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Label Free Microsensor for Total DNA Breast Cancer based on Bioimpedance Spectroscopy

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Abstract. Breast cancer remains one of the leading causes of cancer mortality in women worldwide. Electrochemical biosensors are technologies of interest due to their low production cost and ease of miniaturization. They have been used to detect analytes associated with breast cancer, showing high sensitivity and selectivity. These techniques provide quantitative information about DNA, taking advantage of the electronegative properties of the phosphate groups in its structure, which affect its polarity and electrical properties, including Electrical Bioimpedance Spectroscopy (EBiS). The present research was conducted to characterize total DNA concentration in breast cancer. The biosensor has been fabricated by gold micro-electrode array enbibed on a crystal surface. The total DNA was quantified by UV-Vis spectrophotometry, using an optical density between 260 and 280 nm to determine the total concentration of DNA and its purity ratio of 260/280. Subsequently, each sample was subjected to EBiS measurements using an impedance spectrometer in the frequency range of 100Hz to 10MHz. The findings show absolute reactance (Xc) values with significative statistic correlation to DNA concentration at 3.3 MHz ($R=0.821$, $p<0.05$) for concentrations from 0 to 100 ng/µl. The bioimpedance of biological analytes in suspension at frequencies above 1 MHz can be affected by how dipolar water molecules interact with structural environments. Thus, DNA concentration could intensify this interaction as the amount of nucleotide double helix structures increases, which is reflected in the reactive response associated with their electrical properties. The use of a label free microsensor based on EBiSmeasurement is a feasible technique to estimate BC DNA concentrations, and future research to determine its potential use to detect genetic BC markers is warranted.

Keywords. Breast cancer, DNA, biosensor, bioimpedance.

1 Introduction

Breast cancer (BC) is a relevant public health problem worldwide, as it is the second cause of cancer death in women [1]. BC classification is performed using a molecular and genetic profile

[2]. This is crucial to understand cancer biology and develop specific diagnoses, prognoses and therapies, since genetic and epigenetic changes are associated with the overexpression of oncogenes. [3,4]. Although early detection of genetic and molecular markers associated with the disease using label-free microsensors remains a technological challenge to date.

Deoxyribonucleic acid (DNA) is a repository of genetic information in the cell. The DNA chain forms a helical structure consisting mainly of phosphate-deoxyribose that connects nitrogenous bases; this double helix structure is attributed with properties associated with conductivity [5].

Likewise, the DNA molecule has an electronegative charge and an implicit viscosity with respect to the number of double helix molecules; It is hypothesized that such electrical and physical properties generate an impact on volumetric bioimpedance as a function of DNA concentration. Some research considers that DNA is a molecular cable capable of conducting charge, while others conclude that it behaves like an insulator [6].

In this regard, the development of new simple and inexpensive detection methods for DNA quantification and gene detection, mainly for developing countries, is of great importance and stands out for its numerous biomedical applications. Electrochemical biosensors are technologies of interest due to their low production cost and ease of miniaturization. They have been used to detect breast cancer-associated analyses, demonstrating high sensitivity and selectivity. These techniques provide quantitative information about DNA, taking advantage of the electronegative properties of phosphate groups in the DNA structure that contribute to the polarity and electrical properties of the molecule, including EBiS [7, 8].

Several studies explore the ability of EBiS to determine different concentrations of human DNA highlighting the advantages of impedance such as free labeling of the sample and free functionalization of total DNA using multifrequency measurements to evaluate the characteristic spectra obtained [9]. In addition, EBiS measurements are reported to be associated with DNA concentration [10]. The aim of this study is to propose a label-free biosensor of BC DNA concentration based on EBiS measurements.

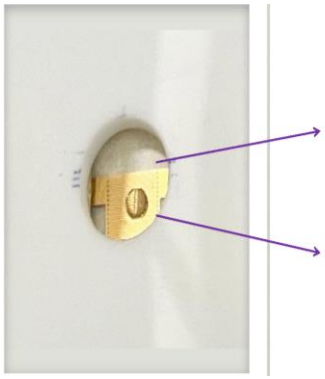

Fig. 1. Gold film microelectrodes

2 Materials and Methods

Thirty samples previously extracted from biopsies of patients with BC were quantified by UV-Vis spectrophotometry, then evaluated through EBiS measurements.

