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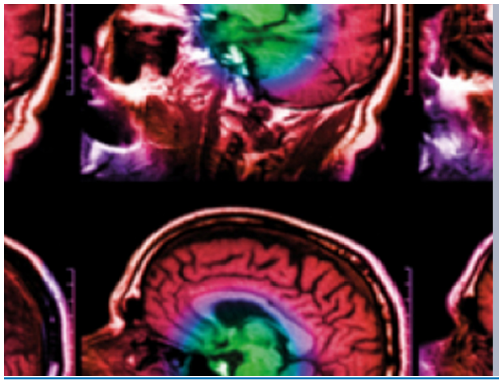

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Sensitivity analysis of electrical bioimpedance patterns of breast cancer cells labeled with magnetic nanoparticles: forming the foundation for a biosensor of circulating tumor cells

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Keywords:— gene expression, bioimpedance, breast cancer cells, magnetic nanoparticles, biosensor

Abstract

Objective: To determine the sensitivity of electrical impedance spectroscopy (EIS) measurements for the detection of a small concentration of breast cancer cells in suspension, previously labeled with magnetic nanoparticles (MNPs) and separated. Additionally, the relation of electrical impedance to the expression of molecular markers was established. *Approach*: MCF-7, MDA-MB-231 and SK-BR-3 breast cancer cell lines at different concentrations (50, 500 and 5000 cells/500 μl) were labeled with a magnetic nanoparticle–antibody (MNP-ab) bioconjugate that recognizes the corresponding molecular markers (EpCAM, MUC-1 and HER-2, respectively). Electrical bioimpedance spectra (100 Hz to 1 MHz) were recorded in each case. *Main results*: At the frequency centered at 100 KHz, EIS displayed a greater sensitivity for magnitude when using 50 cells/500 μl (4.11 ± 0.23, 8.81 ± 1.73 and 17.5 ± 3.61 ohms for MCF-7, MDA-MB-231 and SK-BR-3, respectively). There were no significant differences between the phases of impedance tested at the greatest sensitivity of 1 KHz (-78.05 ± 0.53, -79.23 ± 0.93 and -75.26 ± 0.72 degrees for MCF-7, MDA-MB-231 and SK-BR-3, respectively). *Significance*: Under the present conditions, EIS was sensitive enough to detect a low concentration of breast cancer cells (50 cells/500 μl) and classify the distinct cells lines with a signature impedance pattern, as long as the MNP concentration was very low ($\leqslant$0.125 mg MNPs/50 000 cells). The relative expression of the molecular markers on each cancer cell line was related to the magnitude of the electrical impedance.

1. Introduction

Breast cancer is one of the major worldwide public health problems today. Its high mortality rate is due to a lack of early diagnosis and poor response to therapy, frequently followed by metastasis (Torre *et al* 2017, Arvelo *et al* 2016, Fitzmaurice *et al* 2015). Since the process of metastasis is accompanied by the presence of circulating tumor cells (CTCs) (Giuliano *et al* 2014), the accurate evaluation of such cells subsequent to chemotherapy is of vital importance. Additionally, a reliable tool for the detection of CTCs could serve as an initial test in patients with primary breast cancer (Ebeed *et al* 2012, Gorges *et al* 2012).

Magnetic nanoparticles (MNPs) have already been employed to label molecules or organelles for monitoring (Kouassi *et al* 2005, Reddy *et al* 2012, Wei *et al* 2015), and after being coupled with antibodies, to target molecular markers on cancer cells. In 2001, there was a report on the advantages of isolating SK-BR3 breast cancer cells with a magnetic-nanoparticle/antibody (MNP-ab) bioconjugate (Liberti *et al* 2001).

An efficient methodology for the immunomagnetic separation from whole blood of circulating SK-BR3 breast cancer cells was published in 2011 (Xu *et al* 2011). After an MNP-ab bioconjugate binds to a surface protein overexpressed on a particular type of cancer cell, these cells can be immunomagnetically separated from the blood, and then differentiated from normal cells and quantified by using electrical impedance spectroscopy (EIS) (Schwan 1999, Han *et al* 2006, Qiao *et al* 2012, Zheng *et al* 2013). The technical feasibility

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of identifying cancer cells in whole blood with immunomagnetic separation and EIS has been demonstrated with BT-474 breast cancer cells (Silva *et al* 2014). Our group recently described the successful detection of small concentration of cancer cells by following this immunomagnetic separation procedure, in which MNPs were functionalized with antibodies that recognize surface proteins (EPCAM, MUC-1 and HER-2) differentially expressed according to the stage of breast cancer (Huerta-Nuñez *et al* 2019).

To our knowledge, there are no reports on the degree of sensitivity of EIS employed at a range of frequencies to detect the presence of CTCs at distinct concentrations. Determining the frequency with the greatest sensitivity would facilitate the characterization of the molecular expression of surface proteins and therefore the capability of identifying and distinguishing between types of cancer cells. Another important factor is the relation of EIS to the genetic expression of surface proteins that are targeted to isolate such cells, which is also a relevant and unexplored topic.

In a previous study, we performed RT-PCR to examine the relative expression pattern of three surface proteins, EPCAM, MUC-1 and HER-2, each being characteristically overexpressed on one of the three breast cancer cell lines herein evaluated (MCF-7, MDA-MB-231 and SK-BR-3, respectively). The binding of the MNP-ab bioconjugate to the cancer cell membrane was scrutinized and confirmed with Fourier-transform infrared spectroscopy (Huerta-Nuñez *et al* 2019).

