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Electrochemical immunosensor for anti-tissue transglutaminase antibodies based on the in situ detection of quantum dots

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ABSTRACT

A miniaturized electrochemical biosensor array with in situ detection of quantum dots (QDs) was developed for the detection of anti-transglutaminase IgG antibodies (a celiac disease biomarker) in human sera. For the fabrication of the sensor, a 8-channel screen-printed carbon electrochemical arrays were used as transducers and modified with tissue-transglutaminase by adsorption. The immunologic reaction was carried out in a few simple steps: reaction with human serum, which contains the analyte of interest, followed by the immunoreaction with anti-human IgG labeled with CdSe/ZnS QDs and electrochemical detection of Cd^{2+} released from QDs. All steps were performed on the screen-printed arrays as the solid support, and the detection of Cd^{2+} was performed in situ after acid attack of the QDs without a transfer step by voltammetric stripping. The electrochemical response was correlated with the anti-transglutaminase IgG concentration. The developed electrochemical immunosensor is a trustful screening tool for celiac disease diagnosis discriminating between positive and negative sera samples with high sensitivity.

1. Introduction

Nowadays, electrochemical biosensors are playing an important role as clinical diagnostic platforms [1,2]. Due to the versatility and high sensitivity, electrochemical biosensors are placed in the lead to become a future detection platform at different medical centers for disease diagnosis. To achieve this goal, the development of new detection labels that improve the ones employed so far, which are mainly enzymatic, is a constant concern in this scientific field [3–6].

Quantum dots (QDs) are semiconductor nanoparticles widely used in several applications, mainly on optical and electrochemical biosensing [7–9]. QDs are used in bioassays with fluorescence detection due to their interesting luminescent properties [10]. Metallic components of QDs can be measured electrochemically after an acid attack to break the nanoparticle and release metal cations to the solution. Normally, both the bioassay and the subsequent acid attack are all performed outside of the detection platform [11–14]. Our research group has developed an innovative methodology in which the bioassay as the acid attack and the detection are performed directly on the screen-printed electrodes [15]. This methodology is much easier and similar to performing bioassays with enzymatic labels. Furthermore, using screen-printed electrodes as the biosensor platform provides important benefits over the use of other kind of electrodes, such as low sample volume, low cost device, the ability to perform in-situ analysis, besides being disposable avoiding tedious cleaning steps of the electrodes. The combination of the biosensor methodology developed by our group and the use of QDs as electrochemical label results in an attractive platform capable to compete with enzymatic labeling systems but saving the time of the enzymatic reaction and reagents.

Celiac disease (CD) is a gluten-sensitive enteropathy triggered by dietary gluten in genetically susceptible individuals. CD patients, normally, experience immune reaction and the body produces autoantibodies causing the destruction of intestinal mucous [16]. The main criteria for the diagnosis of CD is a biopsy, but in recent years, the serological tests for the detection of biomarkers are being imposed, allowing to avoid the more invasive analysis [17]. Some of the autoantibodies produced in a CD patient react specifically with tissue transglutaminase (tTG), being an important biomarker of this autoimmune condition. Although, anti-tTG IgA antibodies seems to be a more specific biomarker, sometimes when the patient has IgA deficiency, the determination of anti-tTG IgG antibodies is crucial in order to make a diagnosis. Therefore, the detection of anti-tTG IgG is also conducted in medical facilities for the serological detection of CD. The most common methodology for the detection of CD autoantibodies is an enzyme-linked immunosorbent assay (ELISA). ELISA tests have been used for the detection of anti-tTG antibodies with good sensitivity and specificity [18,19]. An alternative to ELISA tests are the electrochemical immunosensors that have some

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advantages such as increased sensitivity due to the electrochemical detection, besides the use of lower sample volumes, resulting in cost savings [20–23].

Among the published electrochemical immunosensors for the determination of anti-tTG IgG, this is, to the best of our knowledge, the first work employing QDs as electrochemical label. Moreover, in this work we use an innovative methodology where both the bioassay and the detection of QDs are performed in the same platform (i.e. screen-printed carbon electrodes). As explained above, this methodology highly simplifies the ones previously published using QDs electrochemical detection, being easier to perform with less number of steps. Another advantage of this work is the use of 8-channel screen-printed electrochemical arrays ($8 \times$ SPCEs), which further simplifies the procedure and drastically improves the time of analysis.

2. Materials and methods

2.1. Apparatus and electrodes

Voltammetric measurements were performed with a μStat 8000 (DropSens, Spain) potentiostat interfaced to a Pentium 42.4 GHz computer system and controlled by DropView 8400 2.0 software. All measurements were carried out at room temperature. 8-channel screen-printed electrochemical arrays were purchased from DropSens (Spain). Each array is formed by eight 3-electrode electrochemical cells (30 μL volume) with carbon-based working and counter electrodes, whereas pseudoreference electrodes and electric contacts are made of silver. This device has dimensions of $4.0 \times 7.9 \times 0.06$ cm^3 (length × width × height) and the diameter of the working electrodes is 2.56 mm. 8-channel arrays were connected to the potentiostat through a specific connector, DRP-CAST8X.

