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TEMPO-based immuno-lateral flow quantitative detection of dengue NS1 protein

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ABSTRACT

The development of a rapid, affordable, and sensitive diagnostic kit for point-of-care is important in most healthcare settings. In this follow-up paper to our previous work on quantification of dengue NS1 protein via impedimetric measurement, our present technology aims to provide quantification by utilizing proprietary stabilized and improved electroactive immunonanoparticles that bind to the target biomarker and subsequently move along toward the biofunctionalized screen-printed gold electrodes (SPGE) to generate an amperometric signal. The SPGE functions simultaneously as a signal transducer and a solid-state support for a sandwich ELISA-like immunoassay. The successful immunocomplex formation is then recorded electrochemically using a potentiostat, whereby the signal was contributed by the presence of a more hydrophilic redox label than ferrocene, namely radical TEMPO (TEMPO•), on the formulated nanoparticles. In this paper, a bifunctional ligand, thiolated polyethylene glycol (PEG-thiol) polymer, was used to stabilize 20 nm gold colloidal nanoparticles (AuNPs) in the formulation. PEG was incorporated to not only prevent the salt-mediated AuNPs aggregations, but also provide an anchor for antibody and redox species conjugation. To-date, we have successfully miniaturized a 3D-printed prototype device able to sensitively detect and quantify dengue NS1 protein with only 0.6 μL human clinical serum samples diluted in a volume ratio of 1:100 (PBS diluent) in less than 30 min with a simple cyclic voltammetry analysis. The positive and negative samples were also tested with ELISA, a gold standard validation method, which means that the development of the prototype is a promising start as a point-of-care diagnostic technology.

1. Introduction

Dengue affects 2.5 billion of the world's population, with an annual addition of 50–100 million infections of which 500,000 dengue haemorrhagic fever (DHF) cases and 22,000 deaths occur, mostly among children. The World Health Organization (WHO) has classified dengue virus as one of the most prevalent arthropod-borne viruses in the world [1,2]. Dengue is endemic in over 100 countries in Asia-Pacific, America, Africa, and the Caribbean [3]. All four dengue serotypes (DENV1, DENV2, DENV3, and DENV4) are able to cause dengue fever worldwide, with the induction of a primary immune response leading to lifelong protection against infection with the homologous serotype. However, when a secondary infection with a heterologous serotype happens, antibody response is often non-neutralizing and in some cases, can lead to the more severe complications such as DHF or dengue shock syndrome (DSS). [4].

Recently, several dengue diagnostic tests based on the detection of NS1 protein have become commercially available. NS1 is a highly conserved glycoprotein of flaviviruses that possesses both group-specific and type-specific determinants [5,6], including dengue, Japanese encephalitis, yellow fever, and tick-borne

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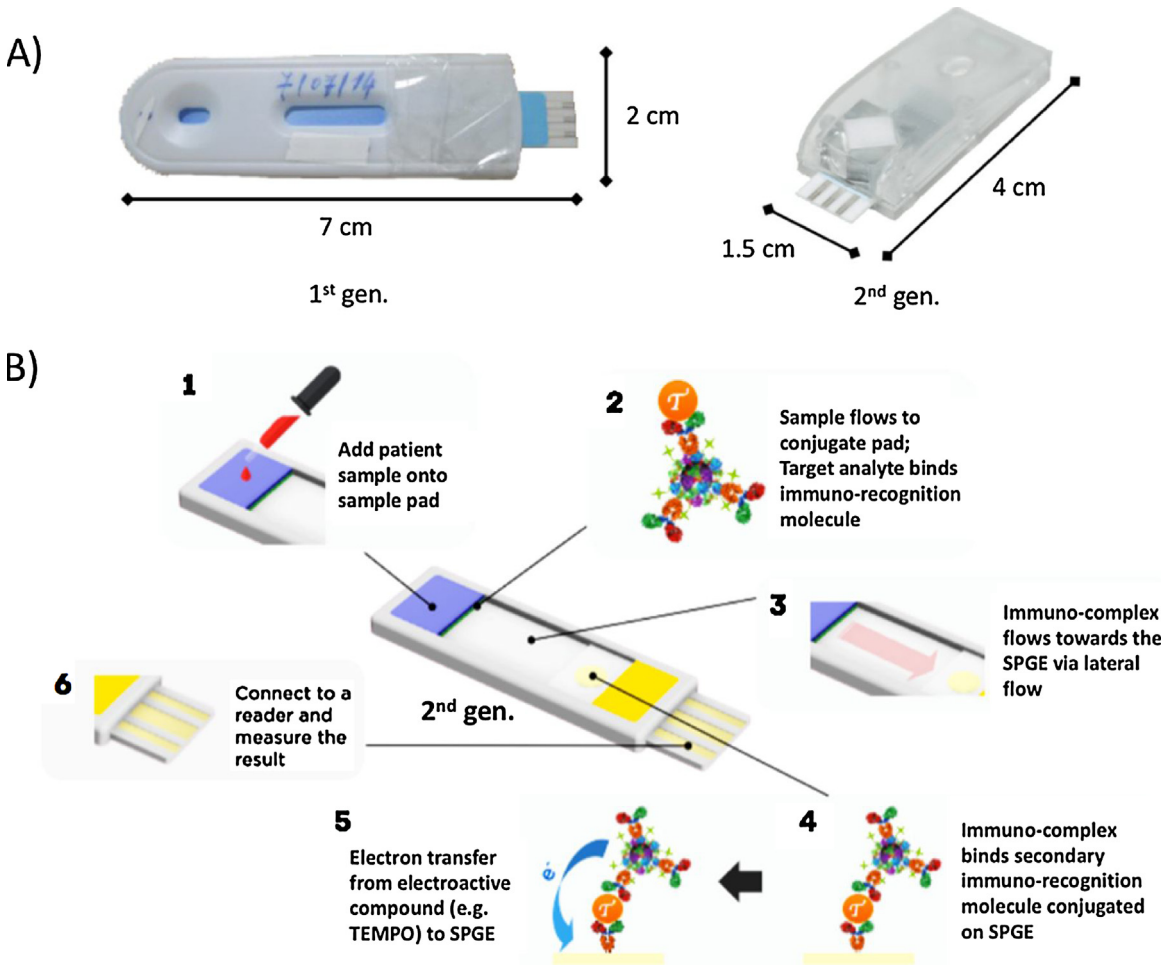