The aim of the current contribution was to take another step towards the development of an EIS methodology that can hopefully serve as a practical tool for clinically detecting and classifying CTCs in blood (after labeling them with an MNP-ab bioconjugate). Accordingly, the sensitivity of electrical bioimpedance measurements was tested at a range of frequencies to identify the presence of breast cancer cells in suspension at different concentrations and to determine the specific type of these cells. Subsequent to labeling and separating the breast cancer cells, the electrical impedance patterns were recorded for each cell type and concentration. The information on the relative expression of the surface protein and the electrical impedance of the cancer cells established a recognizable pattern for the characterization of the molecular markers by EIS. The evaluation of the sensitivity of this spectrographic technique at distinct frequencies lays the groundwork for optimizing a technique capable of detecting and differentiating breast cancer cells in suspension after labeling them with magnetic nanoparticles. The results support the potential of such a biosensor to serve the purpose for which it was designed.

2. Materials and methods

2.1. Cell cultures

Three breast cancer cell lines, MCF-7, MDA-MB-231 and SK-RB-3 (ATCC®, American Type Culture Collection, Manassas, VA), were incubated in 100 mm Petri dishes maintained at 37 °C, 5% CO_2 and 95% humidity. The culture medium consisted of 94% Dulbecco's Modified Eagle Medium F-12, 5% bovine fetal serum and 1% penicillin/streptomycin/amphotericin B (Invitrogen™, Waltham, MA). Independent incubations were carried out for each cell line in triplicate.

2.2. Relative expression examined by RT-PCR

Total RNA was isolated three times from each breast cancer cell line by the phenol-chloroform technique, then quantified in a spectrometer at a wavelength of 260 nm. The integrity of RNA was assessed by denaturing gradient gel electrophoresis with agarose gel (Invitrogen™, Waltham, MA) containing 1% ethidium bromide stain. The samples were treated with DNase (RQ1 RNase-free DNase, Promega Inc., Madison, WI) to avoid any contamination by genomic DNA that may have been in the RNA obtained. Synthesis of the complementary DNA corresponding to each biological replicate was performed with the Cloned AMV First-Strand cDNA Synthesis kit (Invitrogen™, Waltham, MA).

Oligonucleotides were designed on Primer Express version 3.0 software (Applied Biosystems™, Waltham, MA) to determine the expression of EPCAM, MUC-1 and HER-2, utilizing actin as the endogenous gene. The genes were amplified by the RT-PCR with ABI PRISM™ 7000 Sequence Detection System version 1.1 (ThermoFisher Scientific Inc., Waltham, MA), using SYBER Green as the detection system and the SYBER Green PCR Master Mix kit (Applied Biosystems™, Waltham, MA) for monitoring the amplification of the product during each reaction cycle, in which optical plates were submitted to the following conditions: 95 °C for 10 min, 40 cycles of 95 °C for 15 s and of 60 °C for 1 min.

2.3. Functionalization of the MNP-ab bioconjugate

Nanoprobes for targeting cell surface proteins were generated by utilizing fluidMAG-ARA MNPs (Chemicell™, Berlin, Germany, Cat #4115-5) functionalized with a monoclonal antibody. The particular antibody applied depended on the surface protein targeted, being CD36 for EpCAM, CD227 for MUC-1 and CD340 for HER-2 (all from BioLegend™, San Diego, CA, Cat #324 202, 355 602 and 324 402, respectively).

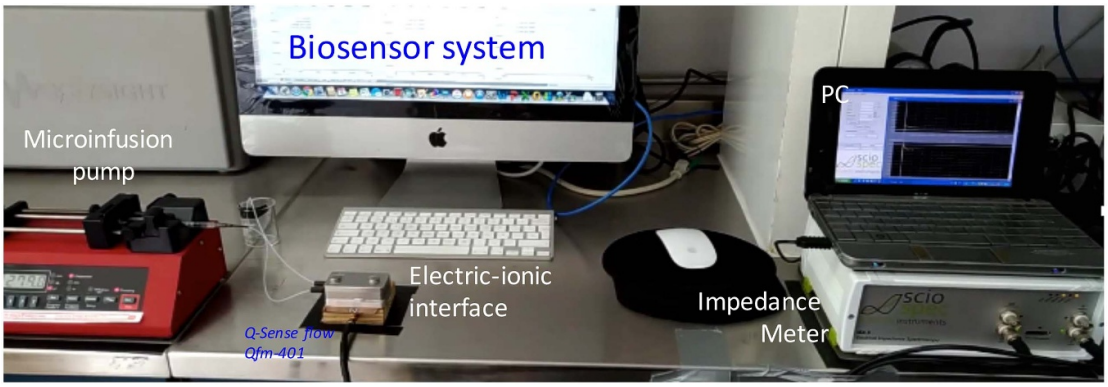

Figure 1. Experimental setup for electrical impedance spectroscopy (EIS) measurements, consisting of four elements: (1) a microfluidic infusion pump, (2) an electric–ionic interface, (3) a Sciospec™ impedance meter and (4) a personal computer (PC).