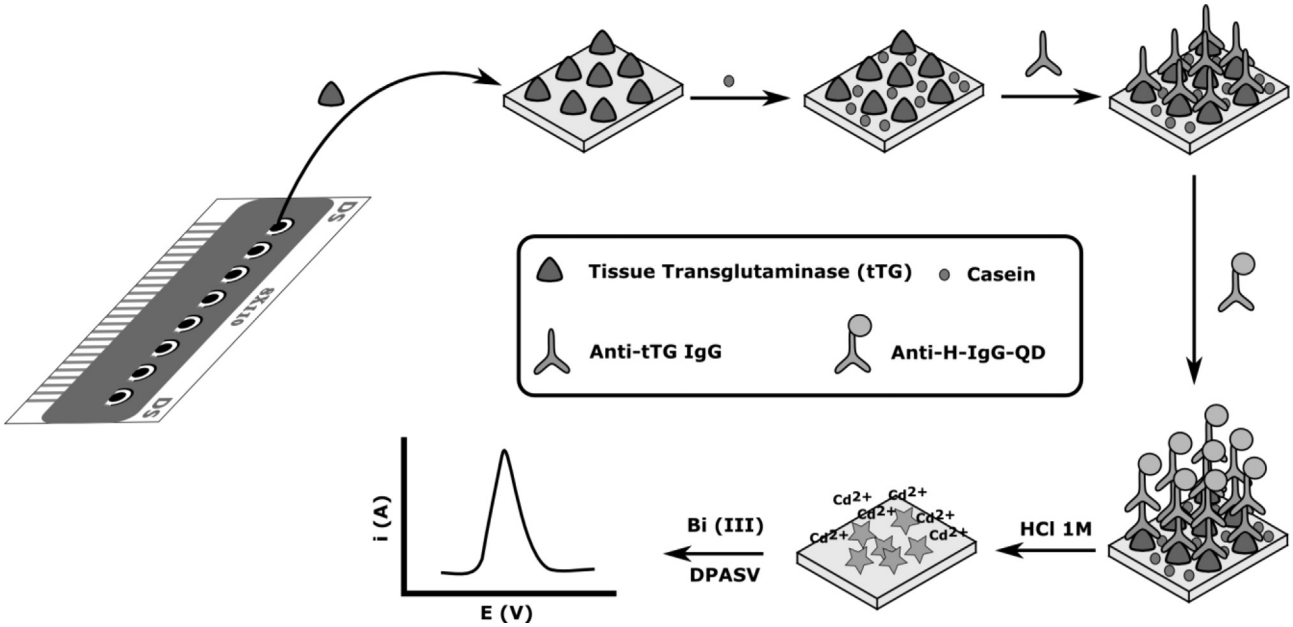
2.2. Reagents and solutions

Sodium hydroxide, acetic acid (100%), fuming hydrochloric acid (37%) were purchased from Merck. Bismuth(III) standard, Tris(hydroxymethyl)aminomethane (Tris), bovine serum albumin fraction V (BSA) and β-casein (98%, from bovine milk) were purchased from Sigma-Aldrich (Spain). Human tissue transglutaminase (recombinantly produced in insect cells) was purchased from Zedira (Germany). Qdot® 655 goat F(ab′)$_2$ anti-human IgG Conjugate (H+L) (anti-H-IgG-QD) was purchased from Life Technologies (Spain). Varelisa Celikey IgG ELISA kits were purchased from Phadia (Germany). Each kit contained six standard serum samples (0, 3, 7, 16, 40, 100 U mL^{-1}) and a positive and a negative control. Ultrapure water obtained with a Millipore Direct Q5™ purification system from Millipore Ibérica S.A. (Madrid, Spain) was used throughout this work. All other reagents were of analytical grade. Working solutions of tTG, anti-H-IgG-QD, BSA, and casein were prepared in 0.1 M pH 7.4 Tris–HCl buffer.

2.3. Immunoassay procedure

An aliquot of 4 μL of tTG solution with a concentration of 0.1 mg mL^{-1} was dropped on the surface of each working electrode and was left overnight. After washing the screen-printed electrode with 0.1 M pH 7.4 Tris–HCl buffer, a blocking step was carried out placing 25 μL of casein blocking buffer (20 μg μL^{-1} of casein in 0.1 M pH 7.4 Tris–HCl) solution for 45 min. The detection of anti-tTG IgG antibodies was accomplished by incubating the immunosensor with human serum samples for 60 min followed by a washing step with 0.1 M Tris–HCl pH 7.4 buffer. Finally, 25 μL of anti-H-IgG-QD (10 nM in terms of QDs) solution (with 1 μg μL^{-1} of BSA) was dropped on the modified electrode for 60 min. A last washing step was carried out with ultrapure water. Then, the electrode was connected to the potentiostat for the electrochemical analysis. In the Scheme 1, a diagram of the immunoassay using the biosensor array is presented.

The measurement step was performed according to a methodology previously developed[15]. After the biological reaction, 1 μL of HCl 1.0 M was added on the working electrode to release Cd^{2+} from QDs, followed by the addition of 25 μL of 0.1 M acetate buffer solution pH 4.5 with 1.0 mg L^{-1} Bi (III). A constant potential of +1.00 V was applied for 60 s to activate the working electrode. Cadmium was preconcentrated on the electrode surface by applying a potential of −1.10 V for 300 s, simultaneously a bismuth film was also formed during this step. The potential was swept from



Scheme 1. Schematic diagram of the electrochemical biosensor array. The bioassay is carried out using the working electrodes of the array as transducers and in situ electrochemical detection of QDs.