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Mini Review

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Redox Probe-Free Immunosensors Based on Electrocatalytic Prussian Blue Nanostructured Films Fabricated in One Step for Zika Virus Diagnostics

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Abstract

Zika virus (ZIKV) is a major global health concern due to its high transmission rate, including spread via blood, saliva, urine, semen, and vertical transmission. In some cases, ZIKV is associated with microcephaly, neuropathy, and Guillain-Barre syndrome. There is no vaccine and the disease is difficult to control, especially given the co-circulation of dengue viruses. The similarities between the two parvoviruses cause severe cross-reactivity. Considering that it is an established highly sensitive and practical tool for, in this study, we developed a sensor platform with intrinsic redox activity that facilitates the readout of measurements. Prussian blue (PB) has an excellent ability to form an electro catalytic surface and provide a redox probe solution in voltammetric measurements. Here, PB is incorporated into a chitosan-carbon-nanotube hybrid to form a Nano composite that is drop-cast onto a screen-printed electrode (SPE). The immunosensor detected the envelope protein of HeZIKV in a linear range of 0.25–1.75 μg/ml ($n = 8$, $p < 0.01$) with a detection limit of 0.20 μg/ml. The developed immunosensor is a novel method of electrochemical measurement without additional redox probe solutions and is suitable for use in point-of-care diagnostics.

Keywords: Zika virus; Immunosensor; Prussian blue; Envelope protein; Point-of-care diagnosis

Introduction

Due to its high prevalence in tropical regions of Africa, the Americas, and Asia, Zika virus (ZIKV) is endemic and considered an emerging disease of public health importance [1]. In addition to vector transmission by Aedes mosquitoes and other arthropods, ZIKV can be transmitted through sexual intercourse, blood transfusion, saliva, urine, and vertical transmission, making its spread difficult to control. ZIKV infection is a self-limiting disease. Most cases range from no symptoms to mild symptoms such as fever, joint pain, headache, malaise, and rash, but can progress to a more severe form. In 2016, a correlation between severe congenital microcephaly and potential mechanisms of infant malformations was identified during an outbreak in the Americas, particularly in north-eastern Brazil. In addition, spontaneous abortion and documented male fertility decline are also associated with ZIKV infection currently; the gold standard for ZIKV diagnosis is a molecular method that uses reverse transcription technology (PCR) to detect viral RNA. However, molecular testing is limited in some endemic countries due to high analytical costs and the need for skilled laboratory personnel to prepare samples and manage results [2]. Furthermore, molecular testing is only suitable for the acute phase, when the virion replication stage is still very active. After the viraemic stage, serological assays to detect ZIKV antibodies or virion proteins are more appropriate. ZIKV consists of seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and a capsid (C), envelope (E) and precursor membrane (prM) Serological detection of ZIKV can discriminate either of these proteins or antibodies from humoral responses. However, multiple viruses are endemic in many countries, including dengue, Zika, yellow fever and West Nile. Due to the similarity of these viruses, it is difficult to exclude cross-reactivity between them, especially to distinguish between dengue and Zika. Recently, the ZIKV-E protein was identified as a good biomarker for his ZIKV. ZIKV is the largest virion and is involved in many aspects of the viral cycle, such as membrane fusion and binding mediation, making it an excellent choice for serological diagnostics. The ZIKV-E protein stands out among the structural proteins as it offers an excellent ability to discriminate between ZIKV and other flaviviruses Immunochromatographic testing (ICT) has been identified as an attractive tool for rapid diagnosis, especially during outbreaks, as recently demonstrated in the coronavirus pandemic from March 2020 [3]. ICT has the advantage of providing results quickly (within about 15-30 minutes) and in most cases a more user-friendly point of care supporting countries with limited or poor resources. You can use as a test. Several ZIKV assays using chromatographic techniques are now commercially available, such as the Chembio DPP Zika IgM system (DPP Zika ICA; Chembio, Medford, NY, USA) and others that detect antigens or antibodies in blood and saliva. Although ICTs have many advantages, they are limited in terms of sensitivity compared to laboratory immunoassays that perform detection using enzymatic reactions (ELISA) or electrochemiluminescence (ECLIA); in addition, these analyzers can provide quantitative results, and their sensitivity and specificity are higher than those of ICT-based devices. However, they also have several disadvantages, such as high infrastructure costs, additional steps for sample processing, the longer time required to obtain results, and the need for sophisticated equipment that substantially increases the cost per test, restricting their use in ZIKV-endemic areas and their distribution in places with limited resources. In contrast, immunosensors based on nanomaterial's can mitigate all these drawbacks while achieving the same sensitivity as ELISA immunoanalyzers, and they have the advantage of being a portable technology, thus facilitating therapeutic management. In recent years, several immunosensors for ZIKV have been developed with optical and acoustic mechanisms, using multimode interference waveguides and electrochemical transduction. Immunosensor technology has made remarkable progress, but several challenges and shortcomings must be overcome for clinical application. It requires many steps and

