Method and Instrumentation of Serologic Diagnostics: An Electrochemical Enzyme-Linked Immunoassay for the Diagnosis of Mouth and Foot Disease

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Abstract—The development of an inmunosensor for the point-of-care detection of the mouth and foot disease is presented. The detector is based on an ELISA method with amperometric detection. A non-structural protein, the 3ABC, is used to selectively detect anti-3ABC antibodies. The biological test is performed directly onto screen printed electrodes. A dedicated small, portable potentiostat is employed for the control of the sensors, as well as data acquisition and storage.

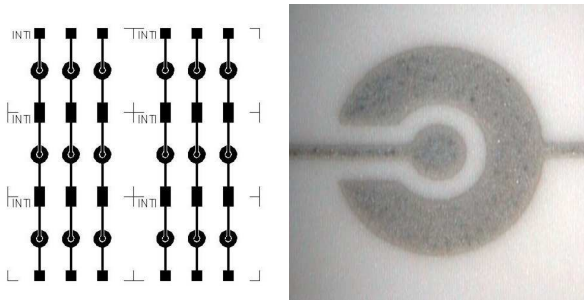
I. INTRODUCTION

Biosensors are compact analytical devices which employ a biological element in order to detect a specific substance (i.e. the analyte). After the analyte has been detected by the biological recognition element, a signal of some sort is produced which then is converted into an electrical signal by means of a transducer. In the case of electrochemical biosensors, a chemical signal generated by the interaction between the biological recognition element and the analyte is converted into an electrical current via an electrochemical reaction at an electrode surface [1]. In this paper, we present the methods and instrumentation for an electrochemical enzyme-linked immunoassay for the diagnosis of the mouth and foot disease. This presentation covers all aspects of the development of the biosensor: the production of gold electrodes by thick film technology and electrochemical cells with a numeric control device, the chemical modification of the gold electrodes with the biological recognition elements, which are antigens of proteinaceous nature, the electrochemical transducer via the action of a redox mediator (a chemical substance which acts as the electrical connection between the enzymes and the electrode), and finally the electronic instrumentation required to control the electrochemical system and process the resulting signal [2,3]. Results obtained for the diagnosis of foot and mouth disease are presented.

II. EXPERIMENTAL DETAILS

A. Electrodes design and manufacture

A three-electrode cell was designed and constructed. Ring-disk geometry was defined for the working and counter electrodes. Disks with a diameter of 1000 μm were employed as working electrodes, while counter electrodes were designed to have ten times as much area as the working electrodes. The inner and outer diameters of the rings were 1600 μm and 3600 μm. The resulting gap between electrodes was 300 μm. Track width was 300 μm and pad size was 2 mm × 2 mm for both electrodes. A 3 × two-electrode array was designed, so they could be evaluated together or individually (Fig. 1).



a) b)

Fig. 1. a) Matrix of 18 sensors. b) Photograph of a thick film two-electrode configuration showing active circular area with 1000 μm diameter for the working electrode.

The two thick film electrodes were printed onto α-Al_2O_3 substrates by conventional screen printing technology; the third electrode was a silver chloride reference electrode. Commercial Au organometallic paste (ESL D8083) and 96 % α-Al_2O_3 substrates were employed. Electrode layout was transferred by means of photolithography to a stainless steel mesh (400 wires per inch) with a negative photosensitive film (Ulano CDF-2). Au ink printing was performed by an EKRA Microtronic-II printer, dried at 125ºC during 15 min and finally fired at 580°C. The electrodes were integrated in an electrochemical cell, constructed in PMMA using a numeric control device from a CAD layout.

B. Electronic instrumentation

A portable potentiostat was developed to allow point-of-care measurements. The instrument can control the reference electrode in the -2.5 V to +2.5 V range, allowing its use for this and other biosensors. The allowed working electrode current is in the -100 μA to +100 μA range.

The circuit uses a low offset operational amplifier (op-amp) to control the counter electrode (OP07). To avoid loading the reference electrode an LT1056 op-amp was selected, providing an input impedance of 10^{12} ohms. The current to

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voltage conversion, carried out in the working electrode circuitry, was also implemented using an LT1056 op-amp. This op-amp introduces a very low current error, typically in the range of 10 pA, and guarantied to be under 0.34 nA for the full operation range.

The potentiostat is controlled by a microcontroller connected to a PC using the Universal Serial Bus (USB). The microcontroller has a 10 bits A/D converter, used to measure the working electrode current, and a PWM output, used to control the reference voltage.

C. *Immobilization antigens onto the electrode surface*

3ABC, a non-structural protein from the foot and mouth disease virus, was immobilized on screen printed Au electrodes employing 3-mercaptopropionic acid and a carbodiimide as molecular linkers between the gold electrode and the protein. Electrodes were cleaned with H_2SO_4:H_2O_2 30% (2:1) and thoroughly rinsed with water (miliQ quality), immersed overnight in a solution containing 40 mM 3-mercaptopropionic acid and 75 % ethanol + 25% water. The electrodes were treated during 30 min with 20 µl of a solution containing 0.1 M 1-ethyl-3(3-dimethyl aminopropyl) carbodiimide and 25mM N-hydroxysuccinimide in 0.1 M PBS buffer of pH 7, and then 20 µl of 3ABC 0.22 µg/µl, also in a 0.1M PBS buffer of pH 7, were added onto each electrode. After 45 min, the electrodes were rinsed again with high quality water and immersed overnight in quenching buffer (0.262% glycine an 1% gelatine). Then the electrodes were rinsed again and ready to be used.

For the serologic tests, 3ABC coated electrodes were incubated with different sera during one hour and rinsed with 0.1% Tween 20 in PBS buffer by means of a controlled flux syringes. Then the electrodes were incubated with anti-Ig conjugates during an hour, rinsed with 0.1% Tween 20 in PBS buffer and finally the electrochemical measurements were carried out.

