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Biosensor based on lectin and lipid membranes for detection of serum glycoproteins in infected patients with dengue

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ABSTRACT

In this work, we developed a biosystem based on Concanavalin A (ConA) and lipid membranes to recognize glycoproteins from the serum of patients contaminated with dengue serotypes 1, 2 and 3 (DENV1, DENV2 and DENV3). The modified gold electrode was characterized using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and atomic force microscopy. Morphological analyses of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), DPPC-ConA, DPPC-ConA-DENV1, DPPC-ConA-DENV2 and DPPC-ConA-DENV3 revealed the existence of a non-uniform covering and large globules. EIS and CV measurements have shown that redox probe reactions on the modified gold electrodes were partially blocked due to the adsorption of lipid-ConA system and reveal the interaction response of the immobilized ConA to the presence of glycoproteins of dengue serum. The biosystem exhibited a wide linear response to different concentrations of sera of dengue serotypes 1, 2 and 3. A higher impedimetric response to glycoproteins present in dengue serotype 3 was observed. Our results demonstrate the applicability of lectin and lipid membranes to the development of biosensors for dengue infections.

1. Introduction

Dengue virus (DENV) is considered a global public health problem and approximately 40% of the world population is at risk of contracting this infection (Organization, 2009). The four serotypes of the dengue virus (DENV1, DENV2, DENV3 and DENV4) are transmitted to humans and cause two clinical syndromes: classical dengue fever (DF) and hemorrhagic fever/dengue shock syndrome (DHF/DSS) (Gubler, 2002; Guzman and Kouri, 2002). The viral particles consist of the three structural proteins (capsid, premembrane and envelope glycoprotein) and the seven nonstructural ones (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) important for dengue virus replication (Lindenbach and Rice, 2001).

Among the techniques available for the diagnosis of dengue infections, reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM antibody capture enzyme immunoassay (MAC-EIA) have been commonly used (Guzman and Kouri, 2004). The RT-PCR rapidly detects the viral RNA of all serotypes from the patient's serum in the viremia phase while MAC-EIA is suitable for distinguishing between primary and secondary dengue infections based on the IgM/IgG ratio, despite not providing a specific diagnosis for the dengue virus, which can be easily confused with other febrile illnesses. The combination of these two approaches is more appropriate for accurately detecting dengue (Poersch et al., 2005). Despite the individual benefits of each method, both have limitations, such as high technological support demands and possible false negative results (Teles, 2011).

Currently, new methods for dengue diagnosis are required to provide specific and rapid detection in real-time, early states of the disease, allied to lower cost. Such methods would enable an appropriate protective management, surveillance and future immunization strategies to restrain the spread of the disease. In addition, the clinical symptoms are often confused with a variety of viral and bacterial diseases, including other flaviviruses infections such as West Nile virus, Japanese encephalitis virus, yellow fever virus, tick-borne encephalitis virus, Murray Valley encephalitis virus and St. Louis encephalitis virus (Gould and Solomon, 2008; Ross, 2010).

In this context, point-of-care biosensors can provide a good performance in diagnosing for high sensitivity and selectivity, handling

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facility and rapid detection (Seah et al., 1995). Electrochemical impedance spectroscopy and cyclic voltammetry are effective techniques to investigate changes in the electrode/electrolyte interface in the development of biosensors for real samples (Mantzila et al., 2007; Prodromidis, 2010). Electrochemical biosensors have also been used in the development of virus biosensors (Caygill et al., 2010), including the dengue virus (Binh et al., 2012; Cavalcanti et al., 2012; Cheng et al., 2012; Fang et al., 2010).

The use of carbohydrate recognition in the development of biosensors is the key point in the identification of various pathologies (Zeng et al., 2012). It is known that in the early stages of dengue virus infection there is an increased expression of specific glycoproteins in the serum (Xu et al., 2006; Dussart et al., 2006). Recently, lectin-based biosensors obtained by us have shown distinct patterns of response for glycoproteins in the serum of patients infected by DENV1, DENV2 and DENV3 (Andrade et al., 2011). In addition, a biosensor for patients with DF and DHF has also been developed by our group (Oliveira et al., 2009).

Concanavalin A (ConA), extracted from jack bean seeds (*Canavalia ensiformis*), is one of the most widely studied lectins. At pH 7.4 it exists as a tetramer with a higher degree of organization, molecular weight of 110 kDa and an overall size of 6.7 nm × 11.3 nm × 12.2 nm (Ballerstadt et al., 2006; Bouckaert et al., 1996). It is known that under neutral conditions each subunit of ConA contains one binding site for glucose- and mannose-specific carbohydrate structures, one for calcium and manganese cations, which activate the binding site of protein for carbohydrates, and a third one for hydrophobic recognition (Liu et al., 2007). Thus, ConA is extensively used in protein-carbohydrate interaction (Pei et al., 2005; Mori et al., 2009; Pedroso et al., 2008), including studies for the diagnosis of dengue (Andrade et al., 2011; Pereira et al., 2008).

