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Synthetic peptides-based SPR biosensors towards visceral leishmaniasis diagnosis

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Peptides are versatile molecules that can be explored in biosensors construction, being promising due to their great stability, standard synthetic protocol, cost-effectiveness, and tunable physio-chemical properties, all of which make it possible to synthetically produce them to substitute proteins or crude antigens as bioreceptors in antibody screening.[1] Thus, considering the transducing element in biosensing, devices based on surface plasmon resonance (SPR) are powerful techniques for biomolecular interaction investigations, allowing analysis at real-time, and without the necessity of chemical or biological probes. SPR is an optical system that explores the excitation of surface plasmons on metallic substrates, which allows highly sensitive analysis according to refractive index changes in the close medium. Another important factor to be considered is the biofunctionalization step, which can intrinsically affect the biosensor's performance, since the environment of the biocomponent and the condition of its immobilization directly affects the capacity of interaction of the receptor with the analyte. For this reason, covalently attaching the bioreceptor on the sensor surface by forming a self-assembled monolayer (SAM) is one of the most used techniques, allowing good control of the receptor orientation, high surface homogeneity, and satisfactory stability.[2] Hence, peptides-based SPR biosensors play an important role in biosensor development, associating the easy quantity fabrication and better stability of the artificial peptides with the ability of a real-time, sensitive and label-free analysis of SPR.[3] In this work, two synthetic antigenic peptides were applied for the first time in SPR-based biosensors for serological diagnosis of Visceral Leishmaniases (VL). VL is an endemic neglected disease that affects humans and dogs around the world, affecting more than 200.000 people each year.[4] Consequently, diagnostic methods are essential to obtain better infection control. The sensors construction consisted of a previous SPR gold disk functionalization, utilizing a mixture of mercaptoundecanoic and mercaptopropionic acid, forming a mixed SAM to covalent binding of two different peptides (PEP1 and PEP2) or the blend of them (PEP1:2). This biofunctionalization step (peptide immobilization) was characterized at real-time via SPR. The biosensors developed were also electrochemically characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), in which due to interfacial shifts caused by each of the steps, changes in the conductivity, resistivity, and capacitance were observed. The three bioreceptor conformations (PEP1, PEP2, and PEP1:2) were evaluated accordingly to sensibility and stability, for the detection of specific antibodies in rabbit serums. Additionally, the ability of detecting positive and negative cases of VL in dog serums was evaluated for each of the arrangements. Summarily, this study explored new and promising biostructures, contributing to a better understanding of their applicability via SPR biosensors, consisting of an important screening tool in an urgent matter such as VL.

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