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A new carbon-paste immunosensor for quantification of tumor necrosis factor-alpha (TNF-α) in human serum

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UM NOVO IMUNOSSENSSOR CONFECCIONADO EM PASTA DE CARBONO PARA QUANTIFICAÇÃO DE FATOR DE NECROSE TUMORAL ALFA HUMANO (TNF-α) EM SORO HUMANO. Fator de necrose tumoral-α (TNF-α) é uma citocina que desempenha um papel importante na ativação de respostas inflamatória e imune. Níveis anormais dessa citocina estão associados a doenças como Artrite Reumatóide (AR), Lúpus Eritematoso Sistêmico e doença de Crohn. A quantificação do TNF-α no soro humano em laboratórios clínicos e de pesquisa é baseada em técnicas imunoenzimáticas, como o ELISA. Este estudo descreve o desenvolvimento de um imunossensor para quantificação do TNF-α circulante usando espectroscopia de impedância eletroquímica (EIS). O sensor pode ser usado para monitorar TNF-α em amostras sanguíneas de pacientes com doenças autoimunes, auxiliando na seleção da abordagem terapêutica adequada. Um eletrodo de pasta de carbono modificado foi produzido com camadas automontadas contendo carbono, óleo mineral e glutaraldeído e passivado com leite desnatado Molico®. O anticorpo monoclonal infliximab (Remicade®) foi usado para imobilizar o TNF-α. A especificidade do imunossensor foi testada com as citocinas TNF-α, IL-1β e IL-6 como antígenos. Utilizando EIS, a resistência de transferência de carga do sistema imunossensor foi comparada em concentrações de 1 a 1000 pg.mL^{-1} e após sucessivas adições do analito à solução na célula eletroquímica. Os resultados mostraram que o eletrodo foi seletivo, pois produziu apenas leituras para o TNF-α. O valor de R2 para a curva analítica foi em torno de 0,9350, confirmando sensibilidade do sensor para TNF-α. Duas amostras de soro humano: uma de um indivíduo sem AR e a outra de um portador de AR também foram testadas no imunossensor e as concentrações séricas de TNF-α foram de 10,8 pg.mL-1 e 21,9 pg.mL-1, respectivamente..

Palavras-chaves: biossensores, quantificação de citocinas, citocinas, doenças auto-imunes, terapia com anticorpos

Tumor Necrosis Factor-α (TNF-α) is a cytokine that plays an important role in activating inflammatory and immune responses . Abnormal levels of this cytokine are associated with diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus and Crohn's disease. Quantification of TNF-α in human serum in clinical and research laboratories is based on immunoenzymatic techniques such as ELISA. This study describes the development of an immunosensor for quantitation of circulating TNF-α using electrochemical impedance spectroscopy (EIS). The sensor can be used to monitor TNF-α in blood samples from patients with autoimmune diseases, assisting in selecting the appropriate therapeutic approach. A modified carbon paste electrode was produced with self-assembled layers containing carbon, mineral oil and glutaraldehyde and passivated with Molico® skim milk. Infliximab monoclonal antibody (Remicade®) was used to immobilize TNF-α. Immunosensor specificity was tested with cytokines TNF-α, IL-1β and IL-6 as antigens. Using EIS, the charge transfer resistance of the immunosensor system was compared at concentrations from 1 to 1000 pg.mL-1 and after successive additions of the analyte to the solution in the electrochemical cell. The results showed that the electrode was selective because it produced only readings for TNF-α. The R2 value for the analytical curve was around 0.9350, confirming sensor sensitivity for TNF-α. Two human serum samples: one from an

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individual without RA and the other from an RA carrier were also tested on the immunosensor and serum TNF-α concentrations were 10.8 pg.mL-1 and 21.9 pg.mL -1, respectively .

Keywords: biosensors, cytokine quantification, cytokine, autoimmune diseases, antibody therapy.

1 Introduction

Tumor necrosis factor-α (TNF-α) is a cytokine produced by different defense cells, including macrophages, monocytes, neutrophils, NK cells, mast cells, lymphocytes and endothelial cells (IBL, 2012). It activates a variety of cell responses, such as apoptosis, survival, differentiation, proliferation and migration. One of the proinflammatory cytokines produced during infection, in tissue lesions or in inflammatory processes, TNF-α acts in a paracrine manner in endothelial cells by activating expression of adhesion molecules, and in an endocrine manner in, for example, the hypothalamus, where it induces fever, as well as in the liver, where it is involved in the synthesis of acute-phase proteins (KONGSUPHOL et al., 2014; BRADLEY, 2008; IDRISS; NAISMITH, 2000). However, when released in high concentrations, TNF-α can induce sepsis.

