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Multiplexed electrochemical immunosensor for detection of celiac disease serological markers

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ABSTRACT

Celiac disease (CD) is a gluten-induced autoimmune enteropathy characterized by the presence of antibodies against gliadin (AGA) and anti-tissue transglutaminase (anti-tTG) antibodies. A disposable electrochemical dual immunosensor for the simultaneous detection of IgA and IgG type AGA and anti-tTG antibodies in real patient's samples is presented. The proposed immunosensor is based on a dual screen-printed carbon electrode, with two working electrodes, nanostructured with a carbon–metal hybrid system that worked as the transducer surface. The immunosensing strategy consisted of the immobilization of gliadin and tTG (i.e. CD specific antigens) on the nanostructured electrode surface. The electrochemical detection of the human antibodies present in the assayed serum samples was carried out through the antigen–antibody interaction and recorded using alkaline phosphatase labelled anti-human antibodies and a mixture of 3-indoxyl phosphate with silver ions was used as the substrate. The analytical signal was based on the anodic redissolution of enzymatically generated silver by cyclic voltammetry. The results obtained were corroborated with commercial ELISA kits indicating that the developed sensor can be a good alternative to the traditional methods allowing a decentralization of the analyses towards a point-of-care strategy.

1. Introduction

Celiac disease (CD) is a disorder of the small intestine caused by an inappropriate immune response to wheat gluten and similar proteins of barley and rye in genetically susceptible individuals [1]. The humoral autoimmune response leads to an abnormal intestinal mucosa characterized by villous atrophy and crypt hyperplasia, resulting in malabsorption related problems [2]. Common serological changes of this condition include the appearance of antibodies against gliadin (AGA) and anti-tissue transglutaminase (anti-tTG) antibodies; which are specific serological markers of the disease. Is reported that immunoglobulin A (IgA) anti-tTG antibody detection has sensitivity higher than 90% and specificity higher than 95%; IgA AGA detection presents a sensitivity of about 80% and a specificity ranging from 80 to 90% [3]. The IgA isotype is considered to be the most specific; however, selective IgA deficiency affects about 2–5% of patients diagnosed with CD [4]. In these cases, the determination of the immunoglobulin G (IgG) class of antibodies is considered [5]. The gold standard diagnosis for CD which relies on an intestinal biopsy has been changing over the last few decades, especially due to the advent of serological tests with high sensitivity and specificity [1]. Serological markers can be used in front-line detection strategy to rule out the pathology. Nowadays, analytical techniques are moving towards the development of more simple, fast, and point-of-care (POC) analyses. Therefore, electrochemical biosensors are playing a growing part in many fields in which a more accurate, sensitive, fast, low cost and, specially, in situ analysis is required [6]. Electrochemical biosensors are in many cases the best option for diagnostics given their high selectivity and sensitivity which allows early detection of many diseases. Among the different types of electrodes that are employed in the construction of electrochemical biosensors, screen-printed electrodes (SPEs) are of special interest. Their properties, such as simplicity and low cost, versatility of design, small dimensions and possibility

Abbreviations: CD, celiac disease; EIs, electrochemical immunosensors; AGA, anti-gliadin antibodies; Anti-H-IgA-AP, anti-human IgA antibodies conjugated with alkaline phosphatase; Anti-H-IgG-AP, anti-human IgG antibodies conjugated with alkaline phosphatase; SPCEs, screen-printed carbon electrodes; MWCNTs, carboxylated multiwalled carbon nanotubes; NPAus, gold nanoparticles.

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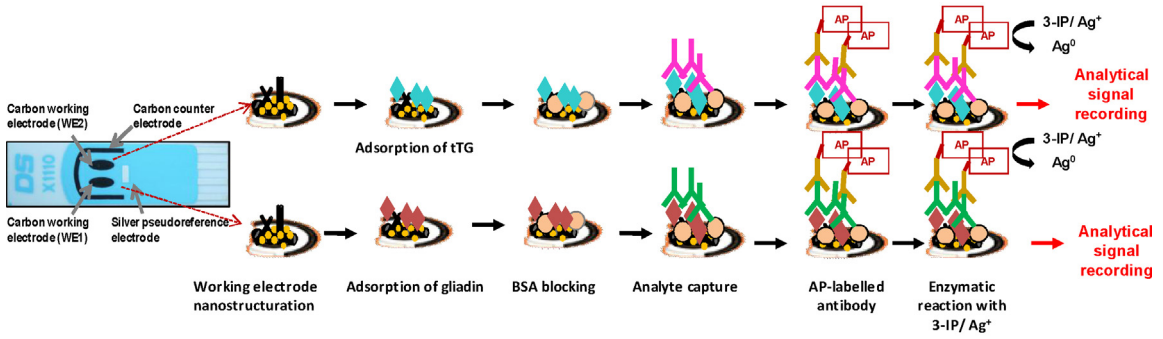

Fig. 1. Schematic representation of the immunosensing strategy followed for the analytical detection of CD serological markers.