2.1 Preparation Steps of the Biosensor

Figure 1 refers to the electrodes used in this research. Microelectrodes composed of interdigitated gold film embedded in a glass surface were used as working electrodes, with characteristics of 10x10 mm.

Interdigitated gold microelectrode with dimensions of 10x10 mm. Embedded in a glass surface. In which, using the drop cast dimple technique, EBiS measurements were performed in a final volume per DNA drop of 2 µl.

The experiment to evaluate the potential of the biosensor for total DNA It is described as follows and a schematic representation of the experimental setup is showed in figure 2:

1) Cleaning the system with purified water (Milli-Q water) and drying the microelectrodes with

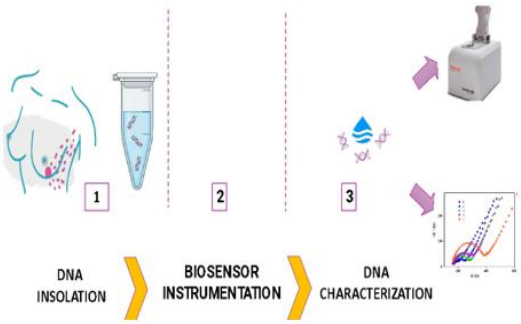

Fig. 2. Diagram of the experiment design. The construction process of the proposed label-free biosensor of BC DNA concentration based on EBiS measurements

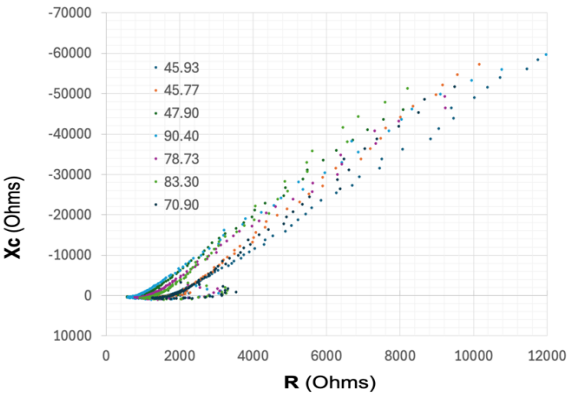

Fig. 3. Scatter plot Resistance (R) vs Reactance (Xc) for total DNA concentrations

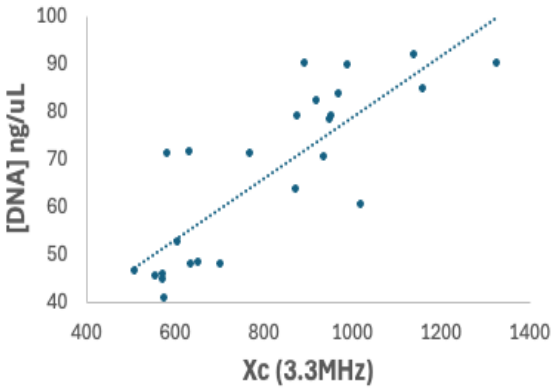

Fig. 4. Dispersion diagrams of DNA concentration vs bioimpedance. (R = 0.821, p < 0.05)

specialized cleaning wipes (Kimwipes, Kimtech Science brand S-8115 kimberly-Clark México).

2) For control samples, water (Milli-Q) and saline solution were used as reference parameters in EBiS, taking measurements of these samples at 2 µl.
3) Cleaning residues with Milli-Q water and measuring EBiS on the gold microelectrodes.
4) Characterization of total DNA samples by EBIS, previously quantified by UV-Vis. Each sample was evaluated in triplicate, added 2ul by drop cast to the electrode surface.
5) Finally, cleaning and drying the microelectrode with purified water. Every test was performed in triplicate. By obtaining measurements of both the resistive and reactive parts, an average of the three measurements was taken and the Bode diagram was determined.

Previously we made calibration curves of the electrode as a function of the concentration of the analyte. In the previous experiment, saline solution and milli q were used as blank samples, which give us reference parameters of the biosensor.