Synthesis of this cytokine by lymphocytes and macrophages is very closely linked to inflammatory processes in autoimmune diseases such as rheumatoid arthritis (RA) (SILVA et al., 2003; CASTRO et al., 2005). As in RA, TNF-α production can be deregulated in diseases such as cancer, systemic lupus erythematosus (SLE), inflammatory bowel disease (Cohen's disease and ulcerative colitis), psoriasis, lung diseases (cystic fibrosis and asthma), ankylosing spondylitis, transplant-associated diseases (graft-versus-host disease and allograft rejection), atherosclerosis, arterial calcification and neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease, prion disease and Parkinson's disease (CICONELLI, 2005; LIMA; GIORGI, 2008; LIU et al., 2008; IBL, 2012).

The incidence of these diseases has increased gradually in recent years. The prevalence in the population at large is now estimated to be over 2%, and RA alone has an estimated prevalence of 0.5% to 1% worldwide depending on the population studied. The link between TNF-α and autoimmune diseases is well established, and a key treatment for autoimmune diseases involves inhibiting or blocking proinflammatory cytokines, such as TNF-α (TOBÓN et al., 2010; CASTRO-LÓPEZ et al., 2014).

Therapeutic monoclonal antibodies, which were developed as a result of biotechnological research efforts to inhibit proinflammatory cytokines, are recommended for the control of autoimmune diseases. Notable among these are antibodies that neutralize TNF-α or target its receptor, which are known as anti-TNF-α antibodies. These biological agents have a number of advantages over other medications, such as DMARDs (disease-modifying anti-rheumatic drugs), because they are specific for defined therapeutic targets and play an established role in the treatment of some diseases (MARTINS; ANDRADE, 2005; CRAVO; TAVARES; SILVA, 2006; MARTIN, P.; MEDEIROS; SCHAINBERG, 2006; MACHOLD et al., 2006; SOCIEDAD CHILENA DE REUMATOLOGIA, 2008; MORENO et al., 2006; CRUVINEL et al., 2008; CARVALHO JUNIOR et al., 2009).

Although they are efficient, the biological agents used in anti-TNF-α therapy can trigger undesirable effects, such as the onset of syndromes similar to drug-induced lupus (FURST et al., 2005), or lead to the production of antinuclear antibodies (ANA), a common occurrence in patients being treated with infliximab (BRAUN; SIEPER, 2004; ABREU; CICONELLI, 2005, RUSSO; KATSICAS, 2005; GOLDSCHMIDT, 2008).

Quantification of human serum or plasma TNF-α levels in clinical and research laboratories is based on immunoenzymatic techniques such as ELISA, the gold standard for this type of analysis (SHRIVASTAVA et al., 2014). Although there is a vast range of commercial ELISA kits, all of which are very sensitive and effective, the technique is expensive to use and requires a well-equipped laboratory as well as trained personnel. Furthermore, it involves multiple steps, and at least four hours are required before the results are available.

Despite issues related to the reproducibility of the results obtained with electrochemical biosensors and the stability of enzymes and other biological agents, these sensors are currently the subject of much research. In an effort to overcome these problems, many studies on new transducer materials and immobilization techniques have been

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carried out (GIL; KUBOTA; YAMAMOTO, 1999; CARVALHO; KUBOTA, 2003; BRAHIM et al., 2003; WANG, 2006; GIL and MELO, 2010; RICCI; ADORNETTO; PALLESCHI, 2012; GOPINATH et al., 2014).

There is a demand for fast, highly specific quantitative tests to measure TNF-α in the blood of patients with chronic inflammatory diseases, such as autoimmune diseases, who require therapies to suit their extremely variable clinical condition. Development of a high-performance, high-quality, practical immunosensor for use in medical diagnosis and measurement of circulating TNF-α with the potential for miniaturization could therefore yield significant benefits (MORGAN; NEWMAN, PRICE, 1996; LAZCKA; DEL-CAMPO; MUÑOZ, 2007; MOHAMED; DESMULLIER, 2011; GOPINATH et al., 2014).

As indiscriminate use of anti-TNF-α favors diseases caused by opportunistic parasites in patients with low concentrations of this cytokine, development of an immunosensor is of fundamental importance to quantify serum TNF-α levels in real time and determine whether the patient needs to be treated with the monoclonal antibody. There is therefore clinical and commercial interest in using new electrobiochemical methods to quantify TNF-α. A portable, easy-to-use, highly sensitive, specific immunosensor would help to ensure effective treatment targeting, reducing indiscriminate use of anti-TNF-α therapy and the consequent onset of pathologies and infections during treatment (SONG; XU; FAN, 2006; LIU; KWA; REVZIN, 2012; LIU; ZHOU; REVZIN, 2013).

This study describes the development of an immunosensor for quantifying circulating TNF-α using electrochemical impedance spectroscopy (EIS) to allow TNF-α in blood, serum or saliva to be measured more efficiently and to help in the diagnosis and therapeutic management of patients with autoimmune and other chronic inflammatory diseases.

