Biomedical device for detection of colorectal cancer focused on the hybridizations of MicroRNAs 21 and 224.

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Introduction: Colorectal cancer (CRC) is one of the malignancies with the highest incidence in Brazil and, in most cases, has little noticeable symptoms until the disease is advanced. Thinking about simplifying the detection and make a faster diagnosis for patients with CRC, this work aims to develop a simplified diagnostic process based on an electrochemical biosensor in which single strands of MicroRNAs are immobilized on the surface of gold electrodes to make the complementary strand detection by electrochemical impedance spectroscopy (EIS). MiRNAs are small sequences containing between 20-25 bases, playing a major role in the regulation of various cellular processes. Many studies indicate that the overexpression of MiRNA-21 has important roles in cancer initiation, progression and metastasis, controlling its volume and survival. Multiple records have found that other microRNAs were also altered by CRC, including miRNA-224. [1]
Methodology: Gold electrodes (Metrohm) were polished with three different alumina particle sizes, and summited to an electrochemical cleaning with H_2SO_4 (0.5M). It was used synthetic DNA sequences (Sigma-Aldrich) corresponding to the MicroRNA-21 (5'-TCAACATCAGTCTGATAAGCTA-3') and MicroRNA-224 (5'-CAAGTCACTAGTGGTTCCGTT -3'), marked with a thiol group in one of the extremities to perform a covalent bonding with the gold. The electrode was than co-immobilized with different proportions of the DNA sequence and 6-mercaptohexanol (MCH), used as a spacer molecule. The electrochemical characterization was performed through EIS using 2mM Fe2+/Fe3+ (redox marker) in 50mM PB + 100mM K2SO4 (EIS buffer). For each tested concentration of complementary strands, the electrode was than incubated for 30 minutes in a solution of complementary DNA strands in EIS buffer, so that it could hybridize to the probe. The negative charge of the DNA probe produces an electrostatic barrier to the negatively charged redox marker, which hinders the charge transfer processes between the redox marker in solution and the electrode.[2]
Results: It was possible to notice an increase of the charge transfer resistance (Rct) values, shown in the Nyquist diagram (Fig1 (a)), according to the concentration of complementary strands that are added to the electrode's surface, suggesting that the hybridization happened for both tested MicroRNAs. It was than possible to build a curve to understand the behavior of the impedance on the electrodes when the concentration of complementary strand added to it changes (Fig 1 (b)).

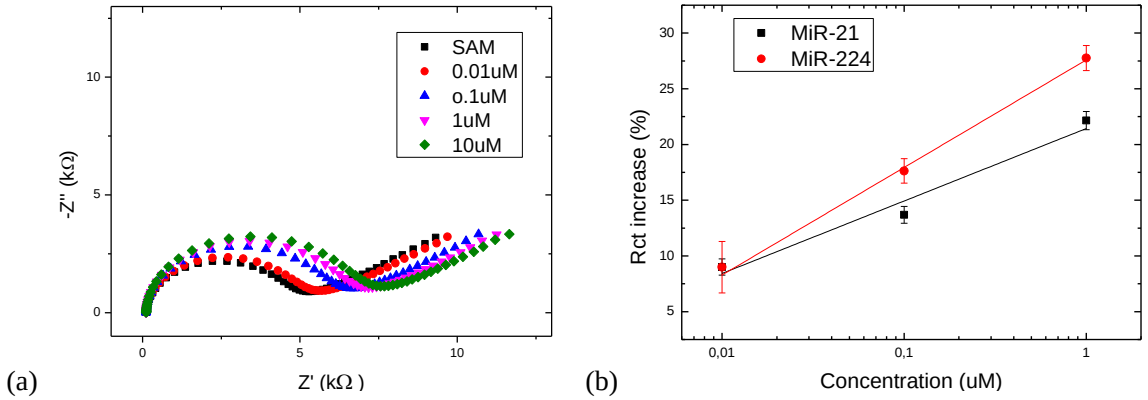

Fig 1. a) Results of imaginary x real impedance acquired using EIS for different concentrations of complementary strands of DNA. b) Percent of Rct variation compared to the electrode containing the probe.

Conclusion: In this study, it was possible to see an increase in the Rct according to the hybridization of complementary strands of DNAs corresponding to MicroRNAs that indicate the incidence of CRC and to characterize the biosensor through the EIS technique.

References: [1] Schetter A.J., Okayama H., Harris C.C. The Role of microRNAs in Colorectal Cancer. Cancer Journal (Sudbury, Mass) 18(3), 2012, 244-252.

[2] Keighleys. D., Li P., Estrela P., Migliorato P. Optimization of DNA immobilization on gold electrodes for label-free detection by electrochemical impedance spectroscopy. Biosensors and Bioelectronics 23, 2008, 1291–1297