2.2 DNA Extraction

Thirty samples extracted from biopsies of patients with BC were selected for DNA isolation, with Flexi-Gene® DNA kit for purification of DNA (Flexi-Gene DNA-Kit QIAGEN, Catalog no./ID: 51206 Germantown, Maryland EE. UU). The nucleic acid solution was extracted and purified.

2.3 UV-Vis Quantification

DNA was quantified by spectrophotometry (Themo Scientific-Themo Fisher, Microvolume UV/Vis Spectrometer Nanodrop 2000c, EE.UU.) using an optical density between 260 and 280 nm to determine the total DNA concentration and its purity ratio of 260/280.

DNA samples with concentrations below 100 ng/ µl were selected as income criteria.

2.4 Ethical Conditions

The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by both institutional Research and Bioethics Committees. It was approval by the Institutional Ethics in Research Committee (Reg. No. Pro 10/16) of Instituto Jaliscience de Cancerología, Guadalajara, Jalisco, México.

2.5 Bioimpedance Spectra

In this study, samples in a volume of 2 µl. were evaluated, EBiS measurements were performed using an impedance spectrometer in a frequency range of 100 Hz to 10MHz. The Sciospec ISX-3 bioimpedance analyzer (Sciospec Scientific, Leipziger, Bennewitz, Germany) was used with a maximum voltage of 100 mV. Programming and data storage of ScioSpec was performed using a personal computer.

2.6 Data Processing

Data processing and analysis was performed using Excel (Microsoft Excel). Pearson correlation coefficients for EBiS measurements across the frequency range in relation to DNA concentration were calculated using SPSS Statistics software (version 21.0).

3 Results

The experiment shows a relationship between the changes of the EBiS patterns in the complex plane, the characteristic spectra depend on the DNA concentration of the samples.

The correlation between two variables was used to examine the possible relationship of EBiS (DNA concentration and reactance measurements at each frequency across the entire bandwidth).

In response to the findings, a Spearman correlation was performed to examine the possible relationship of EBiS measurements to total DNA concentration which showed a statistically significant correlation for the absolute reactance (Xc) values data with a statistically significant correlation with DNA concentration at 3.3 MHz ($R = 0.821$, $p < 0.05$), see Figure 4 which shows the scatter plot of DNA concentration versus bioimpedance absolute reactance (Xc) measurements.

4 Discussion

Recently, EBiS has been used for single cell cancer detection and analysis or in the characterization of specific biomarkers using biosensors, highlighting the advantages of EBiS as a label-free and non-functionalizing technique. It is a potential technique for monitoring and quantifying DNA by means of the electrical properties of the molecule.

Although the DNA structure is loaded with phosphate groups that give the material the ability to conduct electricity, which in turn could influence the ability to conduct electric current and therefore these characteristics influence the bioimpedance spectra, obtaining as a response that heterogeneous parameters that are reflected in the resistance and reactance values inversely proportional to the DNA concentration.

Previous studies from our research group report that impedance measurements at frequencies below 10 kHz indicate a more limited sensitivity, which could be associated with the influence of the interface capacitance between the electrode and the electrolyte.

However, at high frequencies a greater relationship has been found as a function of DNA concentration.

These findings agree similarly with the previous analysis of EBiS measurements in amplicons ,where the electronegative charge of DNA, referring to a sensitivity in the range of 2 to 10 MHz and its impact on electrical impedance at high frequencies in the molecular interaction [10,11,12].

Bioimpedance above 1MHz of biology analytes in suspension could be influenced by the interaction of water dipole molecule with structural environments, thus DNA concentration might promote such interaction as the amount of double helix nucleotide structures increase, and it is reflected on the reactive response associated to its electrical properties.

Therefore, the exploration of the parameters will be relevant for the design of calibration curves to estimate the total DNA concentration, justifying additional studies that allow confirming the observations, to corroborate the information produced.

5 Conclusion

The concentration values of the genetic material were correlated with the bioimpedance measurements at each frequency point.

