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Evaluation of a hypothetical protein via conducting polymer/AuNPs – based impedimetric immunosensor for the diagnosis of Visceral Leishmaniasis

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Neglected tropical diseases (NTDs) are a set of infectious diseases that primarily affect tropical and subtropical countries. Due to the lack of investment in public policies for prevention and control, ends up contributes to greater social inequality significantly affecting populations who live in a context of expressive poverty. Visceral Leishmaniasis (VL) is one of these diseases with a high incidence, which, even in the 21st century, represents a serious public health problem.[1-3] Currently, the most employed methods in clinical laboratories for the serological diagnosis of VL are the enzyme-linked immunosorbent assay (ELISA), and the direct and indirect immunofluorescence essays. However, these methods have still presented low sensitivity to detect asymptomatic cases, and low specificity, which generates a considerable number of false-negative results and cross-react with other parasites. In addition, several recombinant proteins have been evaluated and revealed improvement, especially in terms of specificity in VL diagnosis. In order to develop a portable, low-cost, and highly sensitive method for detecting VL antibodies, in this study, a novel impedimetric immunosensor based on conducting polymer polypyrrole (PPy) doped with poly(sodium-4-styrene sulfonate) (NaPSS) and decorated with gold nanoparticles (AuNPs) was developed. As a sensor recognition element a new recombinant protein, namely C1 protein, was used. Protein C1 has shown a high immunogenic character with a strong binding affinity. However, the function of this protein in the protozoan *L. infantum* is still unknown, constituting a very interesting material for scientific research purposes. Then, a comparison in terms of analytical responses of the proposed C1 protein with a well-established recombinant protein in VL immunodiagnostics, rK39, was realized.[4] Regarding the experimental steps necessary for the development of the impedimetric immunosensors, firstly disposable stainless-steel mesh electrodes were functionalized towards the electrodeposition of PPy:PSS and subsequent incorporation of AuNPs, followed by mercaptopropionic acid (MPA) binding to obtain a self-organized monolayer (SAM). Afterwards, each impedimetric immunosensor was constructed by covalently linking the respective recognition element, C1 protein and rK39 protein, onto the polymeric platform. Scanning and transmission electron microscopies, IR-spectroscopy, and electrochemical techniques were used for the characterization of each step involved in the immunosensor construction process. From the proposed immunosensors, it was possible to establish a comparison profile between the proteins (C1 and rK39) when compared to their reactions against specific antibodies (anti-C1 and anti-rK39). The results showed linear response for different polyclonal antibody concentrations, good sensibility, and very satisfactory limits of detection (in the order of femtomolar) and quantification. In addition, the proposed impedimetric immunosensors were evaluated toward canine serum samples, presenting good analytical performance, which allowed for discrimination between positive and negative samples in a wide range of antibody concentrations. In summary, the proposed platform in this work presented a satisfactory performance, both in terms of sensitivity and selectivity without the necessary electrochemical probe, as a disposable impedimetric immunosensor proposing a new alternative diagnostic tool for VL.

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