The MNPs (100–300 nm) were in an aqueous dispersion composed of iron oxide, a magnetite nucleus (Fe_3O_4) and a covering of hydrophilic polymers. The latter coating protects the MNPs from external ions. On their outer surface, the MNPs had a carboxyl functional group of glucuronic acid that allowed them to covalently bind to biomolecules. The MNP-ab bioconjugate was functionalized as a nanoprobe by employing the A-10 protocol (Chemicell™, Berlin) and the carbodiimide method, based on the reaction of the carboxyl groups of the ferromagnetic MNPs. These groups become highly reactive to the intermediate products of O-acylisourea, reacting quickly with amino groups in the constant region of heavy chains of monoclonal antibodies.

2.4. Breast cancer cells tagged with an MNP-ab bioconjugate

Breast cancer cells were cultured under the aforementioned conditions until a confluence of 80% was obtained, at which point they were mechanically separated and quantified to afford a master sample of 50 000 cells from each line. Subsequently, each of the cell lines (MCF-7, MDA-MB-231 or SK-BR-3) at a controlled quantity was independently incubated with the proper amount of its nanoprobe in 500 µl of PBS. The amount of the respective nanoprobe was established in accordance with the antibody manufacturer's recommendations (BioLegend™, San Diego, CA). For control samples, the nanoprobe was studied alone (in the absence of cancer cells) in a solution of 500 µl PBS. Hence, the following concentration of the corresponding antibody per number of cells was employed: $\leqslant$0.5 µg ab/10^6 cells plus the nanoprobe to target the EpCAM surface protein on the MCF-7 cancer cell line; $\leqslant$1.0 µg ab/10^6 cells plus the nanoprobe to target the MUC-1 surface protein on the MDA-MB-231 cell line; $\leqslant$0.125 µg ab/10^6 cells plus the nanoprobe to target the HER-2 surface protein on the SK-BR-3 cell line. Subsequently, dilution was performed to obtain concentrations of approximately 5000, 500 and 50 cells in 500 µl of PBS.

2.5. Electrical impedance spectroscopy (EIS)

The modular design of the system for bioimpedance measurements consisted of four elements (see figure 1): (1) a microfluidic infusion pump, (2) an electric–ionic interface, (3) a Sciospec™ module and (4) a personal computer (PC). The microfluidic infusion pump (*NE-1002X,* New Era Pump Systems, Inc., Farmingdale, NY) was adapted to a 1 ml insulin syringe to infuse the analyte in a controlled manner through a capillary tube towards the chamber of the electric–ionic interface module. In the latter, the cancer cells were isolated by immunomagnetic separation and bioimpedance was evaluated by means of a microfluidic apparatus (Q Sense-Flow Qfm-401, Biolin Scientific™, Gothenburg), a hermetic chamber that housed a quartz crystal embedded with gold electrodes. The Sciospec™ module (ScioSpec™, ISX-3, Sciospec Scientific Instruments Inc., Leipziger) injected an electric potential difference (100 mV peak) at 125 preprogrammed frequencies logarithmically spaced in the range of 100 Hz to 1 MHz, and at the same time measured the current passing through the electrodes to determine the multifrequency bioimpedance of the system. The PC (HP mini 110–1150LA PC, HP Inc., Palo Alto, CA) served to program the Sciospec™ instrument and store the data. Since a resonance effect developed in the impedance meter system as of 200 kHz, the results from higher frequencies were excluded from the analysis.

Impedance was assessed for each cell line coupled to its respective MNP-ab bioconjugate. The three cell lines, MCF-7, MDA-MB-231 and SK-BR-3, were tested at concentrations of 50, 500 and 5000 cells/500 µl. Hence, one of the cell lines at a given concentration was infused for 15 min through the pump and exposed to the magnetic field in order to acquire impedance data. The signal between each of the intervals was

obtained from 1024 average values per second for magnitude and phase. The measurements from three 15 min assays were averaged for each breast cancer cell line and concentration. The bioimpedance spectra were normalized with respect to their greatest value in the whole bandwidth. This procedure assured that all experimental conditions are evaluated as relative (proportional) spectra in the same dynamic range, which allowed signature differences between the three cancer cell lines to emerge naturally.

2.6. EIS: extraction of features

As a first attempt to extract specific features from EIS spectrum, the integrals of the area under the magnitude ($\boldsymbol{A_Z}$) and phase ($\boldsymbol{A_{Phase}}$) curves were calculated in the frequency (f) range of 1–200 KHz, according to the following equations:

$$A_Z = \int_1^{200} Z(f)\, df \tag{1}$$

$$A_{Phase} = \int_1^{200} Phase\,(f)\, df. \tag{2}$$

In addition, the slope ($\boldsymbol{m}$) of a line connecting the maximum and minimum points of the curves was calculated for magnitude ($\boldsymbol{m_Z}$) and phase ($\boldsymbol{m_{Phase}}$) as follows:

$$m_Z = \frac{Z(f)_{max} - Z(f)_{min}}{f_{Z\_max} - f_{Z\_min}} \tag{3}$$

$$m_{Phase} = \frac{Phase(f)_{max} - Phase(f)_{min}}{f_{Phase\_max} - f_{Phase\_min}}. \tag{4}$$

Sensitivity and specificity analyses were carried out by utilizing these values as comparative parameters. The MNP-ab bioconjugates in the absence of cancer cells served as the reference.