In addition, the use of lipid molecules with biosensors reduces the interference of electroactive species, contributes to signal amplification and, furthermore, creates a native environment for protein immobilization and protection from denaturation (Sofou and Thomas, 2003; Ramsden, 1998). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) is one of the phospholipids commonly found as constituents of cells and organelles and is conventionally used in biosensors (Yamamoto et al., 2010; Puu et al., 2000).

In this study, we developed a novel electrochemical biosensor based on ConA lectin and phospholipid membrane association for the specific identification of glycoproteins present in the serum of infected patients with dengue (DENV1, DENV2 and DENV3). The biosensor was characterized by means of cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM).

2. Experimental

2.1. Materials

Concanavalin A lectin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were obtained from Sigma Chemical Co. (St Louis, USA) and used as received. The dengue sera were available in the Laboratory of Virology/FAMERP stocks. In this work we used the sera of patients contaminated with dengue serotypes 1, 2 and 3 (three patients of each serotype) and serum controls (three patients). All sera were previously characterized as patient dengue negative (serum control) and dengue positive (serotypes 1, 2 and 3) using RT-PCR (Mondini et al., 2007). Phosphate buffer saline (PBS) (pH 7.4) containing 0.1 M $CaCl_2$ and 0.1 M $MnCl_2$ was used to prepare the ConA solutions. The ultrapure water was prepared with a purification system (Millipore-Synergy). All chemicals and solvents were of analytical grade and used as received, without further purification.

2.2. Preparation of lipid vesicles

Liposomes were prepared based on a lipid film formed by the evaporation of solvents (Amselen et al., 1990; Andrade et al., 2004). Liposomes were obtained using 1 mM DPPC dissolved in chloroform/methanol solution (3:1, v/v) under magnetic agitation (150 rpm/min, 15 min). Next, the organic solution was submitted to evaporation under reduced pressure (25 min at 40 ± 1 °C) and agitation at 120 rpm to remove solvents. Subsequently, the dried lipid film was hydrated through the addition of 1 mL of 0.2 M PBS (pH 7.4) and kept under magnetic stirring (40 min). Finally, the suspension of liposomes was stored at 4 °C until use.

2.3. Preparation of the biosensor system

The bare gold electrode surface was freshly polished with 0.05 μm α-Al_2O_3 paste, rigorously rinsed with ultrapure water and cleansed ultrasonically in deionized water for 5 min. After this process, the lipid-modified electrode was obtained by dropping liposome solution onto the bare gold electrode surface at 25 °C for 15 min. After the first immobilization, the modified electrode was rinsed with water to remove non-adsorbed lipid, incubated into 1 mL of the ConA (50 μg/mL) in 10 mM PBS (pH 7.4) for 15 min and rinsed with water to remove excess of the ConA. Finally, the lipid-ConA-modified electrode was exposed to negative serum control or serum from patients infected by DENV1, DENV2 and DENV3 diluted in 10 mM PBS solution (pH 7.4) for 20 min at room temperature. The serum dilutions used in this study were 1:10, 1:250, 1:500, 1:1000, 1:1500 and 1:2000.

2.4. Impedance measurements

Electrochemical measurements were performed using a three-electrode system containing an Ag/AgCl as reference electrode, platinum as a counter electrode and a bare gold electrode as the working electrode (ϕ = 2 mm). All the experiments were carried out on a PGSTAT 128 N potentiostat (Autolab, Eco Chemie, The Netherlands), interfaced with an analyzer controlled by a computer. The impedance spectra were then recorded in the frequency range of 100 mHz to 100 kHz. The amplitude of the applied sine wave potential (SWP) was 10 mV, while the direct current (dc) potential was limited at the open circuit potential measured just before the application of the SWP. CV measurements were performed with a potential sweeping between −0.2 to 0.7 V at a scan rate of 50 mV s^{-1}. CV and EIS measurements were carried out at different stages of the preparation of the modified electrode and performed in the presence of 10 mM $K_4[Fe(CN)_6]^{4-}$/$K_3[Fe(CN)_6]^{3-}$ (1:1) solution (used as a redox probe) in PBS (pH 7.4). All electrochemical measurements were performed in triplicate using at least three different sensors at room temperature inside a Faraday cage.

2.5. Atomic force microscopy measurements

Atomic force microscopy (AFM) measurements were performed using a commercial PicoSPM II microscope (Molecular Imaging, USA). Cantilevers with a silicon AFM probe (Multi 75AL, NCHR, resonant frequency = 75 kHz, force constant = 3 N m^{-1}) were used for the noncontact mode AFM in air at room temperature (approximately 25 °C). Lateral resolution was set at 512 × 512 pixels in a scan area of 5 × 5 μm. Images were obtained from at least three macroscopically separated areas to ensure a good distribution and analyzed using AFM Gwyddion software (Necas et al., 2008).

