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Photoelectrochemical immunodiagnosis of canine leishmaniasis using cadmium-sulfide-sensitized zinc oxide modified with synthetic peptides

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ABSTRACT

A photoelectrochemical immunosensor for the detection of anti-*Leishmania infantum* antibodies based on zinc oxide and cadmium sulfide films electrodeposited on an ITO-coated glass slide (CdS/ZnO/ITO) has been proposed. The effects of light/dark conditions and the kinetics of CdS sensitizer regeneration were evaluated by scanning electrochemical microscopy in feedback mode. The platform was modified using two different peptides (PEP 13 and PEP 16) from two different proteins of high specificity and selectivity toward recognition of *L. infantum* antibodies, producing Peps/CdS/ZnO/ITO. This photoelectrochemical immunosensor provides a cheap and promising method of discriminating between positive and negative canine serum samples.

1. Introduction

Interest in semiconductor-based materials has been increasing due to their wide range of applications, including the development of light-energy-harvesting assemblies, photovoltaic solar cells, environmental remediation and photoelectroanalytical devices (PECs) [1–4]. PECs stand out as an interesting alternative to biosensors since they exploit the best characteristics of electrochemical and optical techniques [5–7].

Zinc oxide presents unique chemical and physical properties such as large band gap, high chemical stability, broad radiation absorption range, high photo-stability, high electrochemical coupling coefficient, high electron mobility, among many other attractive properties [8–10]. However, ZnO requires ultraviolet light for excitation, reducing its usefulness in terms of the development of biosensors since UV light can decompose the biological recognition element of the biosensor or the sample. Therefore, it is necessary to couple the ZnO with a narrow or mid-band gap photosensitizer in order to harvest visible light with ZnO. One of the most promising species for the sensitization of ZnO is cadmium sulfide, which shows excellent photoelectrochemical properties [11].

There is considerable current interest in the development of cost-effective, sensitive, selective, and useful methods for monitoring anti-*Leishmania infantum* antibodies in large numbers of people or animals [12,13]. The high interest in monitoring of Leishmaniasis is based on the worldwide dissemination of this disease, which stands out as one of the most dangerous illnesses originating in tropical and sub-tropical regions [14,15]. To address this problem, novel serological tests such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay test (IFAT), and techniques such as surface plasmon resonance, quartz crystal microbalance, and electrochemical impedance spectroscopy have been proposed for detection of anti-*Leishmania infantum* antibodies [16–25]. Although these are powerful tools for studying biomolecular interactions, they are all limited by their high cost in terms of instruments or materials, or their requirement for multi-step detection which is inconvenient for large-scale screening [26,27].

In this context, our work exploits the benefits of the combined use of a wide band gap material such as ZnO with that provided by CdS in the construction of a photoelectrochemical platform able to harvest visible LED light. The CdS/ZnO photoelectrochemical platform was applied for detection of *L. Infantum* antibodies, discriminating between positive and negative samples.

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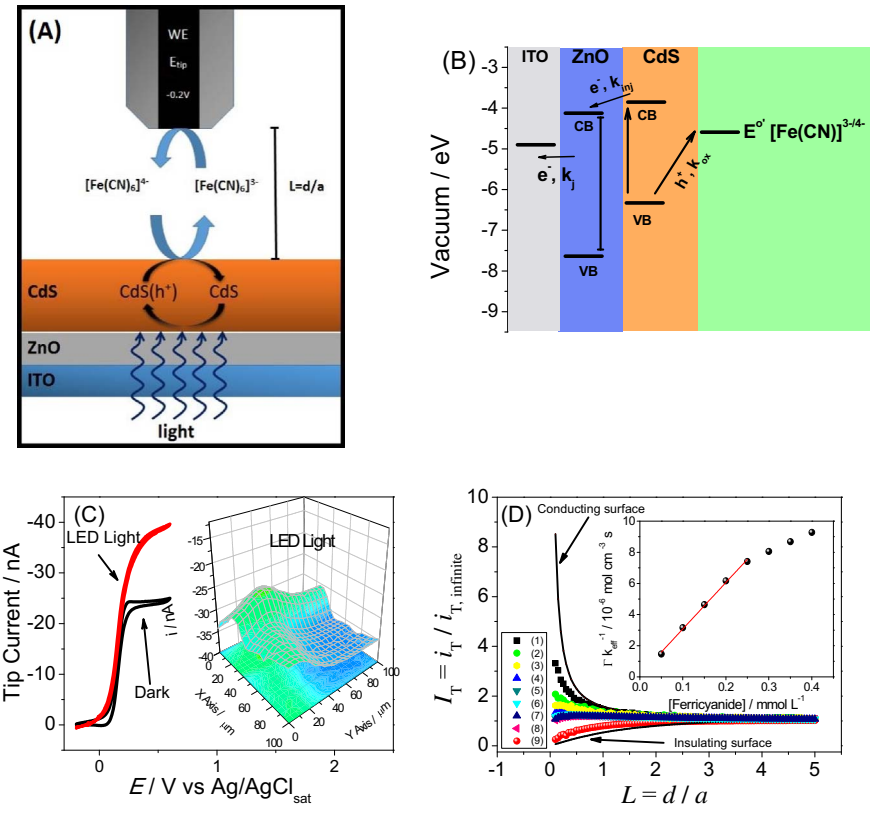