2 Material and Methods

2.1 Chemical and biological reagents

The following materials were used: Vulcan carbon black (CABOT, Brazil), glutaraldehyde (pentane-1,5-dial) (Labsynth, Brazil), mineral oil (União Química, Brazil), potassium chloride (KCl 0.1 $mol.L^{-1}$) (Labsynth, Brazil), potassium ferricyanide ($K_3[Fe(CN)_6]$ 10 $mmol.L^{-1}$) (Labsynth, Brazil), Molico® skim milk 5% (Nestlé®, Brazil) with Tween 20 (5 drops) (Labsynth, Brazil), 0.1 $mol.L^{-1}$ phosphate buffered saline (PBS), Remicade® (infliximab 100 mg) (Schering-Plough, Ireland), cytokine TNF-α from the OptEIA™ Human TNF ELISA Kit II (BD Biosciences, USA), cytokine IL-1β from the OptEIA™ Human IL-1β ELISA Kit II (BD Biosciences, USA) and cytokine IL-6 from the OptEIA™ Human IL-6 ELISA Kit II (BD Biosciences, USA). All the solutions were prepared with ultrapure water produced using a Milli-Q® purification system.

2.2 Human-serum sampling procedure

Venous blood samples were collected from two female volunteers (23 and 30 years old) between 9:00 and 10:00 AM after an 8-hour overnight fast. One of the volunteers had rheumatoid arthritis, and her blood sample was used as a positive control. Samples were collected in tubes without anticoagulants by trained professionals using identical, standardized protocols. Sera were separated by centrifugation (10 min at 3,000 rpm), stored at -20 °C until analysis and used to detect the proinflammatory cytokines TNF-α, IL-6 and IL-1β. Serum levels of these cytokines were quantified using the method proposed here.

2.3 Equipment

The following equipment was used: an analytical balance model ED2245 (SARTORIUS AG, Germany), a Vortex mixer model K45-2820 – 2,800rpm 220V/60Hz with platform (KASVI, Brazil) and a µAutolabIII potentiostat/galvanostat with an FRA2 impedance module (Metrohm Autolab B.V., Holland).

2.4 Construction of the electrode

The base of the working electrode was made from nylon dowels previously drilled in the center to make them hollow. Copper wire and minidisks were then inserted in the hollowed-out electrode. A modified monolayer consisting of 1 g of Vulcan carbon black mixed with 1000 µL of glutaraldehyde 5% and 80 drops of mineral oil was placed on the minidisks. The reference and counter electrodes consisted, respectively, of a silver/silver chloride (Ag/AgCl) electrode and platinum (Pt) electrode, both of which were supplied by Metrohm.

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2.5 Antibody immobilization

The modified electrode was exposed to Remicade ® (infliximab) at an optimized concentration of 125 µg.mL^{-1} (data not shown), which was immobilized by glutaraldehyde on the electrode. Blocking solution containing 0.1% (w/v) Molico® skim milk and 1 drop of Tween 20 (optimized volume and concentration) was then added to fill the vacancies in the glutaraldehyde layer (MAKAAVICIUTE; RAMANAVICIENE, 2013; DE-MOURA et al., 2014; NAIFF et al., 2014; RESENDE et al., 2018).

2.6 Electrochemical test procedure (immunosensor)

2.6.1 Detection of TNF-α in standard solutions and serum samples

In order to determine the detection threshold of the sensor, 50 µL of standard cytokine solution were added to the surface of each electrode for 30 min (BARAKET et al., 2013; BARAKET et al., 2017; BELLAGAMBI et al., 2017; YAGATI, LEE and MIN, 2018) in concentrations in the ranges 1 pg.mL^{-1} to 100 pg.mL^{-1} and 62.5 pg.mL^{-1} to 1000 pg.mL^{-1}. Both procedures were performed in triplicate, and the electrodes were washed three times in PBS pH 7.4 before each test. Cytokines IL-1β and IL-6 were used as negative controls and tested under the same conditions to observe the selectivity of the immunosensor and effectiveness of the blocking (KELLER, WEBB, DAVIS, 2003; HAYS et al., 2005).

Serum samples were analyzed in a similar fashion and in triplicate. A total of 50 µL of sample was added to the surface of each electrode for 30 min. The electrodes were then washed in PBS pH 7.4, and EIS measurements were taken.

2.6.2 Detection of TNF-α in successive additions

Tests were performed with cytokines TNF-α, IL-6 and IL-1β to measure the signal generated for increasing concentrations following successive additions of concentrated standard solution. Constant volumes of standard cytokine solution were added, and EIS readings were taken 30 min after each addition.

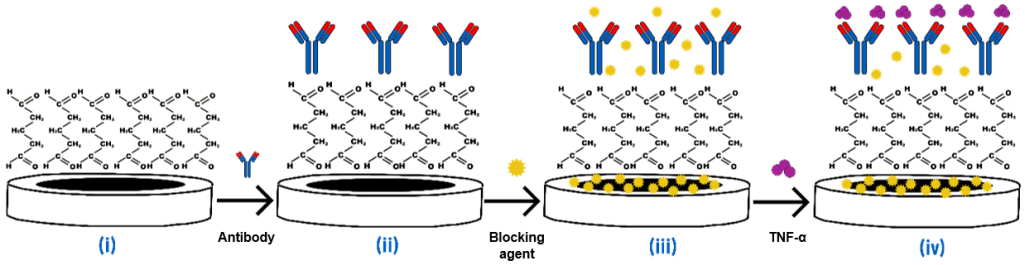

Figure 1. Steps in the production of the immunosensor for quantification of TNF-α using a carbon-paste electrode: (i) carbon-paste electrode modified with glutaraldehyde; (ii) immobilization of the monoclonal antibody; (iii) addition of the blocking agent to prevent non-specific binding; (iv) addition of TNF-α, the cytokine of interest.