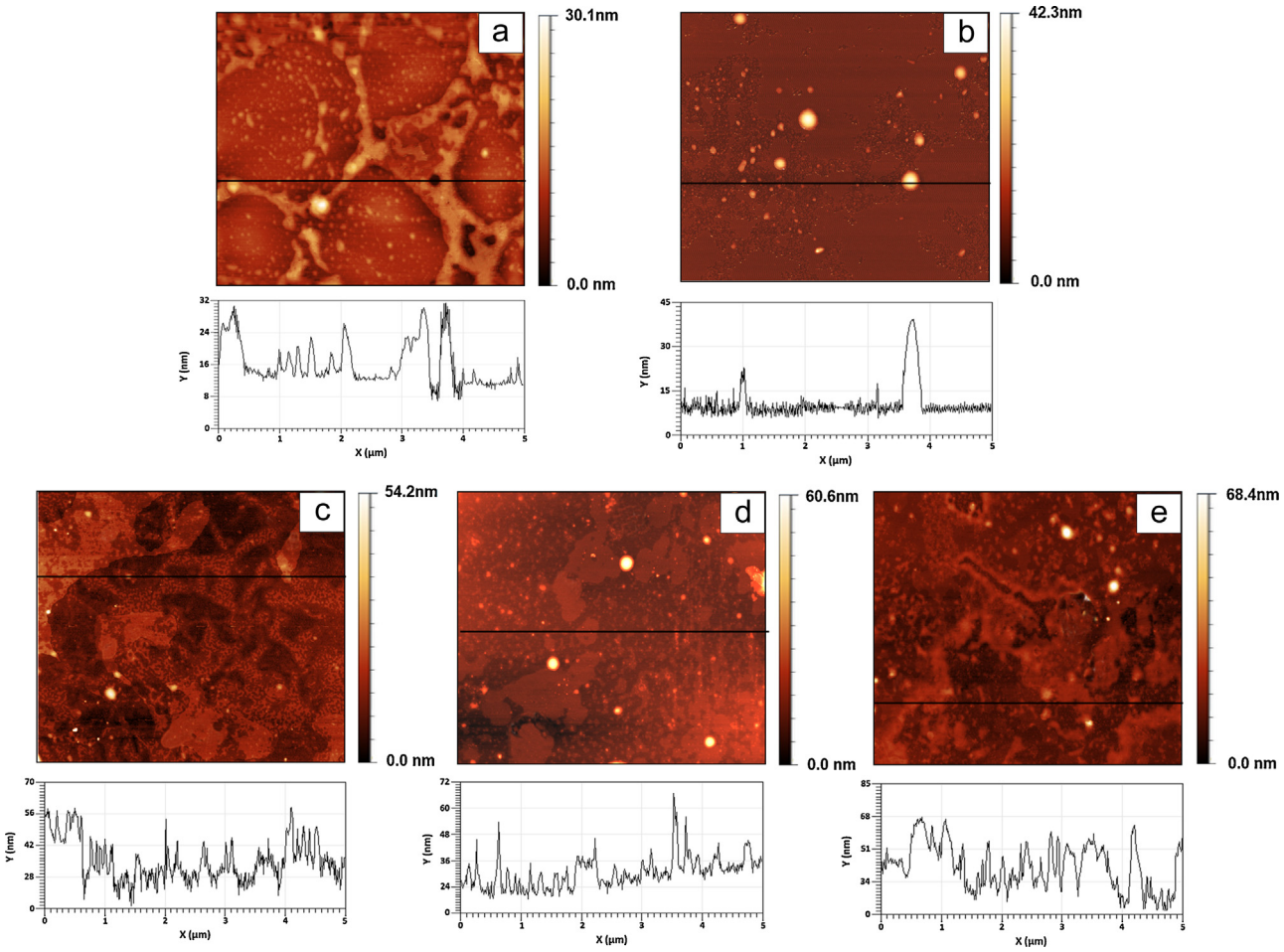

Fig. 1. AFM topographic image obtained for DPPC layer (a), DPPC/ConA (b), DPPC/ConA/DENV1 (c), DPPC/ConA/DENV2 (d) and DPPC/ConA/DENV3 (e) with the corresponding cross section. Scan area of 5 μm × 5 μm.

3. Results and discussion

3.1. Morphological characterization

The architecture of the lipid films and its association with ConA lectin was studied by the AFM technique. Fig. 1 shows AFM topographic images obtained for DPPC (a), DPPC-ConA (b), DENV1 (c), DENV2 (d) and DENV3 (e) and their corresponding cross-sections. Fig. 1a shows the lipid film demonstrating a non-uniform covering of the electrode surface, with a mean height of 30 ± 4.2 nm (see cross-section graph). The AFM images also show the coexistence of fluid and gel domains in the lipid layer (Giocondi et al., 2008). In addition, we also evaluate the association between DPPC and ConA. The insertion of the proteins was performed after absorption of the lipid layer on the substrate.

In Fig. 1b it is possible to visualize large globules (with a mean height of 42 ± 3.6 nm) distributed between fluid and gel domains of the lipid layer corresponding to ConA lectin. Giocondi et al., 2008 also observed that some proteins insert preferentially at the boundary between fluid and gel domains of the lipid layers (Giocondi et al., 2008). Our results are in accordance with previous studies that determined a height of ~12 nm for the ConA tetramer (Bouckaert et al., 1996). Regardless of the serotype, diverse heights were obtained. The fine structured film with thickness on the DPPC-ConA sensor is attributed to adsorbed DENV. In addition, DENV1 showed the weakest interaction with the DPPC-ConA sensor layer (54.2 ± 5.1 nm). The thickest adsorbed layer on the sensor surface was observed for DENV2 and DENV3 with mean heights of 60.6 ± 4.8 nm and 68.4 ± 3.9 nm, respectively (Fig. 1d–e). The difference in the height data may well be due to the specificity of ConA lectin to the DENV.

