Biosensors and Bioelectronics 92 (2017) 47–53

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Piezoelectric immunochip coated with thin films of bacterial cellulose nanocrystals for dengue detection

Cleverton Luiz Pirich[a,b], Rilton Alves de Freitas[b], Roberto Manuel Torresi[c], Guilherme Fadel Picheth[b], Maria Rita Sierakowski[a,b,*]

[a] *Biochemistry and Molecular Biology Department, Federal University of Paraná, Centro Politécnico, CEP 81531-980 Curitiba, PR, Brazil*
[b] *BioPol, Chemistry Department, Federal University of Paraná, P.B 19032, Centro Politécnico, CEP 81531-980 Curitiba, PR, Brazil*
[c] *Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo (USP), CP 26077, 05513-970 São Paulo-SP, Brazil*

ARTICLE INFO

Keywords:
Dengue
Biosensors
Quartz crystal microbalance
NS1 protein
Cellulose nanocrystals

ABSTRACT

Low-cost piezoelectric devices, such as simple frequency monitoring quartz crystal microbalance (QCM) devices, have good clinical utility as fast diagnostic tools for the detection of several diseases. However, unspecific antigen recognition, poor molecular probe adsorption and the need for sample dilution are still common drawbacks that hinder their use in routine diagnosis. In this work, piezoelectric sensors were previously coated with thin films of bacterial cellulose nanocrystals (CN) to provide a more sensitive and adapted interface for the attachment of monoclonal immunoglobulin G (IgGNS1) and to favor specific detection of non-structural protein 1 (NS1) of dengue fever. The assembly of the immunochip surface was analyzed by atomic force microscopy (AFM) and the NS1 detection was followed by quartz crystal microbalance with (QCM-D) and without energy dissipation monitoring (QCM). The CN surface was able to immobilize 2.30 ± 0.5 mg m^{-2} of IgGNS1, as confirmed by AFM topography and phase images along with QCM-D. The system was able to detect the NS1 protein in serum with only 10-fold dilution in the range of 0.01–10 µg mL^{-1} by both QCM and QCM-D. The limits of detection of the two devices were 0.1 µg mL^{-1} for QCM-D and 0.32 µg mL^{-1} for QCM. As a result, QCM-D and QCM apparatuses can be used to follow NS1 recognition and have good potential for more sensitive, fast and/or less expensive diagnostic assays for dengue.

1. Introduction

Dengue fever affects over half of the world's population, causing thousands of deaths every year in urban and semi-urban areas (Antunes et al., 2015; WHO, 2016). This disease is endemic and widespread in subtropical and tropical countries, with higher dissemination rates in South Asia, Africa and South and Central America (Decker, 2012). The dengue virus (DENV) belongs to the *Flavivirus* genus along with members responsible for yellow fever, Zika fever and West Nile fever, all transmitted by arthropods (Kuhn et al., 2002). In particular, DENV is transmitted by *Aedes* sp. mosquitoes and four distinct serotypes are present (DENV I, II, III and IV), all capable of causing dengue fever. After infection with any of the DENV serotypes, the patient acquires long-term immunity, but against only one specific serotype (Gubler, 2006).

The general symptoms of the disease are fever and severe joint pain, though more drastic ones can occur (e.g., shock syndrome) in a second infection caused by distinct DENV serotypes (Kuhn et al., 2002). Unfortunately, the correct diagnosis still represents a problem since the symptoms are nonspecific and can be easily confused with other diseases, such as the simple flu (Bhatt et al., 2013). The currently available diagnostic tools are viral isolation, detection of immunoglobulins by ELISA produced against DENV and the detection of virus molecules (e.g., RNA or DNA) by RT-qPCR. Although these techniques display high accuracy in dengue diagnosis, they are time-consuming, expensive and require highly trained personnel for correct execution (Parkash and Shueb, 2015).

According to the World Health Organization (WHO), 3.9 billion people in 128 countries are at risk of infection, so the development of faster, cheaper and more accurate diagnostic tools is necessary (WHO, 2016).