3 RESULTS AND DISCUSSION

3.1 Characterization by Cyclic Voltammetry (CV)

Cyclic voltammetry tests showed that electrode passivation occurred when glutaraldehyde was used to modify the base. Greater electrode passivation was observed when antibody was added to the previously modified surface, probably as a result of immobilization of the antibody by the glutaraldehyde (MERA et al., 2008; BARAKET et al., 2013; MAKAAVICIUTE; RAMANAVICIENE, 2013; DE SOUZA; FORBICINI and CALG, 2014; RESENDE et al., 2018). When blocking agent was added, even greater electrode passivation was observed and the well-defined oxidation peak was replaced by two oxidation peaks, probably because of the proteins in the skim milk, which act as blocking agents, preventing non-specific binding, and cause complete electrode passivation (DE-MOURA et al., 2014; NAIFF et al., 2014). When the last layer (TNF-α) was added, the oxidation peak extending from about 0.2 V to 0.6 V was replaced by a peak at 0.6 V (Figure 2).

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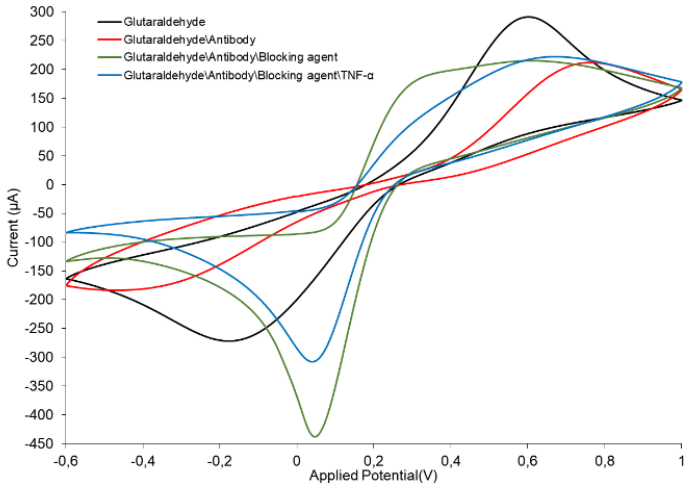

Figure 2. Characterization by cyclic voltammetry. (▬) = glutaraldehyde, (▬) = glutaraldehyde/monoclonal antibody, (▬)= glutaraldehyde/monoclonal antibody/blocking agent, (▬) = glutaraldehyde/monoclonal antibody/blocking agent/human TNF-α.

3.2 Characterization by Electrochemical Impedance Spectroscopy

The Nyquist plots obtained were typical of those produced by a Randles circuit with a Warburg diffusion element, which are characteristic of bioelectrical systems formed of monolayers (BARAKET et al., 2013; SANTOS et al., 2015). In the first layer (▬), the interaction of glutaraldehyde in the system is shown by the formation of an impedance semicircle with a charge transfer resistance (R_{ct}) of 2.1 kΩ and a Warburg diffusion element (Z_w) varying from 2.1 kΩ to 2.7 kΩ. In the second layer (▬), addition of the anti-TNF-α antibody on the surface of the sensor results in greater electrode passivation and a significant increase in R_{ct}, which now has a value of 3.1 kΩ. The Warburg diffusion element (Z_w) varies from 3.1 kΩ to 3.9 kΩ. When the third layer (▬) is added, the signal is significantly reduced and the charge transfer resistance (R_{ct}) is eliminated. This is a result of the addition of the strong blocking agent, which prevents any secondary electron transfer reactions with the surface of the electrode by filling all the empty spaces and passivating the electrode so that no charge transfer to the redox medium can take place (DE-MOURA et al., 2014; NAIFF et al., 2014). The last layer (▬) corresponds to TNF-α, which can cross the blocking agent and interact with anti-TNF-α antibody, showing that the sensor is effective (YANG; RAIRIGH; MASON, 2007; DONG, JING et. al., 2013).