It is noteworthy that dengue viruses have conserved two potential N-linked carbohydrate sites at N-67 and N-153, which are glycosylated (Zhang et al., 2004). These carbohydrates are high-mannose glycans with importance for viral attachment and immune response (Hacker et al., 2009). In addition, has been reported to lack glycosylation at N-153 for DENV2 (Johnson et al., 1994). According to Pereira et al., 2008, the attachment of dengue virus particles to ConA lectin is probably driven by recognition of the mannose-binding site. However, the differences in the number and type of carbohydrate moieties on the virion surface proteins in according to the serotype and their role in virus host interactions remain poorly understood (Herrero et al., 2013).

3.2. Electrochemical characterization of the lipid-ConA-Au electrode sensor

The electrochemical method was used in this work owing to its effectiveness for the development of biosensors for real samples (Mantzila et al., 2007). Fig. 2a shows typical cyclic voltammograms of the stepwise process of the biosensor preparation. We used 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ (1:1) as the redox probe during all experiments. The cyclic voltammogram of the bare gold electrode showed a pair of well-defined redox peaks,

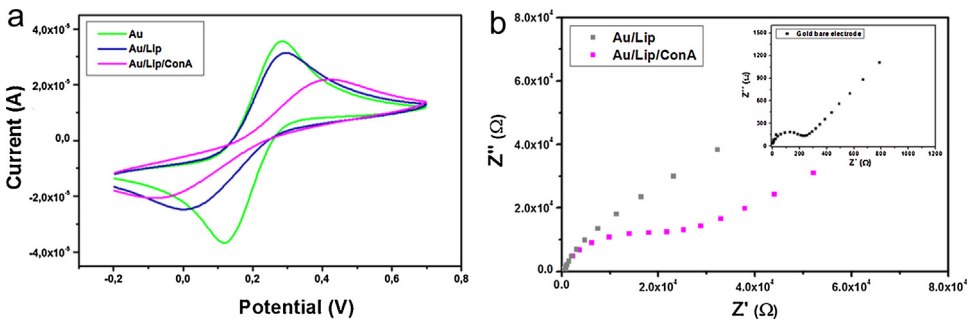

Fig. 2. Cyclic voltammograms (a) and Nyquist plots (b) of the stepwise assembly of the sensor. The voltammetric and impedance spectra were taken in 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ 1:1 + 0.15 M NaCl in PBS 10 mM pH 7.4. Cyclic voltammetry scan was initiated at −0.2 V vs. Ag/AgCl in positive direction at 50 mV s^{-1}. The frequency range for impedance measurements was set on 100 mHz to 100 kHz and the sine wave potential of amplitude equal to 10 mV.

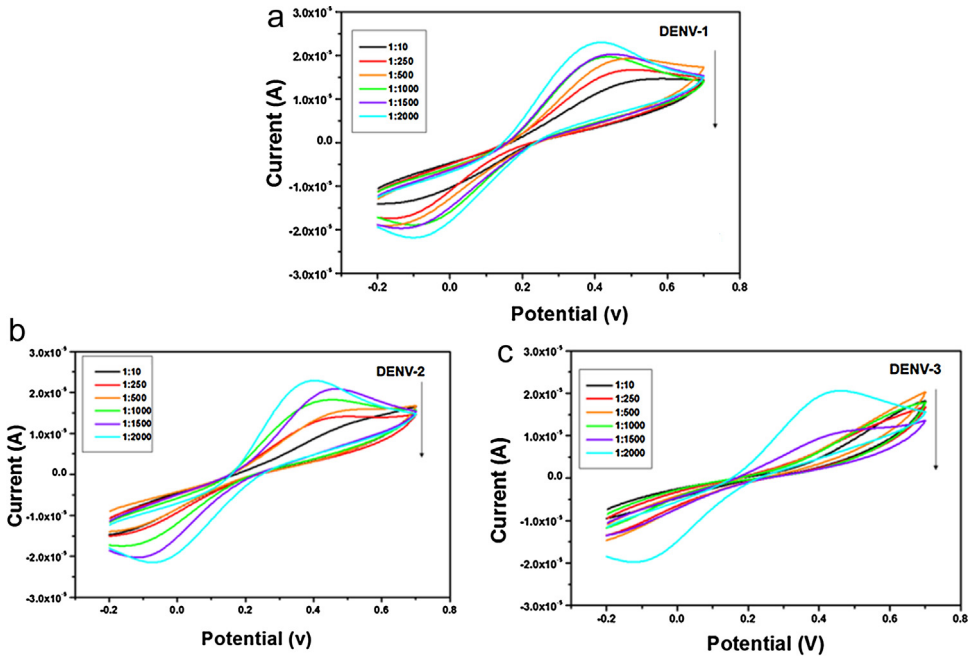

Fig. 3. Cyclic voltammograms of the lipid-ConA system after exposure to different dengue serum: DENV1 (a), DENV2 (b) and DENV3 (c) in dilution folds ranging from 1:10 to 1:2000. Supporting electrolyte: 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ 1:1 + 0.15 M NaCl in PBS 10 mM pH 7.4 solution. Cyclic voltammetry scan was initiated at −0.2 V vs. Ag/AgCl in positive direction at 50 mV s^{-1}.