2.7. Statistical analysis

Data were expressed as the mean ± standard deviation (SD) and examined on the SPSS Statistics version 23.0 program (IBM™ SPSS Statistics, Chicago, IL). The Kruskal–Wallis test was applied to compare the bioimpedance measurements and gene expression of the breast cancer cell lines (based on three independent biological replicates in each case). Receiver operating characteristic (ROC) curves were constructed to determine the sensitivity and specificity of the procedure for differentiating cancer cells from control conditions. The control (basal reference) for the EIS consisted of the MNP-ab bioconjugate in buffer solution (without cancer cells) at the same concentrations used for the cancer cells. Thus, sensitivity and specificity were assessed by comparing the ROC curves of the bioconjugated breast cancer cell lines to the basal reference, with significant differences considered at $p < 0.05$.

3. Results

Images of the three breast cancer cell lines included presently were captured on an optical microscope (figure 2). Morphological differences between the three cell types are evident. Figure 3 shows the electrical bioimpedance spectra normalized in magnitude and phase for each cell line and concentration (MCF-7, MDA-MB-231 and SK-BR-3, at 50, 500 and 5000 cells/500 µl) coupled to its respective MNP-ab bioconjugate and after immunomagnetic separation. Magnitude sensitivity for cell concentration is clear for the case of SK-BR-3, roughly so for MDA-MB-231 and not perceptible for MCF-7 cells. According to the comparative statistical analysis of the raw data from electrical bioimpedance, the frequency centered at 100 KHz displayed a greater sensitivity for magnitude when the solution contained 50 cells/500 µl (4.11 ± 0.23, 8.81 ± 1.73 and 17.5 ± 3.61 ohms for MCF-7, MDA-MB-231 and SK-BR-3, respectively). There were no significant differences between the phases of impedance tested at the greatest sensitivity of 1 KHz (−78.05 ± 0.53, −79.23 ± 0.93 and −75.26 ± 0.72 degrees for MCF-7, MDA-MB-231 and SK-BR-3, respectively). After the relative genetic expression was established and the magnitude of electrical impedance quantified for the three breast cancer cell lines, a comparative appraisal was made (figure 4). As the relative genetic expression of each surface protein decreased, the magnitude of electrical impedance of the corresponding cancer cell seemed to increase (at 100 KHz; figures 4(A) and (B)). Thus, the cells with a greater relative gene expression of the labeled surface protein exhibited a lower impedance. Figure 5 portrays the mean values ± SD of the estimated parameters $\boldsymbol{A_Z}$, $\boldsymbol{A_{Phase}}$, $\boldsymbol{m_Z}$ and $\boldsymbol{m_{Phase}}$ for each cell line at 50 cells/500 µl, indicating significant

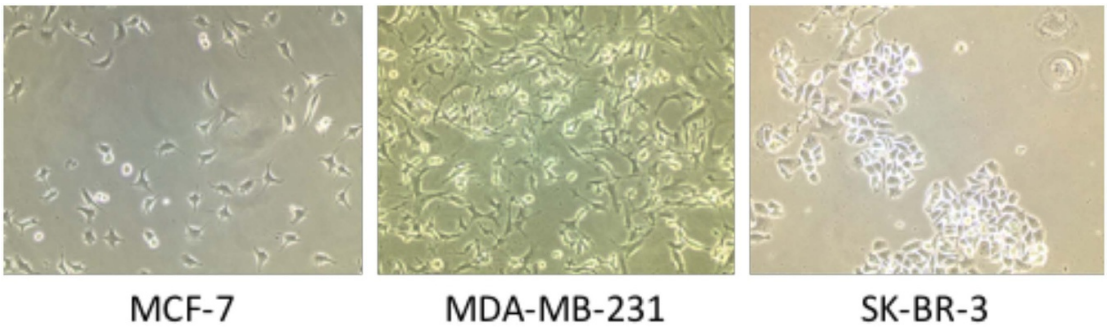

Figure 2. Breast cancer cell lines in the culture medium. The morphological differences between the three types of cancer cells herein evaluated are evident. Optical images were taken at 40×.

differences between groups. Examination of this concentration is relevant due to the extremely small amount of cancer cells found in the blood of patients (~10–50 cells ml^{-1}) (Xu *et al* 2011).

4. Discussion

The findings of the current study represent an important step in the development of a new and practical methodology for the clinical identification and quantification of CTCs by means of bioimpedance measurements. In a clinical setting, such a procedure would take place after labeling cells with an MNP-ab bioconjugate and separating them from patient blood. The bioimpedance signature pattern for the surface protein overexpressed on each of the three breast cancer cell lines presently tested proved to have suitable features for the detection of the cells and classification of their type (considering the lowest concentration analyzed).

The surface proteins EpCAM, MUC-1 and HER-2, characteristic of the MCF-7, MDA-MB-231 and SK-BR-3 cancer cell lines, respectively, were labeled with the appropriate MNP-ab bioconjugate. The possible association between the relative genetic expression of this labeled molecular marker and the electrical bioimpedance data was assessed, finding an inverse relation. That is, the magnitude of the electrical impedance of the corresponding cancer cell line seems to increase as the genetic expression of the surface protein decreases, in agreement with our previous report and with the expected biophysical phenomenon.

Briefly, an enhanced genetic expression of the surface protein is accompanied by a higher concentration of the protein itself on the cell membrane (Huerta-Nuñez *et al* 2019), which implies a larger amount of the bioconjugate bound to these proteins. With a greater concentration of MNPs, the volumetric conductivity of the MNP-cell conglomerate is higher and the impedance lower (see supplementary material stacks.iop.org/PMEA/41/064001/mmedia).