Among the several biomarker methods utilized in dengue diagnosis, the detection of non-structural protein 1 (NS1) of DENV is one of the most employed strategies (Alcon et al., 2002; Gelanew et al., 2015; Lapphra et al., 2008). The NS1 antigen is the best known and studied biomarker of dengue and is present in the blood serum at 1–

* Corresponding author at: Biochemistry and Molecular Biology Department and BioPol, Chemistry Department, Federal University of Paraná, Centro Politécnico, CEP 81531-980 Curitiba, PR, Brazil.

E-mail address: mariarita.sierakowski@gmail.com (M.R. Sierakowski).

http://dx.doi.org/10.1016/j.bios.2017.01.068
Received 2 December 2016; Received in revised form 27 January 2017; Accepted 31 January 2017
Available online 03 February 2017
0956-5663/ © 2017 Elsevier B.V. All rights reserved.

10 μg mL^{-1} on the first day after infection (Darwish et al., 2015). Once infected, the host cell expresses the virus's NS1 protein (M_w ≈45 kDa) and secretes it into the bloodstream (Alcon et al., 2002; Saito et al., 2015). Furthermore, this antigen is highly conserved among the four serotypes, and many anti-NS1 monoclonal antibodies are currently able to distinguish the serotypes in a single test (Parkash and Shueb, 2015).

Recent efforts have been made to perform NS1 recognition through portable, low-cost and accurate biosensors (Sinawang et al., 2016; Wong et al., 2016; Zhang et al., 2015). In particular, piezoelectric biosensors such as quartz crystal microbalance (QCM) sensors offer all of these technical advantages and combine fast analytical procedure with real-time monitoring of biomarker detection, allowing quantitative screening (ng/cm^2) of adsorption phenomena (Bragazzi et al., 2015). More sophisticated QCM analysis is able to follow several overtones, screen energy dissipation (QCM-D) and play an important role in the development and biosensor validation (Bragazzi et al., 2015).

However, DENV diagnosis in complex samples requires the prevention of non-specific interactions along the sensor's surface, a usual drawback found in current QCM analysis. In fact, several studies describe further surface modifications of QCM sensors have been reported, such as improved sensitivity and reduced interference in NS1 recognition of serum samples after coating the sensor's surface with cibacron blue to remove serum albumin and IgG (Cecchetto et al., 2015; Wu et al., 2005). Distinct coating techniques can also provide more sensitivity and reduce sample interaction, such as improved roughness (more antibodies attach per unit of area) or greater surface area (Cheng et al., 2012).

An attractive and functional material for biosensor coating is cellulose nanocrystals (CN). These can be isolated from pristine cellulose (extremely abundant, renewable and biocompatible) by several different treatments (e.g., chemical, enzymatic or mechanical), resulting in nanoparticles with needle shape (e.g., length: 200–1000 nm and width: 5–20 nm) and improved mechanical properties (> 70% crystalline index and > 100 mPa Young´s modulus), providing a versatile surface to attach biomolecules (Mariano et al., 2014).

CN have also been described as forming thin nanofilms with different roughness and organization degrees on piezoelectric sensors by spin-coating techniques, providing a simple and fast way to functionalize these surfaces with different molecules. Furthermore, such films have been reported to exhibit piezoelectric properties comparable to piezoelectric sensors such as quartz (Csoka et al., 2012; Rajala et al., 2015).

In this paper, we propose the functionalization of existing piezoelectric sensors available in the market with thin films of CN to anchor monoclonal immunoglobulin against NS1 dengue antigen. The system was evaluated as a biosensor to recognize NS1 antigen in real time by piezoelectric disturbance. The correlation results between QCM with (QCM-D) and without (QCM) dissipation monitoring were evaluated along with surface analyses by atomic force microscopic (AFM) imaging. We found that thin films of CN present a more suitable platform to perform dengue diagnosis, with lower blood serum dilution than so far reported for piezoelectric sensors. The NS1 antigen was effectively recognized by the proposed platform without the requirement of dissipation monitoring, which can thereby open potential application to design cheaper and simpler piezoelectric devices for clinical diagnostics.

