Chapter 2

Bioimpedance and Cancer Detection

Rafael Gonzalez-Landaeta[1,*] and *César Antonio González-Díaz*[2]

Biological tissues have electrical properties linked to their constitution, size, and shape. Tissues that contain cancer cells present differences in electrical properties due to the structural and histological characteristics of such cells, which differ from healthy cells. This chapter describes the role of Bioimpedance in the estimation of the electrical properties of malignant tissues. The first part describes the fundamentals of Bioimpedance as a useful technique to estimate the electrical properties of tissues/cells; that is, conductivity, resistivity, and permittivity. Secondly, the electrical properties of tissues that contain malignant cells are presented, including some studies where these properties have been estimated in different tissues, namely, breast, prostate, and skin. Thirdly, a short description of the electrodes and the electronic instrumentation used in Bioimpedance for the detection of cancerous tissues/cells is presented. Finally, applications of Electrical Impedance Spectroscopy (EIS) in the detection of Circulating Tumoral Cells (CTC) and breast cancer, as well as an introduction to the Magnetic Induction Spectroscopy (MIS) as a valuable non-contact technique to detect neoplasms in breast tissue have been presented.

[1] Departamento de Ingeniería Eléctrica y Computación, Instituto de Ingeniería y Tecnología, Universidad Autónoma de Ciudad Juárez. Av. del Charro 450 nte. Ciudad Juárez, Chihuahua, México. C.P. 32310.

[2] Escuela Superior de Medicina. Departamento de Posgrado e Investigación Instituto Politécnico Nacional. Plan de san Luis esquina con Diaz Miron, s/n, colonia casco de santo Tomás. Ciudad de México. C.P. 11320.

Email: cgonzalezd@ipn.mx

* Corresponding author: rafael.gonzalez@uacj.mx

2.1 Bioimpedance basics

Since the middle of the last century, the electrical properties of tissues have been studied using Bioimpedance. In the first works done in this field, the intention was to estimate the water content in different tissues. This opened the way to new investigations where electrical properties have been obtained to detect several pathologies, both at the tissue and cellular levels. In this sense, Bioimpedance has been used in the development of various devices ranging from small biosensors to electrical impedance tomographs. Among the diversity of applications, Bioimpedance has been widely used in detecting malignant tissues/cells through *ex vivo* and *in vivo* measurements, where the different electrical properties are evaluated at the microscopic and macroscopic levels. In this sense, here is a brief description of the electrical properties that can be obtained using Bioimpedance, and how these properties allow differentiation between healthy and malignant tissues/cells. On the other hand, the measurement methods and the electronic instrumentation involved in the detection of malignant tissues/cells are also described.

2.1.1 Electrical properties of the cells

Electrical impedance is the term used to estimate the resistance of a given system to electric current when an electric field is applied to it. It is estimated by the ratio between the voltage (V) and the current (I); that is $Z = V/I$. In biological tissues, it is called Electrical Bioimpedance, or simply Bioimpedance, where a voltage is produced due to the distribution of the current injected into the tissue. The Bioimpedance of tissue is determined by the electrical conductivity (σ), the dielectric permittivity (ε), and geometrical factors (length, L, and area, A) of the tissue (see Equation 2.1) and can be used to estimate the electrical behavior of the tissues from a macroscopic point of view (Rigaud et al., 1996).

$$Z^* = \frac{1}{\sigma + j\omega\varepsilon}\frac{L}{A} \tag{2.1}$$

Z^* indicates a complex quantity (combination of real, R, and imaginary, X, components), and $\varepsilon = \varepsilon_r\varepsilon_0$, where ε_r is the relative permittivity and ε_0 is the permittivity of vacuum (8.85×10^{-12} F/m). The conductivity determines the mobility of charge carriers (ions in biological tissues), and the dielectric permittivity is related to the polarization of media as it determines the mobility of bound charges when an electric field is applied (Grimnes and Martinsen, 2015). The permittivity is used to assess the dielectric properties of biological materials. The conductivity and the permittivity of tissues depend on their

composition and structure and are independent of geometrical constraints (Ivorra, 2003).

Considering only one cell suspended in an ionic solution, the ionic solution outside the cell is called the extracellular medium. Inside the cell, there also exists an ionic medium where the metabolic processes occur, and it is called the intracellular medium. Both media are separated by the cell membrane, which acts as a barrier to the ionic flow. The conductivity of both media is higher than that of the cell membrane. So, electrically, the extracellular and intracellular media can be modeled like resistors, and the cell membrane can be modeled like a capacitor.