When a small quantity of nanoprobes was used to recognize the target molecules, there was better sensitivity for the impedance measurements used to detect the cells. This might be explained by the percolation theory, which proposes a percolation effect in the event that the MNPs reach a saturation point in the sample (Sahimi and Yortsos 1990, Berkowitz and Balberg 1993, Hunt 2005). The concentration of MNPs appears to have reached the upper limit when nanoprobe-tagged cell surface proteins were at $\leqslant$0.125 μg ab/10^6 cells, as evidenced by the best sensitivity found with the nanoprobe preparation for the HER-2 molecular marker. In this case, protocol A-10 (Chemicell™, Berlin) for the preparation of nanoprobes indicates a ratio of 50 μg ab/10 mg of MNPs. The calculation established the proper concentration at 0.125 mg MNPs/50 000 cells.

The aforementioned interpretation of the results is supported only by the qualitative evaluation of evident differences in figure 4. The statistical analysis did not show a significant correlation between the degree of genetic expression of the surface proteins and the magnitude of volumetric electrical bioimpedance. Interestingly, the data demonstrate that a decrease in the expression of the molecular marker is reflected in an increase in the magnitude impedance at 100 KHz, which concurs with the biophysical phenomenon contemplated.

The frequency range with the greatest sensitivity to impedance measurements was the beta dispersion bandwidth (Schwan 1957). This is congruent with the dispersion region observed in the magnitude of the spectra (starting at approximately 100 kHz). The normalization of bioimpedance spectra revealed signature differences between the three cancer cell lines. Sensitivity and specificity analysis was carried out with the extracted features as comparative parameters, leading to relevant findings in regard to the integration of $\boldsymbol{A_Z}$. Figure 6 portrays the ROC curves that were instrumental in assessing $\boldsymbol{A_Z}$ sensitivity and specificity for the

