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Electrochemical detection of pathogen nucleic acid for bio-sensing application

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Summary:
Electrochemical impedance spectroscopy (EIS)-based detection of protozoan *Leishmania infantum* (LI) kinetoplastic (k)DNA is presented. Thanks to self-assembled monolayer of oligonucleotide probes on the top of a gold electrode surface, the method allowed to perform a molecular quantification of kDNA copies excluding the time-consuming amplification of genetic target and avoiding the complex procedures of conventional diagnostic methods.

Keywords: Electrochemical Impedance Spectroscopy; *Leishmania infantum* kDNA; cooperative hybridization; molecular sensing.

Introduction

Infectious diseases area major threat for mankind. This health burden challenged all populations, especially those of developing countries, where undernutrition, poor hygiene practice, and underdeveloped health systems do not guarantee access to appropriate treatments. Moreover, the lack of effective monitoring facilities fails to keep infectious diseases epidemic under control. The COVID-19 pandemic has increased this threat even more causing, only in its first year, millions of deaths and impacting dramatically the quality of life worldwide. The COVID-19 outbreak highlights as the availability of diagnostic methods that are, in the meantime, fast, cheap, reliable and able to provide genetic response, is crucial for the management of these diseases. This is not only the case of SARS-CoV-2, as the problem of fast genetic detection is common to all infectious agents, such as viruses, bacteria, and parasites, including those that could potentially be the cause of the next pandemics [1-3].

In this contribution, we present the genetic detection of protozoan *Leishmania infantum* trough an capacitive quantification based on EIS (electrochemical impedance spectroscopy). This paves the way to develop nanobiotechnological platform to address, at the same time, the problem of cost, simplicity, reliability, and sensitivity of the pathogen detection, making a breakthrough advancement in the fight against communicable diseases.

Materials and method

Two oligonucleotide capture probes have been selected and customized for the detection of kDNA target: LI-1 probe → HS-C6-5'-CTTTTCTGGTCCTCCGGGTAGG-3'; LI-2 probe → HS-C6-5'-CCACCCGGCCCTATTTTACACCAA-3'.
Both probes are modified at 5' end with a thiol group that favors the chain grafting on the sensing surface. Moreover, they are complementary to two regions of the same kDNA gene to perform a cooperative hybridization mechanism that increases the yield and stability of the probe/target duplex.
The kDNA target has been extracted and purified from LI cultures and used at the final concentration of 10^6-10^3-10 copies/µL.
Fig. 1 reports the functionalization of the sensing surface that is a gold surface of a 2 mm diameter gold working electrode (from CHInstruments). This surface has been modified by a first a cleaning step performed with alumina slurry and CV cycles with H_2SO_4. Then, thiolate capture probes have been grafted at 25°C for 4 h on the gold surface that, subsequently, has been passivated with 6-mercapto-1-hexanol at 25°C overnight. For the quantification by EIS analysis, the sensing surface has been dipped into three solutions of kDNA at 10^6-10^3-10 copies/µL in PBS and incubated at 50°C for 3h30 to trig the cooperative hybridization.

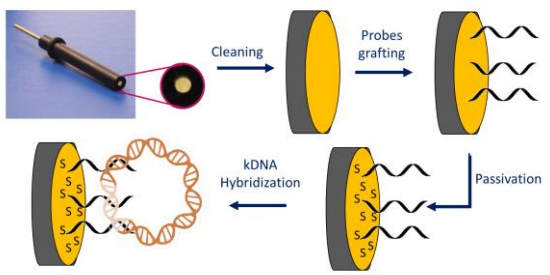

Fig 1. Chemical protocol of electrode sensing surface functionalization.

kDNA detection and quantification has been performed by EIS analysis. For the test, the functionalized gold electrode has been included as working electrode (WE) in an electrochemical cell that has been properly assembled with a Pt wire as counter electrode (CE) and an Ag/AgCl reference electrode (RE). Then, the cell has been exposed to a solution of 5 mM of $[Fe(CN)_6]^{-3/-4}$ that was used as redox mediator. Measurements have been done at 200 MHz to 100 MHz frequency range, 1mA current and 0.234V potential.

Results

Results of EIS analysis are reported in the Nyquist plot of Fig. 2 and Table 1. The analysis has been performed using the sensing WE before and after the functionalization, in order to assay the effectiveness of the chemical protocol, and after the hybridization with the three concentrations of kDNA. As shown, the trend of resistance and capacitance confirm the probes grafting and thiol passivation of the WE gold surface, with an increase of values from the 210 Ω of bare gold to the 663.75 Ω and 1901.6 Ω of probe and probe+thiol modified surfaces (black, green and purple curves in Fig. 2, respectively). This could be due to the electrostatic forces and steric hindrance brought by the grafted oligos and the increase of stationary charges caused by the thiol passivator addition [4]. Once hybridized to the kDNA, the system resistance furtherly increased to 2712.7 Ω, 3895.1 Ω and Ω 13240 Ω consistently with the concentration of the 10-10^3-10^6copies/µL solutions (orange, red and blue curves, respectively) as consequence of the increased number of nucleic acid strands added to the surface. These results proved the sensing performances of the method and its suitability for fast molecular sensing applications.

Tab. 1: Resistance and capacitance values of EIS analysis.

WE surface	R2 (Ω)	C_{DL}(nF/mm²)
Au Bare	210	3,18
Probe	663,75	41,56
Probe+thiol	1901,6	50,1
kDNA (10 cps/µL)	2712,7	51,1
kDNA (10^3/cps/µL)	3895,1	55,5
kDNA (10^6/cps/µL)	13240	79,02

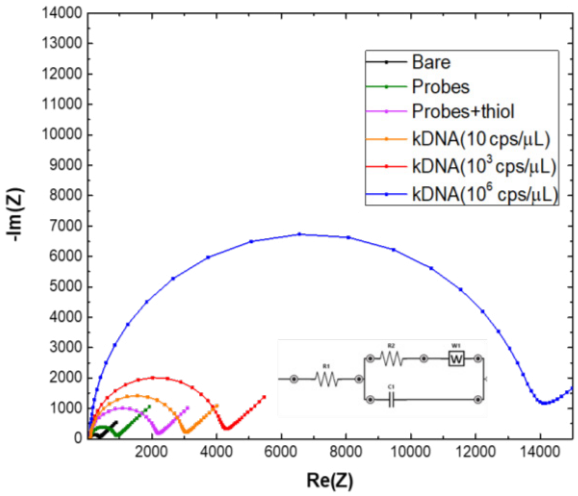

Fig 2. EIS quantification test of kDNA by probe-modified WE.

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