco-friendly and biocompatibility nature. Meanwhile, the nanosized materials with engineered proteins, molecular-imprinted polymers, signalling aptamers, etc., are crucial for the improvement of efficient biosensor. Finally, the innovation in the area of highly selective, long-term stable, environmentally susceptible and cost-effective dengue virus sensing will be extremely important for further commercial applications.

Declaration of competing interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

J.R. Anusha: Conceptualization, Writing - original draft. **Byung Chul Kim:** Validation, Visualization, Writing - review & editing. **Kook-Hyun Yu:** Project administration, Resources, Validation, Writing - review & editing. **C. Justin Raj:** Conceptualization, Supervision, Validation, Visualization, Writing - review & editing.

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