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is not suitable for practical use. In this work, we developed a redox-free electrochemical sensor by investigating Prussian blue (PB) as a stable electro active species intrinsically immobilized on the sensor surface. Species derived from oxidation of PB can generate a faradaic current response, generating a diffusive charge flux to the sensor surface. Building on our previous work and that of other authors using active redox surfaces this new design offers a faster response, a simpler readout system, and a sensor surface It provides fewer steps to avoid passivation in and minimize erroneous responses. PB is a chemically recognized compound that promotes reversible redox reactions. It is called "artificial peroxidase" because it is a chemical substance with high catalytic activity. However, when PB is simply adsorbed onto the sensor surface, loss of reversibility can easily occur as this chemical is sensitive to periodic potential fluctuations and even simple pH. One strategy is to incorporate it into compatible polymers that allow for fixation and stability [4,5] .

Materials and Methods

Iron Ferro cyanide (III)Fe4(Fe(CN)6)3(PB), Chitosan (CHI), Glycine, Potassium ferricyanide (K3(Fe[CN]6)), Potassium Ferro cyanide (K4(Fe[CN]6) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant ZIKV E protein and anti-ZIKV E protein antibody were purchased from EastCoasr Bio (North Berwick, UK). Tissue culture antigen ZIKV Vero E6 was purchased from Bei Resources (Manassas, VA, USA). Multi-walled carbon nanotubes (CNT) and screen-printed electrodes (SPE) were obtained from Dropsens (Oviedo, Spain). A blocking solution containing glycine (100 mmol.L −1) was used in Tris-hydrochloride buffer (Tris-HCl), (100 m/mol.L −1, pH 6.5). Phosphate buffered saline (PBS) (100 m/mol l -1, pH 7.4) was used in all experiments to dilute samples [6,7].

Structural and morphological analysis

For morphological characterization, micrographs were obtained by scanning electron microscopy (SEM) using an AURIGA model (Zeiss, Jena, Germany) with cross-beam scanning. The microscope includes a field emission gun (FEG) electron column and a focused ion column (FIB) to provide superior image quality compared to traditional filament sources. MEVs were operated at potentials between 0.1 and 30 kV with a resolution of 1 nm at 15 KV. The sample was placed on a stub and secured with glassy carbon tape. The stub containing the sample was metallized using a sputter coater (model Q15OR PLUS, Quorum Technologies LTD, Lewes, UK) for 90 seconds before taking the image. A resolution of 200 nm and 1 μm was chosen for acquiring images. Synthesis of CHI-CNTPB Nano composites [8-10].

A CHI-CNT@PB Nano composite solution was prepared by mixing solutions A and B. Solution A was prepared by adding 3 mg CHI to 3 mg CNT dissolved in 2 ml acetic acid (2.5% w/v). , and homogenize for 1 h in an ultrasonic bath at room temperature (~25 °C). Solution B was obtained by dispersing 12 mg of PB in 1 ml of 2.5% (v/v) acetic acid and subjecting the mixture to an ultrasonic bath for 1 hour at room temperature (approximately 25 °C). The final mixture was obtained by adding Solution A to Solution B to a total volume of 3 mL. This mixture was subjected to an ultrasonic bath at room temperature (~25 °C) for 1 h to form CHI-CNT@PB Nano composites with final concentrations of 1 mg mL-1 CHI and 1 mg mL-1 CNT. 4mgmL-1PB [11-13].

Discussion and Conclusions

Synthesis and optimization of experimental conditions

PB is widely used in the development of electrochemical biosensors due to its catalytic activity. However, in most cases, PB and its derivatives become unstable when subjected to cyclic voltammetry, potential fluctuations, and pH changes parameters that affect the diagnostic sensitivity and specificity of biosensors. The search for alternative means to improve PB stability without affecting electro catalytic properties has already been described. CHI polymers were used in this study due to their known biocompatibility and ability to immobilize biomolecules with amine groups. In addition, CHI can increase the stability of films that aggregate on anionic surfaces, and thus can easily bind to PB. Although CHI is considered a non-conducting polymer, it can improve electron transfer when incorporated into highly conductive materials. However, its use in the construction of Nano composites should be carefully considered to provide stability without significantly degrading the electrical signal. Cyclic voltammetry (CV) is an electrochemical technique widely used in research to understand electron transfer processes. In this study [14-17], the electro catalytic activity of CHI-CNT@PB film components was validated by the presence of redox peaks in measurements using 100 m/mol L−1 KCl as the electrolyte support. The electro catalytic activity of PB in CHI-CNT@PB films was evaluated by comparison.

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