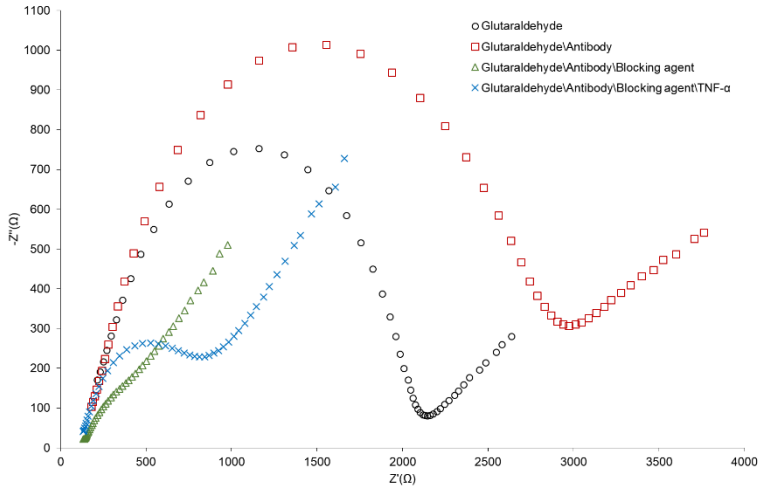

Figure 3. Characterization of the layers by EIS. (×) = glutaraldehyde, (+) = glutaraldehyde/monoclonal antibody, (◇) = glutaraldehyde/monoclonal antibody/blocking agent (○) = glutaraldehyde/monoclonal antibody/ blocking agent /human TNF-α.

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3.3 Results with standard solutions

The tests carried out in triplicate with the experimental electrodes show the interaction between TNF-α and the antibody, as impedance semicircles characteristic of a Randles circuit containing a charge transfer resistance (Rct) and Warburg(Zw) diffusion element were formed. Although quantification of TNF-α was not possible under the conditions tested, the system was able to detect this cytokine even in low concentrations (1 pg.mL^{-1}) and in different mediums, as shown by the Nyquist diagrams in Figure 4.

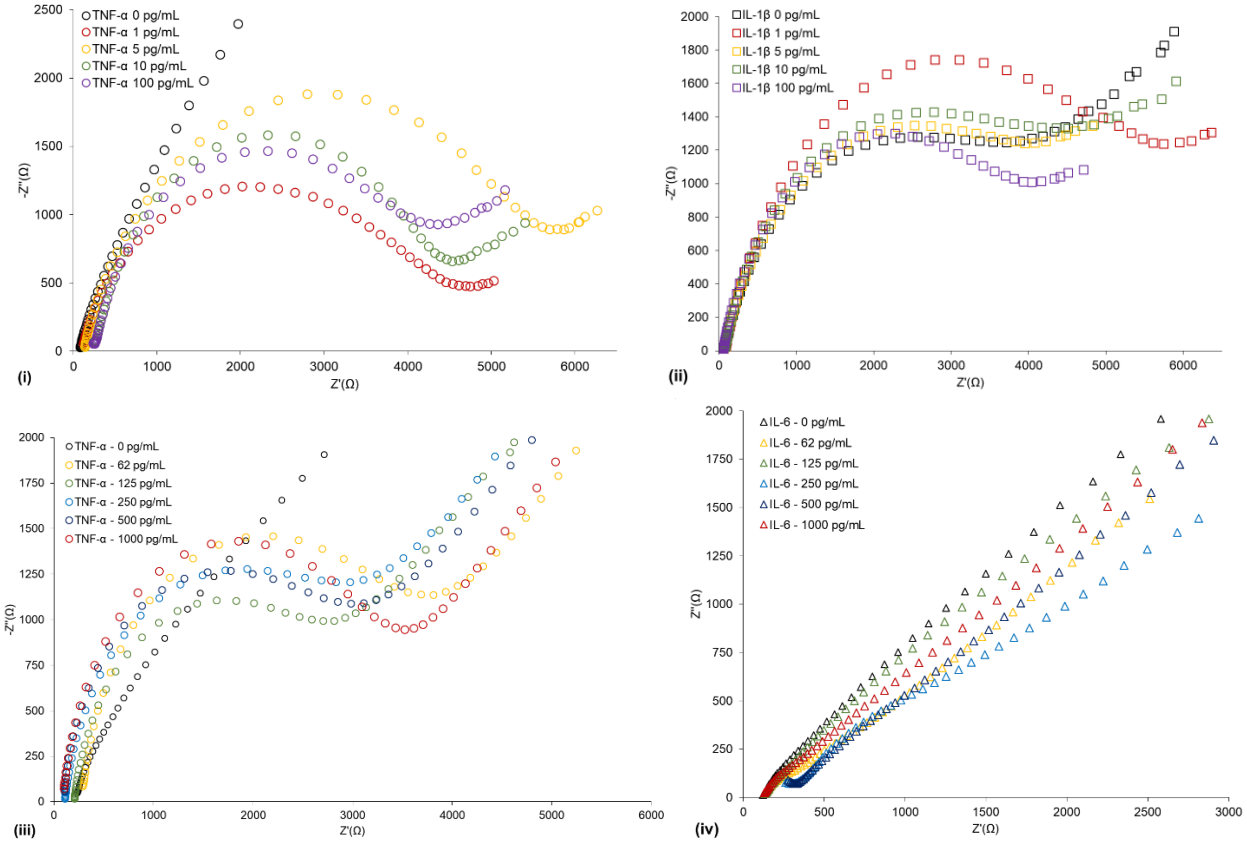

Figure 4. Assays in standard solution: **(i)** assays with TNF-α and **(ii)** assays with IL-1β, both in 3 mol.L^{-1} solution of KCl with concentrations varying from 1 to 100 pg.mL^{-1}; **(iii)** assays with TNF-α and **(iv)** assays with IL-6, both in PBS pH 7.4 and with concentrations varying from 62 to 1000pg.mL^{-1}.

