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Peptide-based electrochemical biosensor for juvenile idiopathic arthritis detection

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ABSTRACT

Juvenile idiopathic arthritis (JIA) is a wide group of diseases, characterized by synovial inflammation and joint tissue damage. Due to the delay in the implementation of biomarkers into clinical practice and the association with severe sequels, there is an imperative need for new JIA diagnosis strategies. Electrochemical biosensors based on screen-printed electrodes and peptides are promising alternatives for molecular diagnosis. In this work, a novel biosensor for detecting juvenile idiopathic arthritis (JIA) was developed based on the immobilization of the PRF + 1 mimetic peptide, as recognition biological element, on the surface of screen-printed carbon electrode. This biosensor was able to discriminate the JIA positive and negative serum samples from different individuals using differential pulse voltammetry, presenting limits of detection and quantification in diluted samples of 1:784 (v/v) and 1:235 (v/v), respectively. Evaluation by electrochemical impedance spectroscopy showed R_{CT} 3 times higher for JIA positive sample than for a pool of human serum samples from healthy individuals. Surface analysis of the biosensor by atomic force microscopy, after contact with JIA positive serum, presented great globular clusters irregularly distributed. The long-term stability of the biosensor was evaluated, remaining functional for over 40 days of storage (after storage at 8 °C). Therefore, a simple, miniaturized and selective biosensor was developed, being the first one based on mimetic peptide and screen-printed carbon electrode, aiming at the diagnosis of the juvenile idiopathic arthritis in real serum samples.

1. Introduction

Juvenile idiopathic arthritis (JIA) is a wide set of autoimmune and inflammatory diseases that affect children and adolescents under the age of sixteen, mainly characterized by synovial inflammation and joint tissue damage (Abramowicz et al., 2016; Barut et al., 2017; Giancane et al., 2017; Hersh and Prahalad, 2015). JIA comprises distinct subtypes, from which oligoarticular, polyarticular and systemic are the most common (Dimitriou et al., 2017; Giancane et al., 2016). Diagnosis is essentially by exclusion, based on medical history, physical examination, imaging and serological tests, but implementation of biomarkers into clinical practice is often delayed (Consolaro et al., 2015; Dimitriou et al., 2017; Giancane, 2016).

The absence of a proper diagnosis and treatment for JIA may result in significant impacts on quality of life, including irreversible damage in the joint tissues, physical limitations, disfigurement and multiple sequels (Abramowicz et al., 2016; Consolaro et al., 2015; Giancane et al., 2016; Wipff et al., 2016). Hence, the development of new tools with potential to aid JIA diagnosis is of a high relevance to improve early detection and treatment.

Electrochemical biosensors emerge in the clinical scenario as promising analytical platforms. They are designed in diverse configurations, including conventional (Li et al., 2013; Liu et al., 2016), nanostructured (Pan et al., 2017; Pandey et al., 2017; Silva et al., 2013), polymer-modified (Castro et al., 2014; Dervisevic et al., 2017; Rodrigues et al., 2014) and screen-printed (Abbaspour and Noori, 2012; Chan et al., 2016) electrodes. In addition, the biomolecules most often employed as recognition elements are nucleic acids (Castro et al., 2014; Souza e Silva et al., 2016), enzymes (Pakapongpan and Poo-Arporn, 2017; Paraíso et al., 2014; Silva et al., 2013), antibodies (Pandey et al., 2017; Rodrigues et al., 2014) and peptides (Hwang et al., 2017; Li et al., 2013; Liu et al., 2016). Peptides have been increasingly employed as recognition elements in biosensors due to their relative stability against denaturation, chemical versatility, specificity and simple acquisition by natural sources, selection in random libraries and chemical synthesis (Liu et al., 2015; Pavan and Berti, 2012).

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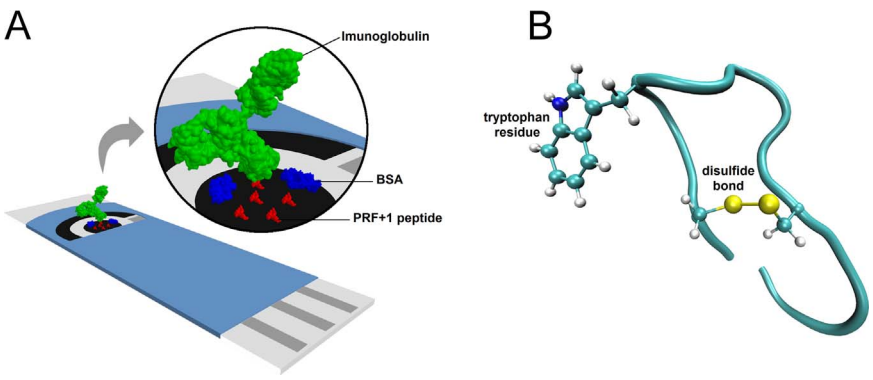

Fig. 1. Three-dimensional representations of the biosensor and its components. A) Biosensor (SPCE/PRF + 1/BSA) after application of positive serum sample, interacting with specific immunoglobulin related to JIA. B) Predicted structure of PRF + 1 peptide (ACSSWLPRGCGGGS), highlighting in balls-and-sticks the sidechains from amino acids reported to oxidize on carbon electrodes.

Screen-printed electrodes are produced by the deposition of a conductive film over an inert substrate and they present advantages such as serial production, low cost, rapid response, potential for automation and portability (Couto et al., 2016; Karunakaran et al., 2015). Their clinical use is increasing, with applications such as cancer diagnosis (Chan et al., 2016; Han et al., 2017) and pathogen detection (Abbaspour et al., 2015; Patris et al., 2016).