Fig. 1. (A) SECM basic principles for investigation of interfacial reaction kinetics involved in heterogeneous reaction at the CdS-sensitized ZnO semiconductor surface, (B) energy band structure of CdS/ZnO and redox potential ($E^{o'}$) of the redox mediator on vacuum scale, (C) cyclic voltammetric response of Pt microelectrode toward redox mediator at a scan rate of 25 mV in 0.1 mol L^{-1} under light and dark conditions, and (D) normalized SECM feedback approach curves obtained on CdS/ZnO/ITO surface under blue LED light for various concentrations of $[Fe(CN)_6]^{3-}$: (1) 0.05, (2) 0.1, (3) 0.15, (4) 0.2, (5) 0.25, (6) 0.3, (7) 0.35, (8) 0.4, and (9) 0.05 mmol L^{-1}. Plot of Γ_{CdS^0}/k_{eff} *vs* concentration of $[Fe(CN)_6]^{3-}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Reagents and chemicals

All chemicals were acquired from Sigma-Aldrich (St. Louis, MO, USA) and Vetec Química Fina LTDA (Rio de Janeiro, RJ, Brazil). All solutions were prepared with water purified in an OS100LXE system from GEHAKA Company. Two different peptides were used in the present work (PEP 13 and PEP 16), which were highly immunogenic in serological diagnostic assays of canine *L. infantum* antibodies with high values of sensitivity and specificity as published elsewhere [28]. The peptides were chemically synthesized using 9-florenyl-methoxy-carbonyl (Fmoc) chemistry [29] in an automated synthesizer model PSSM8 from Shimadzu (Kyoto, Japan). Peptide purity was assessed with reverse-phase high-performance liquid chromatography (HPLC) and mass spectrometry (MALDI-TOF-TOF AutoFlexIIITM, BrukerDaltonics, Billerica, Massachusetts, USA).

2.2. Electrochemical measurements

The PECs experiments were performed in a homemade photoelectrochemical system based on a potentiostat model PGSTAT 128N from Metrohm-Autolab B.V and a box containing a three-electrode cell with an indium tin oxide-coated quartz substrate (ITO) as working electrode. The PEC measurements were performed with a low-cost commercial 20 W LED light. The scanning electrochemical microscopy (SECM) apparatus for photoelectrochemical measurements was a Sensolytics base coupled to the potentiostat and irradiation from a blue LED light of 6 W.

The counter electrode and reference electrode were a platinum wire and Ag/AgCl/KCl (saturated, aqueous solution) ($Ag/AgCl_{sat}$), respectively. The CdS/ZnO/ITO platform was attached to the cell bottom and sealed with an O-ring as substrate. SECM tips were Pt microelectrode of radius, *a*, of 12.5 µm with ratio of the insulating glass radius, *rg*, to that of *a* ($R_g = rg/a$) of 10, which was biased at − 200 mV *vs* $Ag/AgCl_{sat}$ to generate the reduced form of the redox probe. All SECM measurements were performed in the presence of $[Fe(CN)_6]^{3-}$ as an electron donor molecule.

2.3. Construction of the photoelectroanalytical immunosensor

The photoelectrochemical platform was constructed stepwise by electrodeposition of CdS on previously electrodeposited ZnO. Briefly, the zinc oxide was electrodeposited from aqueous solution containing 5 mmol L^{-1} of $ZnCl_2$ and 0.1 mol L^{-1} KCl whose pH was adjusted to 4 [30]. In order to maintain a high availability of oxygen in solution, 35% hydrogen peroxide aqueous solution was introduced into the electrolyte giving a final concentration of 5 mmol L^{-1} [31]. The electrolyte solution was maintained at 65 °C with aid of a hot plate while applying a deposition potential of − 1.0 V *vs* $Ag/AgCl_{sat}$ for 10 min to perform the electrodeposition of zinc oxide. The second step in the construction of the platform was performed by electrodeposition of CdS films on the previously deposited ZnO. The plating solution was prepared with appropriate amounts of $CdCl_2$ (0.02 mol L^{-1}) and sodium thiosulfate (0.1 mol L^{-1}). Before the plating process, the pH of the plating solution used in the electrochemical synthesis was adjusted with hydrochloric acid to 2.3 units. Then, 10 cyclic voltammetric sweeps were carried out from − 1 V to 0.6 V *vs* $Ag/AgCl_{sat}$ for electrodeposition of CdS on the surface of the ZnO/ITO.

After that, appropriate amounts of PEP 13 and PEP 16 were dissolved in chitosan solution to obtain a final concentration of 50 µg mL^{-1} of each peptide. For the photoelectrochemical sensor construction, an aliquot of 10 µL of peptide solution was dropped on the CdS/ZnO/ITO platform and left to dry by evaporation of water under ambient conditions to produce the photoelectrochemical sensor (hereafter denoted as Pep/CdS/ZnO/ITO).