Analysis of the interactions with IL-1β and IL-6 (proinflammatory cytokines secreted and released in plasma in clinical conditions similar to those in which TNF-α is secreted and released) in the same concentrations and conditions as in the tests with TNF-α revealed that for IL-1β the sensor generated an impedance semicircle characteristic of a Randles circuit with a charge transfer resistance (R_{ct}) and Warburg(Zw) diffusion element, although the IL-1β could not be quantified. This may be due to interaction between the layers and the medium tested, as the test medium for IL-1β was 3 mol.L^{-1} KCl, which may have reduced the effectiveness of the blocking agent. However, this was not the case with IL-6, for which only the Warburg diffusion element (Z_w), characterized by a straight line rather than a semicircle, was observed. Such a response is characteristic of diffusion processes, indicating that the sensor is specific for TNF-α under our experimental conditions.

3.4 Response curves for successive additions

Successive additions of the cytokines were made to the test solution with a single electrode, and EIS measurements were taken at 30-minute intervals after each addition. Voltages of 100 mV and 250 mV were tested. When the latter value was used, the sensor was able to quantify TNF-α and the quantification limit based on extrapolation of the standard curve generated was 4.8 pg.mL^{-1}.

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Under the conditions used here, when a voltage of 100 mV (Figure 5) was used the sensor was specific for TNF-α and generated an impedance semicircle characteristic of a Randles circuit with a charge transfer resistance (R_{ct}) and Warburg(Zw) diffusion element. The same was not true for IL-6, as the impedance plot did not include a semicircle and therefore corresponded to a Warburg diffusion element (Z_w) on its own. However, semicircles were generated at concentrations of 562 pg.mL^{-1} or higher when the layers break down, as observed in the test with TNF-α. When IL-1β was used, an impedance semicircle was formed but no variations were observed that would indicate that the value of R_{ct} had changed significantly.