In a previous work, Araujo et al. (2016) selected peptides against antibodies purified from serum of JIA patients and assessed their performances for potential application in diagnosis. After validation, the synthetic PRF + 1 peptide was able to discriminate JIA patients from healthy individuals and patients with other immune-mediated rheumatic diseases with high accuracy. In the present work, we aimed to employ PRF + 1 peptide as a recognition biological element in the development of a simple, selective and sensitive platform for JIA detection. Therefore, screen-printed carbon electrodes (SPCEs) were modified with PRF + 1 peptide and tested for the differential recognition of JIA and healthy control serum samples from different individuals, followed by performance evaluation.

Biosensors applied to rheumatoid arthritis diagnosis have been described in the literature (Ahn et al., 2011; Bertok et al., 2015; Drouvalakis et al., 2008; Pang et al., 2015; Villa et al., 2011). However, they do not refer to JIA specifically or use mimetic peptide as probe immobilized onto SPCE.

To the best of our knowledge, this is the first biosensor based on peptide and screen-printed carbon electrode specifically designed to detect JIA in human serum samples.

2. Material and methods

2.1. Reagents and instrumentation

Reagents Na_2HPO_4, NaH_2PO_4 and NaCl (Neon); $K_4Fe(CN)_6.3H2O$ (Fluka), $K_3Fe(CN)_6$ (Acros Organics), KH_2PO_4 (Vetec), KCl (J.T. Baker) and $HClO_4$ (Reagen) were used as purchased. Solutions were prepared with deionized water (Gehaka, 18 MΩ cm) and bubbled with ultrapure N_2 prior to electrochemical measurements. Percloric acid solution was prepared at 0.5 mol L^{-1}. Buffer solutions were saline phosphate buffer (PBS, 0.01 mol L^{-1}, pH 7.4, containing 10 mmol L^{-1} NaCl) and phosphate buffer (PB, 0.1 mol L^{-1}, pH 7.4) with or without the addition of 10 mmol L^{-1} NaCl. Potassium ferrocyanide/ferricyanide solutions were prepared at 5 mmol L^{-1} or 0.1 mmol L^{-1} containing 0.1 mol L^{-1} KCl.

Previously selected PRF + 1 peptide (ACSSWLPRGCGGGS) (Araujo et al., 2016) was chemically synthetized (GenScript) and diluted in deionized water (1 μg mL^{-1}). Blocking solution was prepared with bovine serum albumin (BSA, Sigma-Aldrich, 96%) at 3% (m v^{-1}) in PBS. A pool of human serum samples from healthy individuals and patients who met the criteria for JIA subtypes (persistent oligoarticular; extended oligoarticular; rheumatoid factor-positive polyarticular; rheumatoid factor-negative polyarticular; and systemic) was obtained with the approval of Research Ethics Committee from the Federal University of Uberlândia, Minas Gerais, Brazil (number 685/09). Serum samples were used without dilution or diluted in phosphate buffer.

All electrochemical measurements were performed with a CHI 760C potentiostat (CH Instruments, USA) and DS110 screen-printed carbon electrodes (Dropsens, Spain). Electrochemical impedance spectroscopy experiments were performed with PGSTAT302N (Metrohm Autolab, The Netherlands) and ItalSens IS-C screen-printed carbon electrodes (Palmsens, The Netherlands). The SPCEs consisted of a carbon counter electrode, a carbon working electrode and a silver pseudo-reference electrode.

2.2. Biosensor preparation

Prior to use, screen-printed carbon electrodes (SPCEs) were electrochemically cleaned by cyclic voltammetry (from 0 to +0.8 V, 50 mV s^{-1}) in percloric acid solution (0.5 mol L^{-1}). Then, their surface was activated through chronoamperometry (−1.4 V, 300 s) in phosphate buffer (0.1 mol L^{-1}, pH 7.4), (adapted from Sahin and Ayranci, 2015). Immediately after activation, 2 μL of PRF + 1 solution (1 μg mL^{-1}) was applied onto the SPCE surface and the system was maintained at room temperature for 30 min. Next, 2 μL of blocking solution (BSA, 3%) was applied onto the SPCE/PRF + 1 surface and maintained at room temperature for 30 min. Finally, 2 μL of healthy control (HC) or juvenile idiopathic arthritis (JIA) serum sample was applied onto the biosensor surface (SPCE/PRF + 1/BSA) and maintained at room temperature for 30 min, in order to test its differential recognition (Fig. 1A). Between each step of preparation, the electrodes were rinsed with phosphate buffer. The specificity of the PRF + 1 probe was confirmed by voltammetry analysis, EIS measurements and AFM images.

2.3. Electrochemical measurements

Electrochemical measurements were performed after each step through differential pulse voltammetry in phosphate buffer solution (0.1 mol L^{-1}, pH 7.4), with potential ranging from +0.2 to +1.0 V, scan rate 40 mV s^{-1}, modulation amplitude 0.05 V, step potential 0.008 V, modulation time 0.06 s and interval time 0.2 s, using diluted sera (1:100, v/v). The same technique was applied using potassium ferrocyanide/ferricyanide solution (0.1 mmol L^{-1}) with 0.1 mol L^{-1} KCl, potential range from −0.2 to +0.5 V, scan rate of 40 mV s^{-1}, modulation amplitude 0.05 V, step potential 0.008 V, modulation time 0.06 s and interval time 0.2 s, using undiluted serum. Electrochemical impedance spectroscopy was conducted using potassium ferrocyanide/ferricyanide solution (5 mmol L^{-1}) with 0.1 mol L^{-1} KCl, open circuit potential, frequencies ranging from 100 kHz to 10 mHz, amplitude 10 mV and serum dilution 1:100.

2.4. Figures of merit of the biosensor

The biosensor performance was evaluated with varying dilutions of