demonstrating the reverse process of the redox probe in the solution phase. After each step of biosensor construction, the current decreases, due to enhanced barriers in the transfer kinetics at the electrode/electrolyte interface (Bard and Faulkner, 2001).

The formation of a lipid layer on the bare gold electrode leads to a decrease in the redox peak currents. Also, after immobilization of the ConA lectin on the lipid layer, the redox peak currents decreased to −8 and 16 mA. In addition, the decrease in the redox peak currents of the sensor layer was attributed to a blocking effect via inhibition of the diffusion of ions such as $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ anions, and indicates successful immobilization at each step.

Fig. 2b shows the Faradaic impedance spectra in the Nyquist plots during the stepwise modification process. The bare gold electrode reveals a very small semicircle domain, indicating a very low electron-transfer resistance (R_{CT}). Successive increases in diameter of the semicircles were observed after electrode modification. This is the result of the increase in charge transfer resistance for ferro/ferri-cyanide redox probe to access the metal surface, owing to the adsorption of biomolecules, which retards the electron transfer kinetics between the redox probe and the electrode surface (Bard and Faulkner, 2001).

3.3. Electrochemical detection of serum glycoproteins in patients infected with dengue

Fig. 3a–c shows the recognition capability of the lipid-ConA system for six different dilutions of each dengue serotype (1:10, 1:250, 1:500, 1:1000, 1:1500 and 1:2000). Fig. 3 reveals a gradual decrease in the current with the increase in the dengue serum concentration. A major decrease in the amperometric response for dengue serotypes 2 and 3 was observed. The reduction in the current is related to the amount of the ConA-glycoprotein complex formed on the electrode surface.

Fig. 4a–c shows the real and imaginary parts of the impedance of the lipid-ConA system in the presence of increasing serum concentrations. EIS results are in accordance with CV analysis. EIS experiments showed higher values for the interfacial electron transfer resistance to DENV2 and DENV3 as compared to DENV1.

Fig. 5a and b presents the CV and EIS results for 1:10 dilution of the infected serum and non-contaminated serum for the evaluation of the specific biosensor response. After the lipid-ConA-Au sensor interacted with DENV serum, a remarkable disappearance of the current peaks occurred (Fig. 5a). In particular, after interaction with DENV3, the sensor shows the disappearance of the anodic and

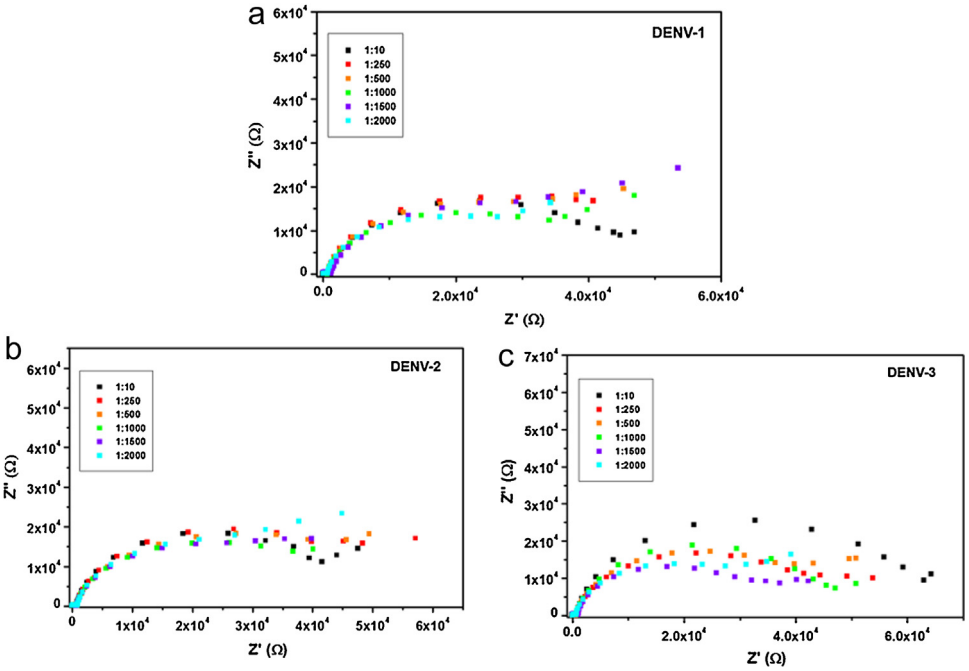

Fig. 4. Nyquist plots of the lipid-ConA system after exposure to different dengue serum: DENV1 (a), DENV2 (b) and DENV3 (c) in dilution folds ranging from 1:10 to 1:2000. Supporting electrolyte: 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ 1:1 + 0.15 M NaCl in PBS 10 mM pH 7.4 solution. The frequency range for impedance measurements was set on 100 mHz to 100 kHz and the sine wave potential of amplitude equal to 10 mV.