Fig. 1. A) Evolution of electrolateral flow immunosensor (ELLI) from a 7-cm long 1st generation prototype to a 4-cm thumb-sized 2nd generation prototype. B) ELLI's 2nd generation assay schematic and rationale: A miniaturized and more compact prototype with chemically modified PEG-stabilized and TEMPO-tagged AuNPs for an amperometric point-of-care dengue NS1 protein detection in less than 30 min.

encephalitis virus [7]. The flavivirus NS1 protein has been recognized as an important immunogen in infections [8] and antibodies elicited against NS1 have been shown to play a role in protection against the disease [9,10]. Moreover, it has been targeted for use in diagnosing dengue in humans because this protein is secreted in the bloodstream and can be detected on day 1 after the onset of fever, even before viral RNA [11–13]. Currently, diagnosis of dengue fever is most commonly performed with serological tests detecting anti-dengue antibodies (IgM and IgG) via either ELISA or rapid lateral flow strip tests while additional biomarkers such as NS1 protein is being added to aid in the accuracy. Therefore, a simple, 1–2 step, point-of-care-format is recommended for disease surveillance in communities as a first line of defense [14], especially when there is no specific treatment for dengue/severe dengue. WHO suggests that early detection and access to proper medical care lowers fatality rates below 1% [15]. Although ELISA provides greater sensitivity in dengue diagnosis, it is more costly, slower (at least 4-h turnaround time and usually much more), and requires trained staff in a laboratory. The rapid lateral flow strip format is more suitable for point-of-care use, and thus is more widely used. However, one should note that although the rapid lateral flow strip has been fittingly satisfactory providing qualitative diagnosis, there is an exigency of NS1 protein quantitation in dengue triage. Individuals are more prone to severe cases when the number of circulating NS1 protein is higher, notwithstanding the fact that the infecting serotype and patients' immune status are equally pivotal [16–18].

There is a growing need for quantitative sensors whereby optical sensors using various methods (surface plasmon resonance, fiber optics, colorimetry, fluorescence, bio and chemiluminescence) have been oftentimes utilized [19–27]. However, their applications are for now limited to laboratory use, mainly due to their higher costs. Moreover, fluorescence-based assays often exhibit optical interference, require filter and light excitation, and pose photobleaching risk [28], while bio- and chemiluminescence-based assays are faced with impractical storage and handling of the probes and reagents which ensure enhanced sensitivity [29]. Many have reported the development of optical paper-based point-of-care devices; however, their field application is hindered by the cost of both reader and probes [30–32]. Therefore, research should be focused on developing a quantitative, yet affordable, rapid, portable, and user-friendly biosensor so as to be in accordance with ASSURED diagnostics rapid test criteria by WHO [33].

Recently, electrochemical sensors have been of utmost interest providing an attractive quantitation means to analyze a biological sample due to the direct conversion of a biological event to an electronic signal and the possibility of system miniaturization so as to be cheap, small, portable, and user-friendly, epitomizing the glucose sensor [34,35]. Since then, fabrication of electrochemical biosensors has been reported for a number of applications employing different reporters [36–44]. However, their major drawback is the multiple washing steps that may amount to error and hence reducing their use for a point-of-care setting. This has been