of incorporation in portable systems, as well as adequate electroanalytical characteristics, have progressively introduced SPEs in fields such as environmental analysis, food quality control, and clinical diagnostics [7,8]. In recent years, the development of electrochemical transducers that make use of electrode surface modifications with nanomaterials became an exciting area of development for modern analytical science [9–11]. The use of nanomaterials extends the potentialities of these electrodes by improving their original electrochemical properties. For this reason, carbon nanostructure/metal nanoparticle hybrid systems have been recently exploited as last generation transducers for the development of biosensors [12–14], because a synergic effect of each material property is obtained [15]. Within the electrochemical biosensors field, electrochemical immunosensors (EIs) combining the specificity inherent to antigen–antibody interactions with the high sensitivity of electrochemical transduction [16], can be an excellent alternative to conventional immunochemical tests. Therefore, a dual immunosensor for the simultaneous detection of AGA and anti-tTG antibodies was developed. A dual screen-printed carbon electrode (SPCE) nanostructured with a carbon–gold hybrid system was employed as the transducer surface. The immunosensing strategy consisted of the immobilization of gliadin and tTG (i.e. CD specific antigens) onto the nanostructured electrode surface followed by the electrochemical detection of the human antibodies present in real serum samples. The antigen–antibody interaction was recorded using alkaline phosphatase labelled anti-human antibodies and a mixture of 3-indoxyl phosphate with silver ions (3-IP/Ag^+) was used as the substrate [17]. The analytical signal was based on the anodic redissolution of enzymatically generated silver by cyclic voltammetry. As far as we are concerned, there is no record of an EI for the simultaneous detection of the referred antibodies. The published works regarding EIs for CD clinical diagnosis that are currently available are focused on the detection of just one of the biomarkers [12,13,18–25].

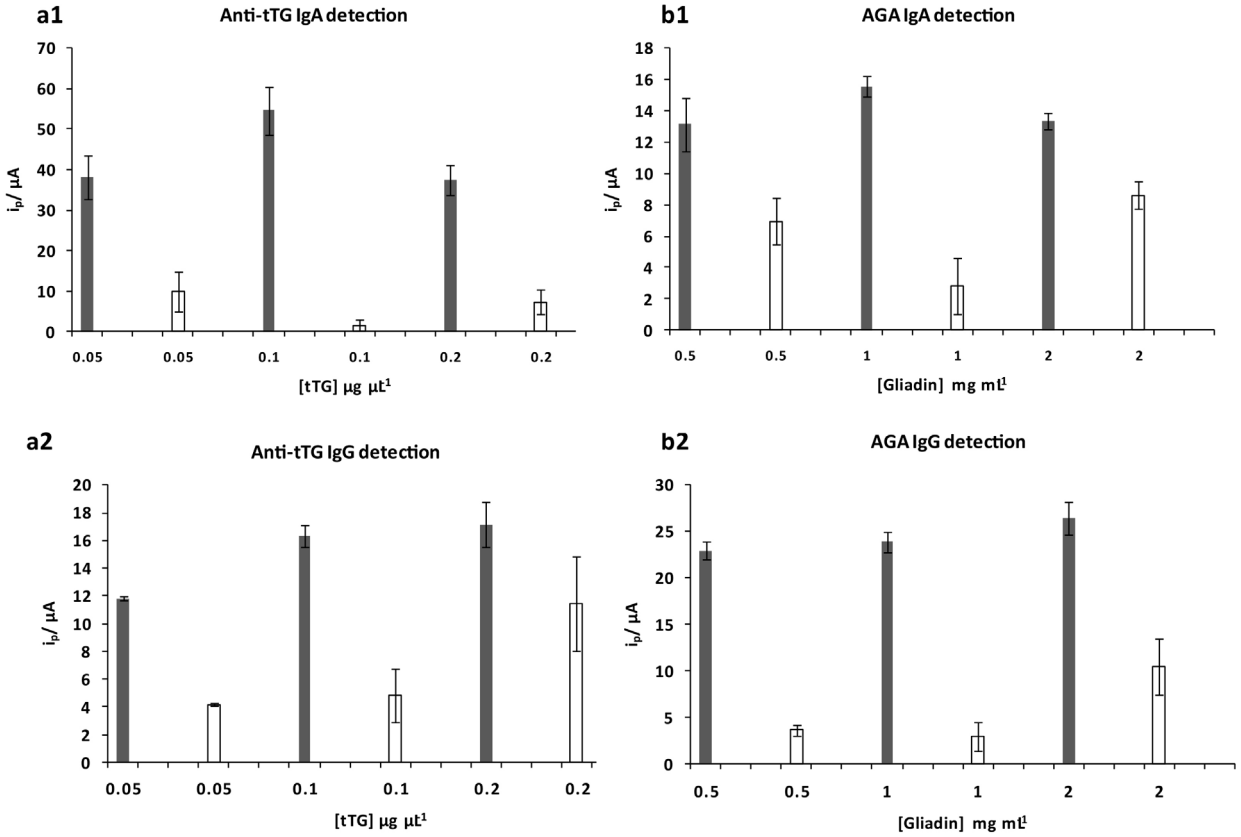

Fig. 2. Effect of the different concentrations of the antigen (tTG: a1 and a2; gliadin: b1 and b2) on the analytical signal for the detection of the corresponding immunoglobulins class A (a1 and b1) and class G (a2 and b2). Experimental conditions: BSA 2%; positive (grey bars) and negative (white bars) control samples (1:2); anti-H-IgA-AP 1:30,000 (a1 and b1) and anti-H-IgG-AP 1:50,000 (a2 and b2); 3-IP 1.0 mM; Ag^+ 0.4 mM. Average data ± standard deviations are indicated ($n = 3$).