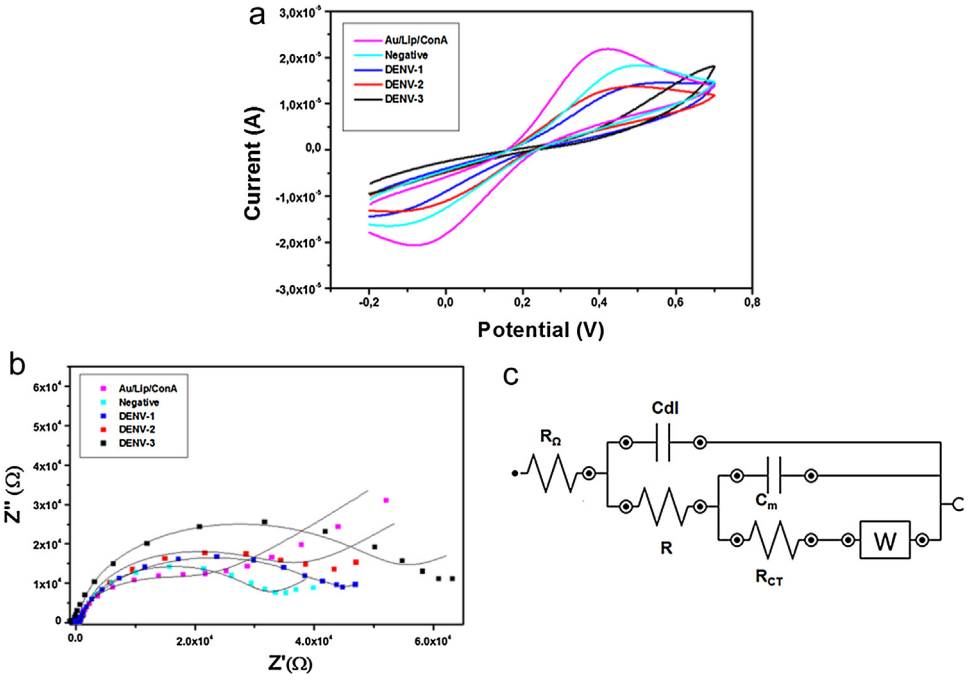

Fig. 5. Cyclic voltammetry (a) and Nyquist plots (b) of interaction lipid-ConA system with different dengue serotypes (DENV1, DENV2 and DENV3) and negative control at a fixed concentration (1:10). The spectra were taken in 10 mM $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$ 1:1 + 0.15 M NaCl in PBS 10 mM pH 7.4. Cyclic voltammetry scan was initiated at −0.2 V vs. Ag/AgCl in positive direction at 50 mV s^{-1}. The frequency range for impedance measurements was set on 100 mHz to 100 kHz and the sine wave potential of amplitude equal to 10 mV. The solid lines correspond to the fitting using the complex equivalent parameters obtained. (c) Equivalent circuit adopted to fit the impedance data in the presence of a redox pair of $K_4[Fe(CN)_6]^{4-}/K_3[Fe(CN)_6]^{3-}$.

cathodic peaks, due to the specific interaction between the ConA lectin and glycoproteins present in the tested serum. The reason for peak reduction is that the lectin-glycoprotein complex acts as an inert electron and mass-transfer blocking layer and hinders the diffusion of ferro/ferri-cyanide toward the electron surface (Oliveira et al., 2009).

Furthermore, the biosensor demonstrated a weak interaction with glycoproteins present in the DENV1 serotype, revealing a small decrease in the amperometric response of the electrode. The lipid-ConA-Au sensor system was able to recognize the dengue serotype in the following sequence: DENV3 > DENV2 > DENV1.

Table 1
Values of the equivalent circuit elements obtained from the fitting of the impedance results.