D. *Electrochemical measurements*

Electrode potential was changed from 0 to –300 mV applying 50 mV steps in a 0.1 M phosphate buffer of pH 7 + 0.1 M KCl. 4 mM hydroquinone was employed as a redox mediator and the H_2O_2 concentration was increased from 0 to 1.5 mM. A wire of 1 mm diameter of Ag | AgCl was employed as a reference electrode.

III. RESULTS

In this electrochemical immunodetectors, an antigen (3ABC) is linked to the electrode surface. If this electrode is placed in contact with a serum containing anti-3ABC antibodies (i.e. a “positive serum”), antigen-antibody complexes are formed, which can be further recognized by a second antibody. The second antibody is labeled with a redox enzyme (horseradish peroxidase, i.e. HRP). Thus, an antigen-antibody-(HRP-labeled anti-antibody) complex is formed on the electrode. The activity of the HRP can be electrochemically detected after the addition of hydrogen peroxidase and a suitable redox mediator, in a similar fashion as in the case of enzymatic electrodes [4]. This process is schematically shown in Fig. 2.

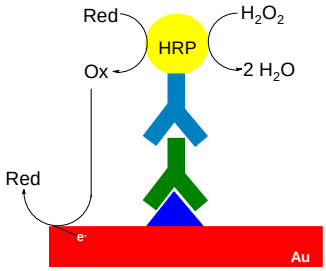

Fig. 2. Schematic representation of enzyme-linked immunoassay with electrochemical detection.

On the other hand, if a 3ABC coated electrode is placed in contact with a serum without 3ABC antibodies (a “negative serum”), no antigen-antibody complexes are formed and, consequently, no HRP activity is electrochemically detected.

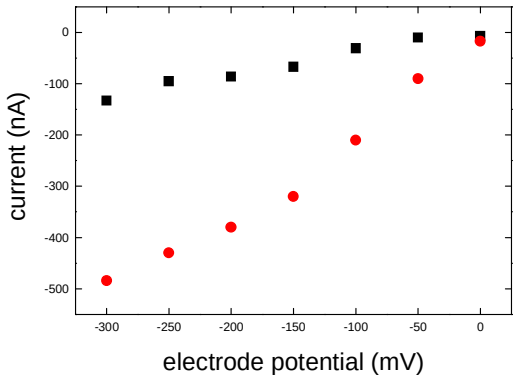

Fig. 3. Current-potential curves obtained under steady state conditions for an enzyme-linked immuno assay for a negative serum (squares) and a positive serum (circles). Hydrogen peroxide concentration: 1.5 mM. The potential of the working electrode was controlled with a potenciostat EG&G PAR 273A.

Fig. 3 shows the current-potential curves obtained for a positive and a negative serum. The difference in current values is high enough as to permit the discrimination of infected and non-infected specimens from this electrochemical enzyme-linked immunoassay.

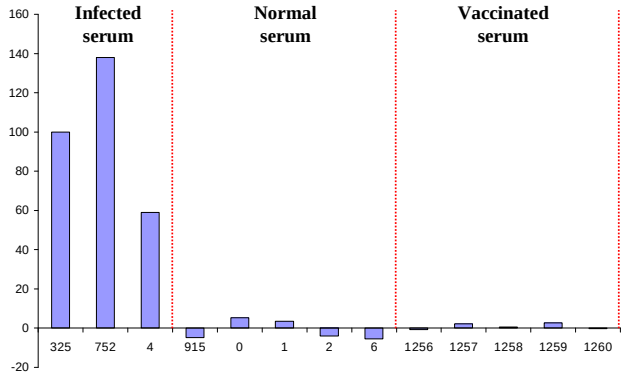

Fig. 4. Results for positive (infected), negative (normal) and vaccinated sera, normalized to serum number 325.

Fig. 4 shows the measurements results for different sera. Abscissas represents each serum with its code identification, while ordinates represent a magnitude proportional to the measured current. This magnitude is corrected by the current measured with an electrode without any immobilized biomolecule and normalized to the current measured with the serum number 325. High values for the ordinates account for positive sera, while small values of the ordinates are obtained for the negative and vaccinated sera.

IV. CONCLUSIONS

The development of an immunosensor for point-of-care detection of the mouth and foot disease was presented. The detection method, based on an electrochemical ELISA test, proved to be as sensible as the standard fluorimetric method. The sera of infected cattle were clearly sorted out from those of vaccinated and non-infected cattle. The detection system is small, portable, and specially suited for the detection of the disease in remote and harsh environments.

REFERENCES

[1] A.J. Bard, L.R. Faulkner, "Electrochemical Methods: Fundamentals and Applications", John Wiley & Sons, 2001.

[2] S.Campuzano, M.Pedrero, J. Pingaron, "A peroxidase-TTF biosensor based on self-assembled monolayer modified Au electrodes for the flow-injection determination of hydrogen peroxide", Talanta 66 (2005) 1310-1319.

[3] M.E. Ribone, M.S. Belluzo, D. Pagani, I.S. Macipar, C.M. Lagier, "Amperometric bioelectrode for specific human immunoglobulin G determination: Optimization of the method to diagnose American tripanosomiasis", Analytical Biochemistry 350 (2006) 61–70.

[4] G. Longinotti, P. Lloret, G. Ybarra, C. Moina, L. Hermida, O. Milano, M. Roberti, L. Malatto, L. Fraigi, L. "Screen-printed electrochemical biosensors based on magnetic core-shell nanoparticles," Proceedings of 6th Ibero-American Congress on Sensors, IBERSENSOR 2008.