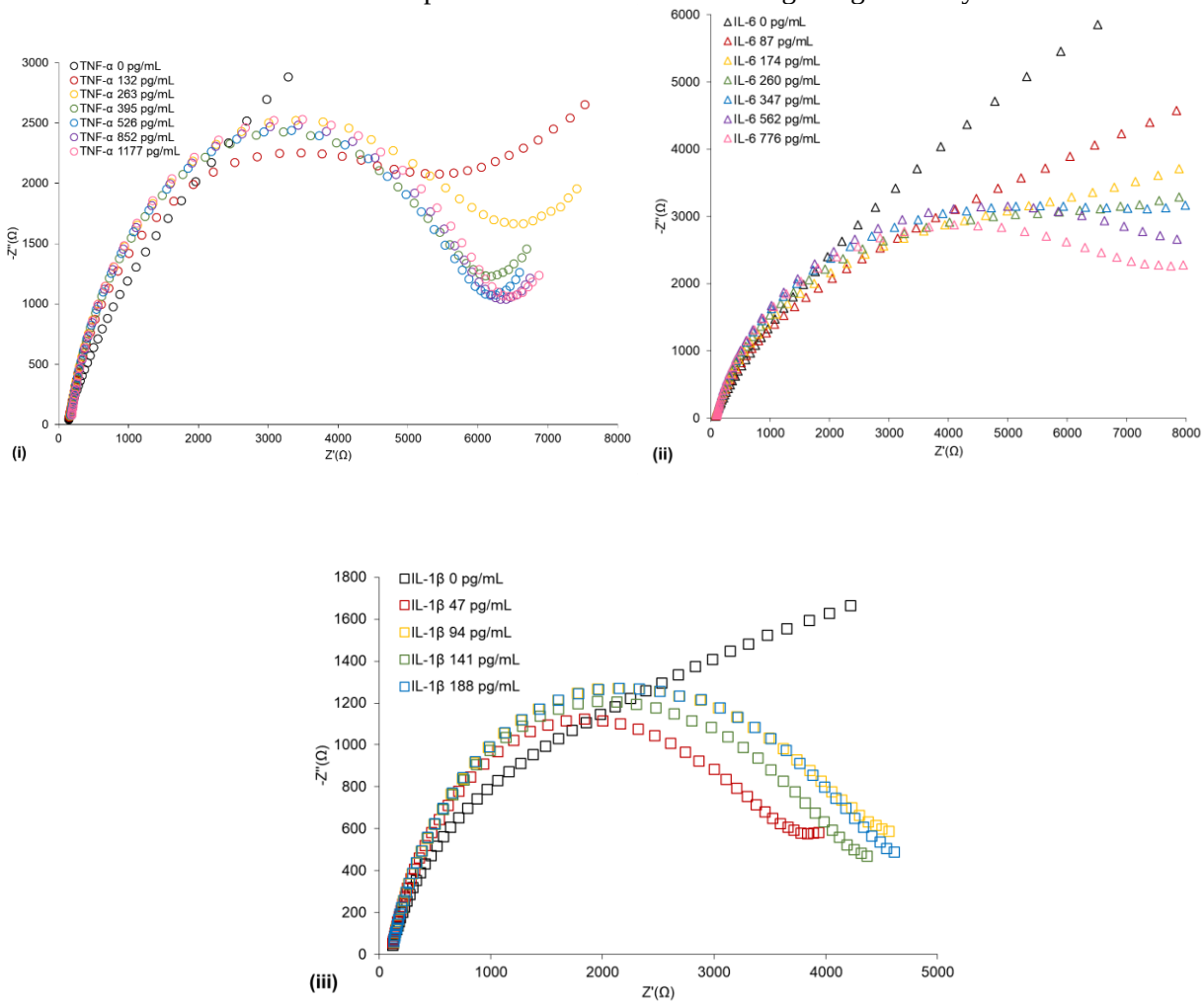

Figure 5. Results of assays using a voltage of 100 mV: **(i)** assays with IL-1β; **(ii)** assays with TNF-α; **(iii)** assays with IL-6;

The response curves for 250 mV under the same conditions (Figure 6) show that the sensor is specific for and can quantify TNF-α in concentrations from 132 to 852 pg.mL^{-1} with a straight line $R_{ct(normalized)} = (R_{ct(TNF-\alpha)}/R_{ct(blank)})$, $R_{ctnormalized} = 0.8746 \ln(\text{TNF-}\alpha) - 1.362$ and a correlation coefficient of 0.9293. Saturation and degradation effects were observed in concentrations equal to or greater than 852 pg.mL^{-1}. In the case of IL-6, an impedance semicircle was generated for concentrations of 562 pg.mL^{-1} or higher. This is due mainly to the breakdown of the layers and the loss of blocking activity because of the long incubation period, as the analysis at a concentration of 562 pg.mL^{-1} corresponds to a period of 3 hours from when the electrode was manufactured to the time when the test was carried out. The results for IL-1β show the formation of an impedance semicircle; however, there was no change in R_{ct}, indicating that the sensor is specific for TNF-α.

A comparison of the biosensor analyzed here with other electrochemical sensors described in the literature is given in Table 1.

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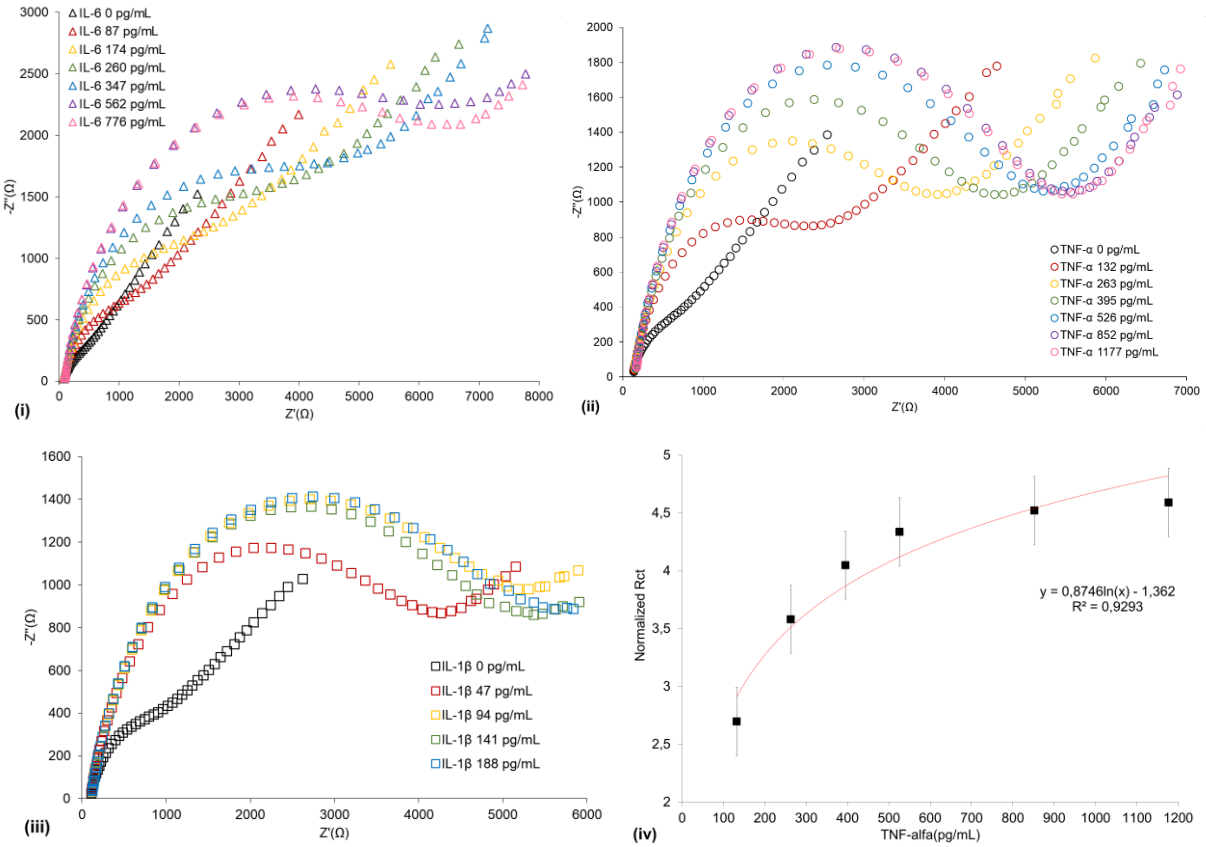