Modified electrode	Cdl (nF)	R_{CT} (K_Ω)	W	R_Ω (Ω)	C (nF)	R (Ω)
Bare gold electrode	230 ± 4.31	0.3 ± 0.05	5.66 ± 1.34	77 ± 3.21	11.30 ± 1.32	728 ± 3.54
Lipid	1160 ± 11.45	13.0 ± 0.94	9.87 ± 1.85	124 ± 7.42	9.36 ± 1.27	740 ± 5.32
Lipid-ConA×	906 ± 13.97	17.5 ± 1.17	27.2 ± 3.05	190 ± 5.93	13.81 ± 2.31	681 ± 6.72
Lip-ConA-DENV1						
Dilution 1:10	775 ± 5.29	30.5 ± 2.74	55.1 ± 4.56	150 ± 4.25	5.32 ± 1.35	782 ± 3.41
Dilution 1:250	560 ± 4.75	24.4 ± 3.85	32.2 ± 4.23	208 ± 3.12	6.18 ± 1.56	712 ± 7.64
Dilution 1:500	564 ± 5.21	23.3 ± 2.01	32.6 ± 2.40	217 ± 6.23	5.11 ± 1.26	733 ± 9.43
Dilution 1:1000	874 ± 6.83	22.7 ± 2.74	35.3 ± 2.57	186 ± 4.72	4.27 ± 1.24	804 ± 5.36
Dilution 1:1500	1070 ± 6.35	20.9 ± 2.54	25.3 ± 2.59	188 ± 5.30	2.98 ± 0.63	687 ± 7.49
Dilution 1:2000	1060 ± 8.36	19.7 ± 1.39	29.0 ± 3.12	120 ± 4.16	13.7 ± 1.64	600 ± 6.28
Lip-ConA-DENV2						
Dilution 1:10	650 ± 6.75	32.3 ± 1.73	56.0 ± 3.27	151 ± 2.34	5.96 ± 0.74	802 ± 9.42
Dilution 1:250	622 ± 9.04	30.8 ± 3.74	44.0 ± 4.14	235 ± 4.03	5.15 ± 1.15	601 ± 13.75
Dilution 1:500	713 ± 8.12	26.3 ± 4.01	36.0 ± 2.94	336 ± 5.92	6.42 ± 1.64	863 ± 18.39
Dilution 1:1000	738 ± 7.55	25.8 ± 3.92	46.1 ± 3.91	311 ± 6.73	9.17 ± 1.73	833 ± 20.34
Dilution 1:1500	755 ± 7.46	21.5 ± 2.79	38.2 ± 2.06	302 ± 5.12	11.2 ± 2.27	865 ± 15.57
Dilution 1:2000	1340 ± 12.73	21.1 ± 4.15	22.4 ± 2.78	104 ± 4.39	9.99 ± 2.03	678 ± 5.23
Lip-ConA-DENV3						
Dilution 1:10	432 ± 8.01	48.4 ± 2.68	61.4 ± 5.12	118 ± 2.63	10.13 ± 1.86	768 ± 8.43
Dilution 1:250	945 ± 9.42	38.3 ± 3.08	40.5 ± 2.01	126 ± 2.74	5.49 ± 1.04	804 ± 13.79
Dilution 1:500	699 ± 10.01	27.5 ± 2.36	52.4 ± 1.98	218 ± 3.65	6.14 ± 1.43	955 ± 17.32
Dilution 1:1000	998 ± 9.32	26.1 ± 2.59	45.8 ± 4.35	240 ± 4.95	11.90 ± 2.57	984 ± 7.31
Dilution 1:1500	1160 ± 13.74	25.4 ± 2.77	44.0 ± 3.64	113 ± 4.54	7.41 ± 1.58	833 ± 7.80
Dilution 1:2000	1080 ± 12.03	22.1 ± 3.05	33.1 ± 2.31	162 ± 4.69	15.96 ± 2.41	643 ± 9.36
Lip-ConA-DENV negative						
Dilution 1:10	474 ± 5.56	24.4 ± 2.63	87.7 ± 6.34	121 ± 2.54	7.15 ± 1.40	698 ± 4.53
Dilution 1:250	987 ± 7.37	24.3 ± 3.04	56.2 ± 2.93	111 ± 3.71	8.95 ± 1.52	744 ± 4.28
Dilution 1:500	948 ± 8.77	23.8 ± 4.26	26.1 ± 3.24	115 ± 2.63	7.53 ± 1.36	860 ± 8.54
Dilution 1:1000	957 ± 6.93	22.2 ± 3.85	28.9 ± 2.74	117 ± 2.68	7.64 ± 0.93	801 ± 6.04
Dilution 1:1500	1070 ± 9.92	20.1 ± 5.92	29.5 ± 1.49	118 ± 2.57	8.50 ± 1.34	741 ± 2.51
Dilution 1:2000	1130 ± 14.05	18.0 ± 7.42	30.9 ± 2.59	113 ± 2.65	8.75 ± 1.62	732 ± 5.63

Fig. 5b shows the Nyquist plot of the data points measured and the corresponding fitting curves (straight solid lines in Fig. 5). In addition, it is clear that the fitting of the data points is in accordance with the complex equivalent circuit (Fig. 5c). An increase in the R_{CT} values is detected after exposure to DENV1, DENV2 and DENV3. The R_{CT} element of the equivalent circuit can therefore serve as the sensor parameter. The determination of its value allows the detection of the dengue serotypes.

The impedance data was fitted with NOVA 1.8 software using the complex equivalent circuit (Fig. 5c), which includes solution resistance (R_Ω), electron transfer resistance (R_{CT}), and the Warburg impedance element (W) as the main elements. Furthermore, double-layer capacitance (Cdl), protein layer resistance (R) and capacitance of the lipid membrane (C_m) were also considered. Weight factors were obtained based on Nyquist graphical data, hence the frequency range elected for analysis was 100 mHz to 100 kHz, while maintaining the parameters fixed in the fit procedure. The R_{CT} depends on the insulating feature at the electrode/electrolyte interface. W and R_Ω represented the bulk properties of the electrolyte solution. R_{CT} controls the electron transfer kinetics of the redox-probe at the electrode surface and it is well known that high R_{CT} values are usually associated with blockage of the electrode surface. In our study, changes in R_{CT} were higher than in the other impedance components.