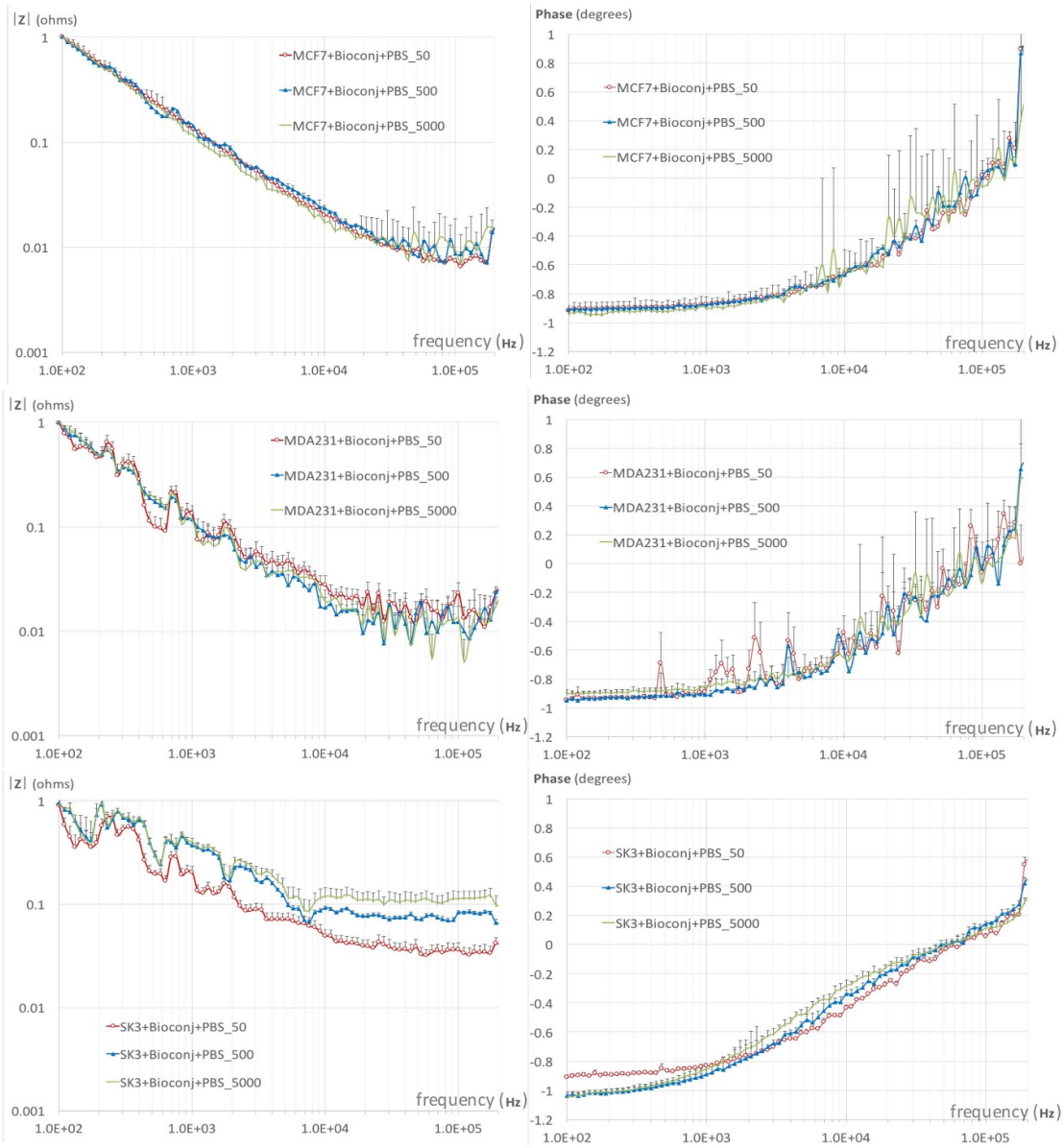

Figure 3. The electrical bioimpedance spectrum (magnitude and phase) determined for each cell line and concentration (MCF-7, MDA-MB-231 and SK-BR-3 at 50, 500 and 5000 cells/500 µl) after its coupling to the respective magnetic nanoparticle (MNP)-antibody conjugate and immunomagnetic separation. Spectra are normalized in relation to the highest value in the whole bandwidth.

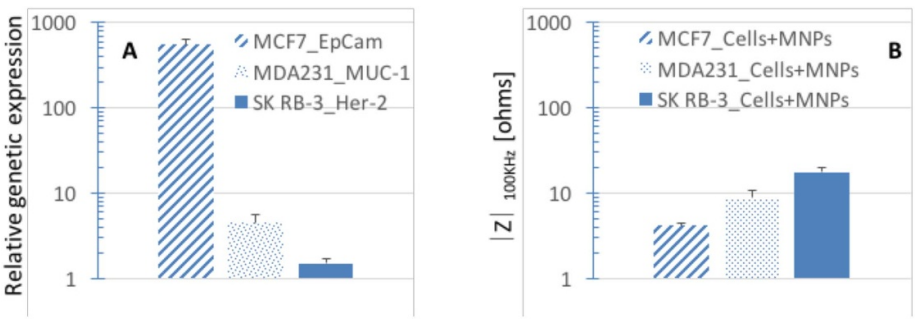

Figure 4. (A) Relative genetic expression and (B) magnitude of electrical impedance in the three breast cancer cell lines. Values represent the mean ± standard deviation.

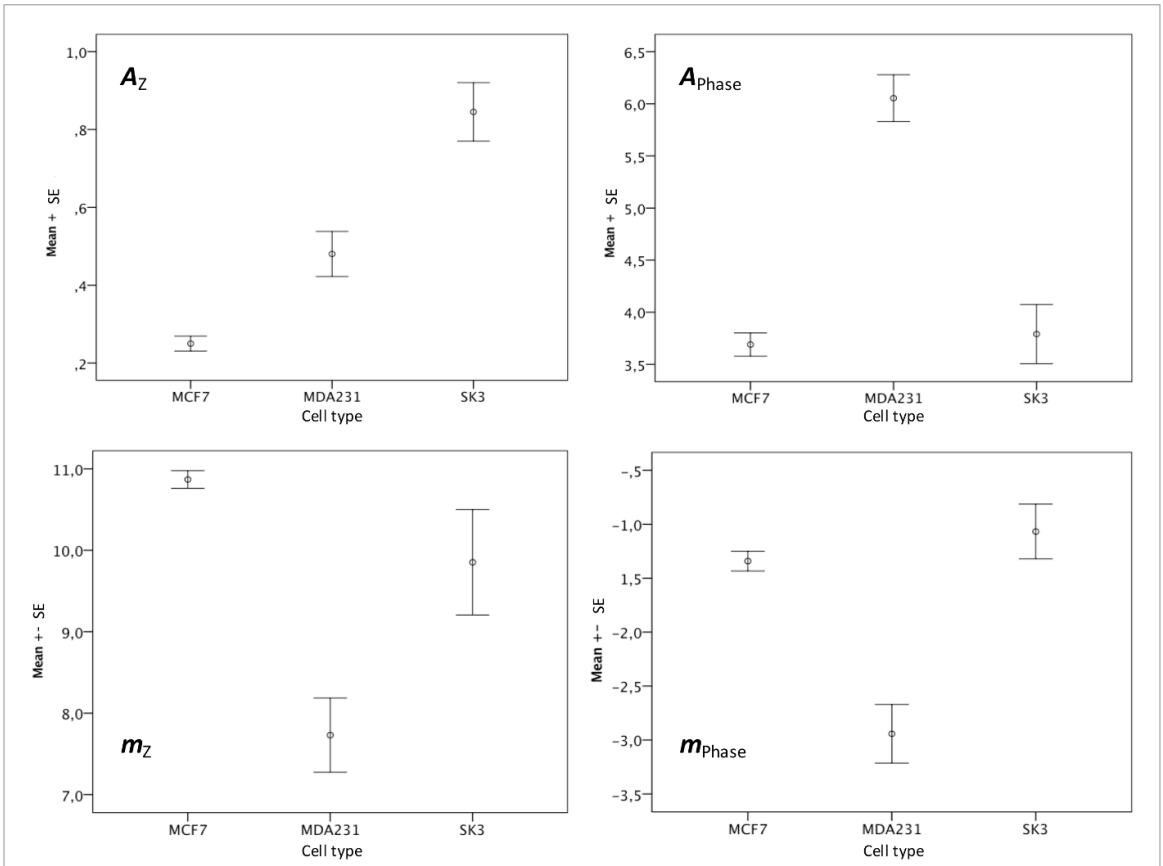

Figure 5. Integration values of the area under the bioimpedance curves for the spectrum magnitude ($\boldsymbol{A_Z}$) and phase ($\boldsymbol{A_{Phase}}$), magnitude slope ($\boldsymbol{m_Z}$) and phase slope ($\boldsymbol{m_{Phase}}$) for each cell line (MCF-7, MDA-MB-231 and SK-BR-3) at a concentration of 50 cells/500 µl. Data are expressed as the mean ± standard deviation.