The findings suggest a relative sensitivity threshold of 0 to 100 ng/ µl. of DNA associated with the concentration of nucleic acids, which in turn is related to the electrical properties of the DNA concentration and changes in multifrequency electrical bioimpedance.

The exploration of the parameters will be relevant for the design of calibration curves to estimate the total DNA concentration associated with impedance, justifying additional studies to confirm the observations, to corroborate the information produced.

While the use of a label-free microsensor based on EBiS measurement is a feasible technique to estimate BC DNA concentrations, future research is warranted to determine its potential use to detect genetic markers of BC.

In this study, we demonstrate that BC DNA concentration based on electrical bioimpedance spectroscopy (EBiS) can be measured by EBiS obtained using a label-free microsensor.

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References

1. **Katsura, C., Ogunmwonyi, I., Kn-Kankam, H., Sunita, S. (2022).** Breast cancer: Presentation, investigation and management. Br J Hosp Med (Lond), Vol. 83, No. 2, pp. 1–7. DOI: 10.12968/ hmed.2021.0459.
2. **Zhang, X. (2023).** Molecular Classification of Breast Cancer: Relevance and Challenges. Arch. Pathol Lab. Med., Vol. 147, No. 1, pp. 46–51. DOI: 10.5858/arpa.2022-0070-RA.
3. **Roy, M., Mahajan, A. (2023).** Molecular Classification of Breast Cancer. PET Clin., Vol. 18, No. 4, pp. 441–458. DOI: 10.1016/j.cpet. 2023.04. 002.
4. **Medford, A.J., Gillani, R.N., Park, B.H. (2018).** Detection of Cancer DNA in Early Stage and Metastatic Breast Cancer Patients. Methods Mol. Biol., Vol. 1768, pp. 209–227. DOI: 10.1007/978-1-4939-7778-9_13.
5. **Mozneb, M., Mirtaheri, E., Sanabria, A.O., Chen-Zhong, L. (2020).** Bioelectronic properties of DNA, protein, cells and their applications for diagnostic medical devices. Biosens Bioelectron, Vol. 167, pp.112–441. DOI:10.1016/j.bios.2020. 112441.
6. **Dekker, C., Ratner, M.A. (2001).** Electronic properties of DNA. Physics World, Vol. 14, No. 8, pp. 29–33. DOI: 10.1088/2058-7058/14/8/33.
7. **Özyurt, C.N, Uludağ, İ., İnce, B., Sezgintürk, M.K. (2023).** Lab-on-a-chip systems for cancer biomarker diagnosis. Journal Pharm Biomed Anal, Vol. 226, pp. 115–266. DOI:10.1016/j.jpba.2023. 115266.
8. **Xin, H., Yuanfang, L., Zhuet, C. et al. (2020).** DNA based label free electrochemical biosensors: From principles to applications. TrAC Trends in Analytical Chemistry, Vol. 133. DOI:10.1016/j.trac. 2020.116098.
9. **Ames, G. et al. (2019).** Gene-sensor on the basis of bioimpedance measurements assisted with nanotechnology: An instrumentation proposal. Journal of Physics: Conference Series, Vol. 1272, 3rd Latin-American Conference on Bioimpedance South America Journal of Physics Conference

Service. DOI: 10.1088/1742-6596/1272/1/012023

10. **Hernández, C.A., Corzo-Cruzet, A., Sánchez-Monroy, V. et al. (2023).** Correlation of the DNA Concentration of Human Samples to Electrical Bioimpedance Measurements: A Pilot study. Journal Electr. Biomp, Vol. 13, pp. 132–135. DOI: 10.2478/joeb-2022-0018.
11. **Park, J.S., Lee, M.S., Park, C.Y. et al. (2020).** Compact DNA droplet concentration detection system based on impedance measurement. Sensors Mater, Vol. 32, No. 7, pp. 2345–2354. DOI: 10.18494/SAM.2020.2806.
12. **Ames, G., Gnaim, R., Sheviryov, J. et al. (2020).** Impedance measurements sensitive to complementary DNA concentrations. IFMBE Proceedings, Vol. 72, pp. 154–157. DOI: 10.1007/ 978-981-13-3498-6_23.

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