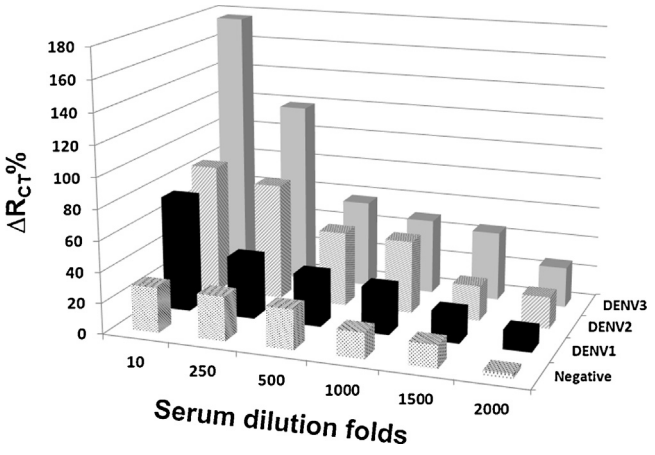

Fig. 6. ΔR_{CT}% for the lipid-ConA system case after exposure of the electrode to different dengue serotypes (DENV1, DENV2 and DENV3) and negative control in dilution folds ranging from 1:10 to 1:2000.

Table 1 shows the equivalent circuit parameters of the fitting curves for the various steps of the biosensor preparation and its subsequent interaction with serum glycoproteins in infected patients with dengue (serotypes 1, 2 and 3). Our results revealed different responses for each dengue serotype. The highest values of R_{CT} were obtained for DENV3, due to the ConA specificity. The performance of the modified electrode for detection of dengue glycoproteins was evaluated through the relative variation of this parameter (ΔR_{CT}). This variation can be calculated according to the following equation:

$$\Delta R_{CT}(\%) = \left(\frac{R_{CT}(\text{ConADENV}_{\text{serotype}}) - R_{CT}(\text{ConA})}{R_{CT}(\text{ConA})} \right) \times 100$$

where R_{CT} (ConA) is the value of the electron-transfer resistance of the lipid-ConA-Au modified electrode. R_{CT} ($\text{ConADENV}_{\text{serotype}}$) is the value of the electron transfer resistance of the lipid-ConA-Au modified electrode after exposure to the patients' sera containing dengue serotypes 1, 2 and 3. The ΔR_{CT} clearly increases with serum concentration, indicating that the interactions between ConA lectin and dengue serum glycoproteins can be sensed by the modified electrode surface (Fig. 6). We observed no significant responses (very low changes in R_{CT}) performing experiments with negative dengue serum obtained from healthy patients. It is worthy of note that the high R_{CT} values are usually associated with blockage of the electrode surface. Thus, we related the different R_{CT} values to the ability of the ConA to recognize glycoproteins in dengue serotypes.

The increase in ΔR_{CT} is different for each serotype studied and the highest response was for DENV3. In addition, the reproducibility of the biosensor system was evaluated from the ΔR_{CT} response of three different patients for each dengue serotype at different biosensors ($n = 3$) and an acceptable R.S.D. values of 5.73%, 5.43% and 4.89% for DENV1, DENV2 and DENV3, respectively.

ConA has the ability to attach to dengue particles (DENV2 and DENV3) of domain III in proteins E of the virus containing two glycosylated potential N-linked carbohydrate sites at N-67 and N-153, whereas most other flaviviruses have only a single N-linked glycosylation site at N154 (Chambers et al., 1990; Heinz and Allison, 2003). Mabalirajan et al. (2005) have shown that DENV1 and DENV3 caused hemorrhagic dengue fever in primary infections, while DENV2 and DENV4 were only associated with DHF in secondary infection. In addition, in response to dengue infection, the production of inflammatory mediators is involved in disease progression. An example of this effect is the activation of the complement system (C3 and C5B) involved in the increasing permeability seen in severe dengue. The levels in complement C3 and C5b as well as other plasmatic glycoproteins, are increased in primary events associated with DENV3. Like other glycoproteins present in dengue sera, C3 contains carbohydrates in both subunits with high-mannose type, (Man)5-9 (GlcNAc)2, which promotes the ConA specific interaction (Miki et al., 1986).

4. Conclusions

In the present paper, we have introduced a simple approach to the development of a biosystem based on the immobilization of Concanavalin A lectin on a gold electrode modified with a lipid monolayer. Cyclic voltammetry and electrochemical impedance techniques were used to investigate the immobilization of the Concanavalin A lectin on lipid film. Subsequently, using the lipid-ConA system a good sensitivity and reproducibility were obtained, revealing the possibility of detecting abnormal glycoproteins for the diagnosis of dengue. The detection of glycoproteins in the blood serum by ConA occurs by recognition of the mannose-binding site.

Conflict of Interest

The authors declare that there are no conflict of interest.

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