Enhancing canine visceral leishmaniasis diagnosis using plasmonic immunosensors: incorporating artificial bioreceptors and artificial intelligence

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Highlights

- Multi-epitope protein (PQ20) enhances leishmaniasis diagnosis, aligning with the 2030 SDG health goals.
- AI-driven automation for precise signal discrimination in SPR-based biosensor data.

Abstract

Developing countries face additional challenges in healthcare management due to neglected tropical diseases (NTDs). Limitations in current diagnostic methods, along with socioeconomic and climatic factors, significantly contribute to the persistence and spread of these illnesses. Addressing NTDs is a key target of the United Nations' Sustainable Development Goal for good health and well-being, and the development of new diagnostic platforms is a crucial measure for disease control. Among NTDs, visceral leishmaniasis remains a major public health concern, particularly in Brazil, where delayed or inadequate early detection in both human and canine populations hinders effective disease control. Another contributing factor is the current climate emergency, which has facilitated the spread of vector-borne diseases, such as leishmaniasis. To propose an enhanced screening tool for canine visceral leishmaniasis (CVL), this study explored the application of multi-epitope proteins as bioreceptors in a surface plasmon resonance (SPR) biosensor. By combining multiple immunodominant epitopes carefully selected through bioinformatics tools, multiepitope proteins offer a robust alternative to traditional diagnostic methods. SPR not only allows the sensitive identification of biomolecules (e.g., anti-Leishmania antibodies) but also serves as a powerful tool for studying biomolecular interactions in real-time and without the necessity of labels, enabling the assessment of key kinetic parameters when exploring novel recognition elements. The biomaterials under investigation were previously developed by mapping B- and T-cell epitopes of immunodominant proteins from *L. infantum*. The novel chimeric protein, named PQ20, incorporated the 20 most reactive peptides identified during epitope screening. The immunosensor was constructed by covalently immobilizing PQ20 onto a thiol-functionalized surface prepared with a mixed self-assembled monolayer of 3-mercaptopropionic acid and 11-mercaptoundecanoic acid via EDC:NHS coupling. Afterward, the deactivation of the remaining active sites on the monolayer was optimized by testing different blocking molecules – bovine serum albumin, ethanolamine, and glycine – with ethanolamine providing the best discrimination between a canine serum pool of positive (n = 10) and negative (n = 10) CVL cases. Electrochemical techniques were essential to characterize and understand each step in constructing the biosensor. For both electrochemical impedance spectroscopy and cyclic voltammetry, the incorporation of new insulating molecules at the electrolyte/electrode interface hindered the faradaic process of the studied redox probe, which was observed through all the steps necessary for the construction of the biosensor. The proposed methodology demonstrated a limit of detection of 5.9 nmol L^{-1} and a limit of quantification of 17.8 nmol L^{-1}. To further evaluate the platform's capacity to identify positive and negative CVL cases correctly, the immunosensor was tested on canine sera (n = 27): 14 confirmed positive cases and 13 negative samples, accurately classified by the PQ20-based biosensor. Moreover, a selforganizing map analysis was performed to further interpret the immunosensor's analytical performance in discriminating positive from negative CVL cases, thereby integrating artificial intelligence with the SPR-based biosensor for automated sensing response interpretation. This study builds on these findings to evaluate the potential of these chimeric multi-epitope proteins to improve diagnostic accuracy and efficiency for CVL, as well as the integration with artificial intelligence towards more reliable diagnostic tools.

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