2. Materials and Methods

2.1. Materials

Bacterial cellulose was provided by Membracel® and converted into pulp by mechanical treatment (Pirich et al., 2015). Ammonium persulfate, acetic acid, sodium acetate, dihydrogen phosphate, hydrogen phosphate, ethanolamine chlorate, *n*-hydroxysuccinimide, n-ethyl-n´-(3-dimethylaminopropyl) carbodiimide hydrochloride, and bovine serum albumin (BSA) were purchased from Sigma Aldrich®. The monoclonal immunoglobulin anti-NS1 (IgGNS1) and NS1 antigen were purchased from ABD Serotec®. All solutions and dispersions were prepared with ultra-purified water (18.2 MΩ.cm^{-1}, Millipore Milli-Q purification system). The pHs of phosphate buffer saline (PBS) and sodium acetate (NaOAC) buffers were adjusted to 7.4 and 4.5, respectively. Blood serum of healthy patients was collected to simulate testing with the addition of purified NS1 antigen in the concentrations of 0, 0.01, 0.1, 1.0 and 10.0 μg mL^{-1}. The serum samples were diluted with PBS, according to necessity.

2.2. Methods

2.2.1. Cellulose hydrolysis

Dispersions of pulp sheets of bacterial cellulose (20 mg mL^{-1}) were ground in a Supermasscolloider mill (Masuko Sangyo MKCA6-2), 20 times at the lowest distance between the discs (~1 μm). The milled cellulose was added to a heated ammonium persulfate solution (1 mol L^{-1}, 60 ± 1 °C) during 120 min. The resulting dispersion was transferred to a cold ultrapure water bath (4 °C) for quenching. Next, the suspension was centrifuged (4,600×g for 15 min) and the supernatant was discarded. The sediment was redispersed in ultrapure water and centrifuged again (repeated 2x). Then, the sediment was redispersed in ultrapure water and submitted to dialysis (dialysis tubing with a molecular weight cutoff of 12–14 kDa) against ultrapure water, until the pH remained neutral for at least 12 h. The resulting suspensions were then sonicated for 10 min and centrifuged at 4,600×g for 15 min, to remove large particles. In this step, the sediment was discarded and the supernatant was collected. The resulting dispersion was stored at 4 °C with $CHCl_3$ (20 μL mL^{-1}).

2.2.2. Conductivity titration

Conductivity titration was employed to determine the oxidation degree (OD) on the surface of the isolated CN. The carboxyl groups of CN dispersion (2.6 mg mL^{-1}) were converted to acidic form with hydrochloric acid to a final concentration of 0.01 mol L^{-1}. Then, the dispersion was titrated with constant addition of NaOH solution (0.1 mol L^{-1}) and the conductivity changes were monitored. The OD was calculated by the following equation:

$$OD = 162(V_2 - V_1)c[w - 36(V_2 - V_1)c]^{-1} \quad (1)$$

where, V_1 and V_2 are the amounts of NaOH (L), c is the NaOH concentration (mol. L^{-1}), and w is the weight of the CN (g), 162 corresponds to glucose molecular weight and 36 is the difference of anydroglucose molecular weight and glucuronic acid sodium salt moiety.

2.2.3. ATR-FTIR spectroscopy

Infrared spectroscopy was used to confirm the oxidation of CN by ammonium persulfate. The spectra were recorded with a Vertex 70 FTIR spectrometer (Bruker Co., Billerica, USA) equipped with an attenuated total reflectance (ATR) accessory. All spectra were obtained using freeze-dried CN in acidic form (pH 2.0), with 16 scans and resolution of 4 cm^{-1} in the range of 400 – 4000 cm^{-1}.

2.2.4. Immunochip surface assembly

MHz AT-cut quartz crystals coated with gold were cleaned at 70 °C with a heated piranha solution composed of H_2O:NH_4:H_2O_2 (7:1:1, v:v:v) for 30 min and rinsed with deionized water and vacuum dried in an oven. Next, the piezoelectric discs were mounted on a homemade spin-coating device and 100 μL of polyethylenimine solution (2 mg mL^{-1}) was added to its surface. The solution was kept static for 10 min for adsorption. Afterward, the piezoelectric disc was

Download English Version:

https://daneshyari.com/en/article/5031575

Download Persian Version:

https://daneshyari.com/article/5031575

Daneshyari.com