Table 1. Sensitivity and specificity analyses based on ROC curves.

Extracted feature	Cell types and *concentration*	ROC curve area	Std. error	*P*	Sensitivity	1-Specificity
$\boldsymbol{A_Z}$	All Cells_*50 cell/500µl*	0.701	0.075	*0.014*	0.667	0.333
$\boldsymbol{A_Z}$	All Cells_*500 cell/500µl*	0.540	0.087	*0.624*	0.667	0.778
$\boldsymbol{A_Z}$	All Cells_*5000 cell/500µl*	0.486	0.083	*0.865*	0.667	0.593
$\boldsymbol{A_Z}$	MCF-7_*50 cell/500µl*	0.519	0.142	*0.895*	0.778	0.778
$\boldsymbol{A_Z}$	MDA-MB-231_*50 cell/500µl*	0.716	0.125	*0.122*	0.778	0.444
$\boldsymbol{A_Z}$	SK-RB-3_*50 cell/500µl*	0.840	0.098	*0.015*	0.778	0.222

ROC: Receiver operating characteristic; ***Az***: integration values of the area under the bioimpedance curves for the spectrum magnitude

recognition of each cancer cell type at 50, 500 and 5000 cells/500 µl. Based on the greatest area under the ROC curves with statistical significance, the best sensitivity and specificity was afforded at a concentration of 50 cells/500 µl (area under the ROC curve = 0.701; $P = 0.014$), which was then employed for further sensitivity analysis. Figure 7 displays the ROC curves for $\boldsymbol{A_Z}$, used to differentiate specific cancer cells lines (MCF-7, MDA-MB-231 or SK-BR-3) at 50 cells/500 µl. The best sensitivity and specificity correspond to the recognition of SK-BR-3 (area under the ROC curve = 0.840; $P = 0.015$; figure 7). As can be appreciated, the proposed biosensor technique was capable of recognizing the lowest concentration presently tested and of identifying the cancer cell type (figure 6). Table 1 shows the sensitivity and specificity results for the three cell lines evaluated.

A 3D dotplot graph of combined extracted features establish clusters for each cell type (figures 8(A) and (B)). The combination of the features of $\boldsymbol{A_Z}$, $\boldsymbol{A_{Phase}}$ and $\boldsymbol{m_Z}$ provided the best definition of the cluster for MDA-MB-231 cells, while the combination of features of $\boldsymbol{A_Z}$, $\boldsymbol{A_{Phase}}$ and $\boldsymbol{m_{Phase}}$ worked better for characterizing MCF-7 cells. The features extracted from the bioimpedance signature appear to be suitable for the detection of a low concentration of each of the cancer cell lines herein examined and the differentiation of one from the others. Hence, the key to be able to distinguish a cancer cell type is the determination of the particular combination of features that constitute a unique signature. Such conditions can be observed by the opposite clustering effect of MDA231 *vs* MCF7 when the $\boldsymbol{m_Z}$ and $\boldsymbol{m_{Phase}}$ axes are interchanged in the 3D plots

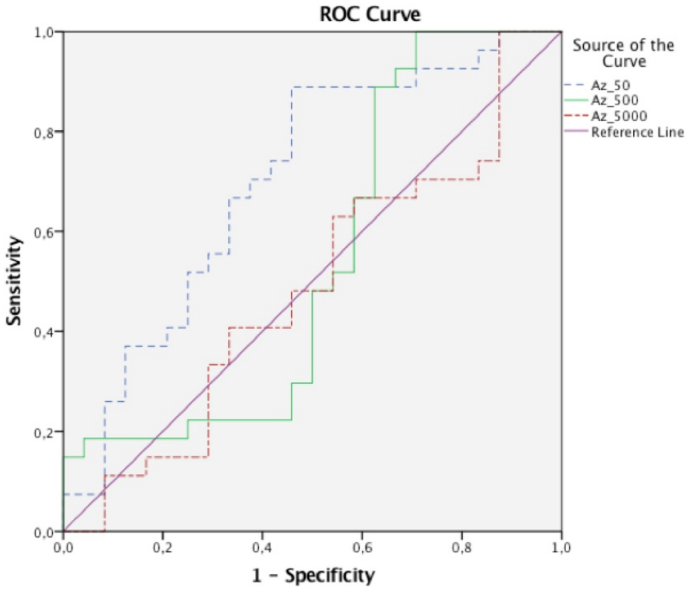

Figure 6. Receiver operating characteristic (ROC) curves that portray the area under the bioimpedance curves for spectrum magnitude (A_Z). Sensitivity and specificity analyses were able to detect the presence of the three types of cancer cells (at 50, 500 and 5000 cells/500 μl), following the coupling of these cells to the respective magnetic nanoparticle (MNP)-antibody conjugate and immunomagnetically separating them.