Figure 6. Results of assays using a voltage of 250 mV: **(i)** assays with IL-1β; **(ii)** assays with TNF-α; **(iii)** assays with IL-6; **(iv)** response curve with TNF-α.

Table 1 – Comparison of the proposed biosensor with other methodologies already described in the literature.

Method	Medium	Detection limit	Detection range	Reference
		------ (pg mL^{-1})------		
Piezoelectric biosensor with a quartz crystal microbalance sensor for determination of TNF-α	Undiluted sample	1.62	–	Pohanka, 2017
Antibody immobilized on a comb-shaped gold electrode microarray for detection of TNF-α	Undiluted sample	60	500 – 100	Arya; Estrela, 2016
Antibody immobilized on screen-printed carbon electrodes modified with carbon nanotubes for simultaneous detection of TNF-α and IL-1	Saliva	0.85	1-200	Sanchés-Tirado et al., 2017.
Antibody immobilized on gold microelectrodes for detection of TNF-α	Artificial and human saliva	3.21	1-100	Bellagambi et al, 2017.
Antibody immobilized on magnetic beads for detection of TNF-α	Undiluted sample	1	1-1000	Kongsuphol et al., 2014
Antibody immobilized on a graphite screen-printed electrode modified with poly-anthranilic acid for detection of TNF-α	Undiluted sample	5	Up to 100	Ardakani et al., 2014
Antibody immobilized on a DSP [(dithiobis)succinimidyl propionate] monolayer for detection of TNF-α	Culture medium: sample at 10%	1	1-100	Pui et al., 2013.
Antibody immobilized on a carbon-paste electrode modified with glutaraldehyde for detection of TNF-α	Serum	1	1-1000	The present study

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3.5 Sensor selectivity and sensitivity

The negative controls using IL-1β and IL-6 (Figure 6) showed no antigen-antibody interaction, confirming that the immunosensor is specific for TNF-α. This agrees with the results of the study by Kongsuphol et al. (2014), who used IL-2 as a negative control.

In a study by Liu et al. (2013) of an electrochemical immunosensor for detecting TNF-α in blood samples, the detection limit was 10 ng.mL^{-1}, while in an ELISA assay the detection limit was 15 pg.mL^{-1} and the detection range was 15 to 1000 pg.mL^{-1}. In our study and in the study by Kongsuphol et al. (2014), the detection limit was 1 pg.mL^{-1} in a range from 1 to 1000 pg.mL^{-1}, indicating that the immunosensor is sensitive as it can detect low concentrations of TNF-α in the sample (Table 1). Furthermore, only one hour and fifteen minutes is required to carry out the tests, much less than for commercial tests with an ELISA kit. For example, according to Sánchez-Tirado et al. 2016, in the case of assays with DuoSet® ELISA Development Systems [www.rndsystems.com] four hours and forty minutes are required to complete all the steps before the analysis.

3.6 Test in a real serum sample

Our results indicate that the TNF-α level in the negative control sample was 10.8 pg.mL^{-1}, a normal serum level similar to that described by Charles et al. (1999), while the corresponding figure for serum from the patient with rheumatoid arthritis was 21.9 pg.mL^{-1}. Both values agree with the figures for patients with autoimmune diseases reported in the literature (11.70 ± 3.22 versus 13.6 ± 53.6 pg.mL^{-1}) (PENESOVÁ et al., 2013). Our findings therefore confirm the functionality of the sensor and its potential for detecting and quantifying circulating TNF-α in human serum (CASTILLO-HERNANDEZ et al., 2017).

4 Conclusions

We have described a new method for manufacturing a sensitive immunosensor that is specific for TNF-α in undiluted serum samples. Carbon-paste electrodes were used for EIS, and the resulting signal varied according to the presence or otherwise of TNF-α. The proposed method had a detection limit of 1 pg.mL^{-1} and could quantify the cytokine in concentrations ranging from 1 to 1000 pg.mL^{-1} even in different mediums.

The results described here suggest that the system would be suitable for biomedical and clinical applications if it were miniaturized through the use of printed carbon electrodes or even low-cost microfluid systems. Our findings also indicate that the methodology is suitable not only for measuring isolated cytokines but also for measuring cytokines in undiluted human serum, suggesting that it could be used in medical offices to direct treatment of patients with autoimmune or chronic inflammatory diseases.

6 Divulgation

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