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PCR-free detection of miRNA biomarkers for neurodegenerative disorders

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Summary:

A PCR-free strategy for microRNAs (miRNAs or miR-) fast and accurate detection is presented. The approach relies on the functionalization of a gold working electrode (WE) with an array of oligonucleotide probes selective for two miRNAs biomarkers that affect some intracellular processes related to human neurodegenerative disorders. The electrochemical impedance spectroscopy (EIS) analysis performed on the modified electrode allowed to directly sense the probes/miRNA duplex formation in a fast and accurate mode that is suitable for Point-of-Care (PoC) diagnostic applications.

Keywords: PCR-free detection; miRNAs; neurodegenerative disorders; solid state hybridization; EIS analysis; PoC.

Introduction

MiRNAs are small non-coding RNA molecules that introduced a new frontier of diagnostics to screen and predict human diseases at an early stage, when therapies could be more effective against the pathological onset. Some miRNAs are involved in various aspects of human neurodegenerative disorders, including the Alzheimer's disease pathogenesis. This is the case of miR-34a, whose dysregulation can affect regulating processes such as neuronal apoptosis, inflammation and Aβ metabolism, and miR-29a, that can conditionate the synaptic plasticity and neuronal survival. Both miRNAs are free to circulate in the bloodstream and easy to be extracted and purified, which makes them potential biomarkers for molecular diagnostics applications and integration into PoC technologies [1,2].

Within this perspective, a PCR-free strategy can be a suitable approach for the development of new point-of-care devices for massive screening [3-5].

In this contribution a PCR-free strategy for the selective and fast detection of miR-34a and miR-29a is proposed. The detection approach is based on the EIS analysis of a gold working electrode that is functionalized with an array of miRNA-specific oligonucleotide capture probes. Thanks to their specific design, probes can hybridize at solid state with the miRNA targets and these are revealed by measuring the resistance and capacitance variations due to the duplex formation, without any PCR amplification and long thermal cycling, thus, simplifying the complexity of analysis and the architecture of the entire detection system towards a PoC format.

Materials and method

The oligonucleotide capture probes for miRNA PCR-free detection have been designed as follows: miR-34a probe → HS-C6-ACAACCAGCTAAGACACTGCCA; miR-29a probe → HS-C6-TAACCGATTTCAGATGGTGCTA. More precisely, a chain of 6 carbon atoms (C6) has been added at 5' end of each sequence, working as spacer for the right orientation of probes once immobilized on the surface. Upstream the spacer a thiol (SH-) group has been added for the probes grafting on the metallic surface of the EC chip. Synthetic ribonucleotide sequence of mature miR-34a and miR-29a have been purchased by MetaBion and used as targets.

As schemed in Fig. 1, the WE surface has been modified by a first a cleaning step performed with alumina slurry and a sonication in a 1:1 solution of ethanol and ultrapure water. Subsequently, a CV voltammetry in H_2SO_4 has been applied to the electrode to complete its surface cleaning. For the functionalization with capture probes, the electrode surface has been incubated in a 1:10 solution of 6-mercapto-1-hexanol and probes in PBS 10 mM (pH=7.4) at 25°C for 4h with 70rpm/min agitation, to graft a self-assembled monolayer of thiolate probes. Lastly, the functionalized electrode has been dipped into a 1µM solution of miRNA targets in PBS 10 mM (pH=5.5) and this has been incubated at 50 °C for 3h30 for the final hybridization.

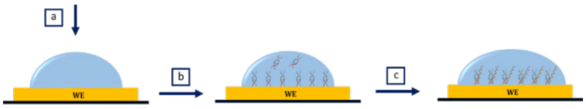

Fig 1. Electrode gold surface preparation: (a) surface cleaning; (b) probes grafting; (c) miRNA hybridization.

The electrochemical Impedance Spectroscopy (EIS) analysis of the solid-state hybridization with the miRNAs has been performed by preparing an electrochemical cell that has been properly assembled including the functionalized WE, a Pt wire as counter electrode (CE), and an Ag/AgCl reference electrode (RE). All electrodes have been dipped into a 5 mM solution of [Fe(CN)6]$^{-3/-4}$ in PB buffer (pH=6.8), used as redox-active analyte. The EIS has been done in the frequency range of 200 MHz to 100 MHz and by fixing current and potential at 1 mA and 0.240V, respectively.

Results

Nyquist plots shown in Fig. 2 have been obtained by the EIS analysis performed after probes grafting and miR-34a and 29a hybridization (Fig. 2a and 2b, respectively) on the WE surface. The resistance values confirmed the effectiveness of the functionalization process, comparing the bare gold WE to the probes-modified WE, and the miRNA PCR-free detection. Indeed, the resistance of 210 Ω measured on the bare surface (black curve) increased to 1338 Ω after the 34a probes grafting and 1377 Ω after the 29a probes addition (green and blue curves in figures), probably due to the steric hindrance and electrostatic repulsion produced between the probes and the formed passivating layer. The resistance furtherly increased up to 1730 Ω and 2061 Ω after the miR-34a and miR-29a hybridization (orange and red curves) as consequence of the perfect match formation and the doubled number of strands added to the surface dielectric, proving the ability of this strategy to directly sense the miRNAs without further amplifications.

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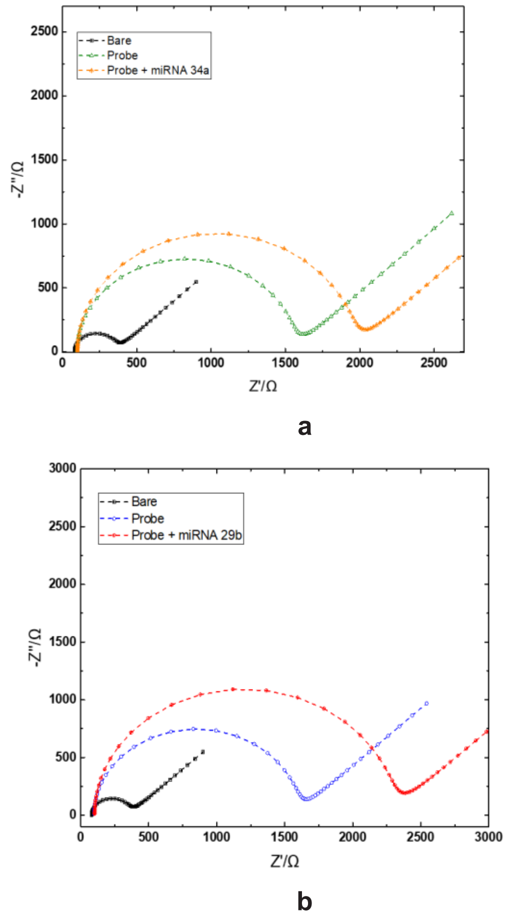

Fig 2. EIS analysis of miR-34a (a) and miR-29a (b) by probe-modified gold WE surface.

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