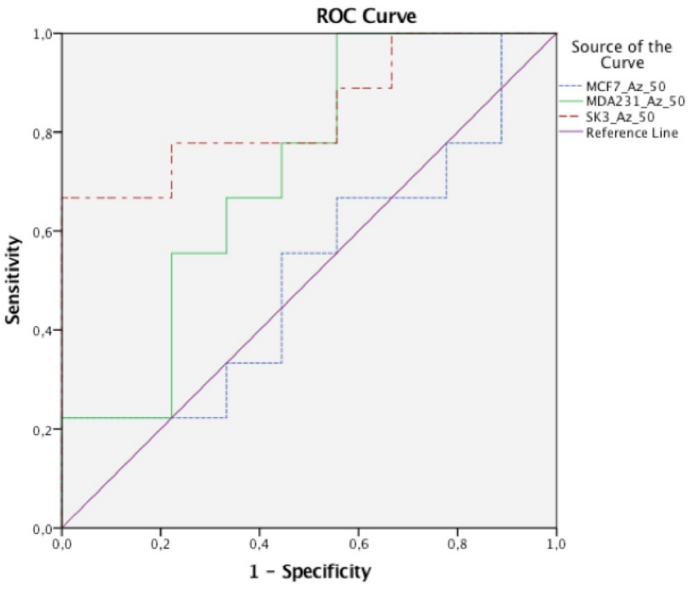

Figure 7. Receiver operating characteristic (ROC) curves that illustrate the area under the bioimpedance curves for spectrum magnitude (A_Z). Sensitivity and specificity analyses were able to distinguish between the three types of cancer cells (MCF-7, MDA-MB-231 and SK-BR-3, examined only at 50 cells/500 μl) following the coupling of these cells to the corresponding magnetic nanoparticle (MNP)-antibody conjugate and immunomagnetically separating them.

(graph A *vs* B). The ability to classify a given cancer cell line based on its unique features evidently requires an analysis of the bioimpedance spectrum in a certain frequency range, found in the current investigation to be 0.1 to 200 KHz. Electrical conductivity analysis at single frequency or with direct current would not be sufficient for the task of producing and extracting specific features leading to a distinct signature spectrum for each cancer cell line.

The results of this study constitute a preliminary characterization of the pattern of expression of the surface proteins targeted for immunomagnetic separation of breast cancer cell lines, and the electrical impedance patterns at a range of frequencies capable of differentiating the cell types. These findings contribute to forming the foundation for a biosensor of CTCs.

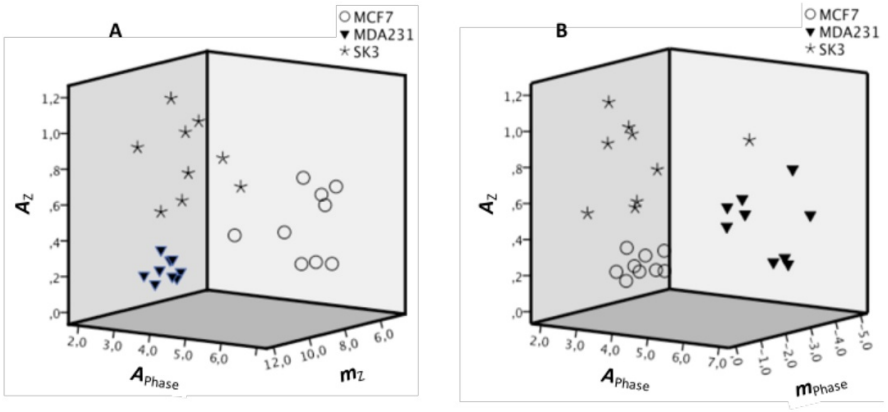

Figure 8. 3D dotplot graphs constructed from two specific combinations of features based on the integration values of the area under the bioimpedance curves: (A) for spectrum magnitude ($\boldsymbol{A_Z}$) and phase ($\boldsymbol{A_{Phase}}$) together with the magnitude slope ($\boldsymbol{m_Z}$), and (B) for spectrum magnitude ($\boldsymbol{A_Z}$) and phase ($\boldsymbol{A_{Phase}}$) together with the phase slope ($\boldsymbol{m_{Phase}}$). These two combinations allowed for the differentiation of the three cell lines (MCF-7, MDA-MB-231 and SK-BR-3), evaluated only at 50 cells/500 µl.

5. Conclusions

The three breast cancer cell lines presently examined (MCF-7, MDA-MB-231 and SK-BR-3) are each characterized by the overexpression of a certain surface protein (EpCAM, MUC-1 and HER-2, respectively). The corresponding surface protein was labeled with an MNP-ab bioconjugate, which targeted and codified it. When applying EIS at the lowest concentration of cancer cells (50 cells/500 µl), the resulting signature pattern provided suitable information to be able to differentiate one cell line from another. When using a small quantity of nanoprobes for the recognition of the target molecules, the sensitivity of impedance for cell recognition improved, suggesting that the genetic expression of the surface protein of a given cancer cell line is related to its electrical impedance pattern. A decreased expression is reflected in an increased magnitude of impedance, which as aforementioned is congruent with the expected biophysical phenomenon. The current results contribute to the foundation of knowledge required for the development of a biosensor of CTCs based on genetic recognition and immunomagnetic isolation. It is necessary to carry out further research with a greater number of assays to enhance the rigor of the present findings, as well as with the blood of cancer patients to test the separation of CTCs.

Acknowledgments

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Patents

The current study is based on the detection of cancer cells through measurements of electrical bioimpedance, for which a patent application has been submitted: González CA 2017 MX/a/2017/010319 and 2018 MX/a/2018/009086.

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