Biological tissues are not perfect conductors or perfect capacitors. They could be considered conductors with capacitive properties and capacitors with conduction properties. Due to these non-ideal properties, once an electric field is applied to a biological tissue (assume a step function), a certain time is required for it to reach a new electrical equilibrium: this process is called *relaxation* and depends on the polarization (perturbation of the charge distribution) mechanism (Grimnes and Martinsen, 2015). Since suspended cells can be emulated as RC circuits, it is feasible to say that the electrical properties of tissues are frequency-dependent.

Dispersions represent the *relaxation* mechanisms in the frequency domain. Three zones of change from a specific value to another specific value define the dispersions called alpha (α), beta (β), and gamma (γ). At low frequencies (< 1 kHz), the α-dispersion describes the accumulation of opposite charges between both sides of the cell membranes, creating a large dipole due to the electric field applied (surface polarization) (Pethig, 1984; Scholz and Anderson, 2000). In the radiofrequency region (1 kHz – 100 MHz), the β-dispersion is attributed to the charging and discharging mechanism of the cell membrane (Pethig, 1984; Rigaud et al., 1996). The γ-dispersion, which occurs at higher frequencies (≥ 1 GHz), is attributed to the relaxation of the water molecules (Schwan, 1957).

The terms "complex conductivity" and "complex permittivity" are mainly used to estimate the electrical properties of the cells from a microscopic point of view (Rigaud et al., 1996), and are defined by Equations 2.2 and 2.3, respectively:

$$\sigma^* = \sigma + j\omega\varepsilon_0\varepsilon_r \tag{2.2}$$

$$\varepsilon^* = \varepsilon_r - j\frac{\sigma}{\omega\varepsilon_0} \tag{2.3}$$

From Equation (2.2), as the frequency increases the conductivity increases, which is attributed to the fact that the cell membrane is short-circuited, so its contribution to the distribution of the injected current is

negligible. In the case of the complex permittivity (Equation (2.3)), it becomes prominent at lower frequencies, where the capacitive effect of the cell membrane prevails. So, when a multifrequency current is injected into a tissue, the path for higher frequency currents includes both intracellular and extracellular media because the capacitive effects of the cell membrane disappear (Grimnes and Martinsen, 2015; Webster, 1990).

The complex conductivity and permittivity can be obtained indirectly by measuring Z^*. However, the geometry of the tissue could achieve misleading results. For this, a scaling factor called "cell constant" is used to relate the complex impedance and the conductivity (or the permittivity) of the tissue (Ivorra, 2003). The cell constant is $K = A/L$, being A and L the area and length of the measurement cell, respectively.

2.1.2 Electrical properties of malignant tissues

Cancer cells have structural differences compared with healthy cells and have histological characteristics that can be reflected in their electrical properties, namely the conductivity, resistivity ($\rho = 1/\sigma$) of the intracellular and extracellular media, and the permittivity of the cell membrane. Bioimpedance has become a useful technique for detecting malignant tissues, where measurements can be performed *in vivo*, and *in vitro* in controlled environments. In the literature, most of the studies are focused on breast, prostatic and, skin cancers (Sarode et al., 2016). However, other experimental studies have used Bioimpedance to study the electrical properties of cancer cells in muscles (Blad and Baldetorp, 1996), lymph nodes (Malich et al., 2002), and colon (Haemmerich et al., 2003).

In vitro measurements of breast cancer have revealed differences in the conductivity and the permittivity, which have been attributed to the structural and cellular inhomogeneities of tumoral tissue (Surowiec et al., 1988). In the range of 499 Hz and 1 MHz, an increment in the low-frequency-limit resistance of carcinoma in comparison with the mammary gland has been revealed (Jossinet, 1998). Nevertheless, these results disagree with those obtained by Surowiec et al. (1988), who obtained an increase in the conductivity and the permittivity in the radiofrequency range. The discrepancies between these two studies can be attributed to the measurement conditions, which may alter the results. In the case of *in vitro* measurement, the conditions of the environment where the cells are immersed define the reliability of the technique. Living tissues have temperature coefficients that affect the conductivity and the permittivity of the cells (Pethig, 1984), so temperature changes could distort the values of the conductivity and the permittivity. Besides, the electrical properties of the excised tissues differ from those of the living tissues because of the lack of blood irrigation. However, in the case of breast tumors, these

differences are considered negligible within the first 30 minutes after tissue removal since the passive electrical properties of the cells remain stable during this time (Heinitz and Minet, 1995).

In vivo measurements are less common but provide more reliable results. Morimoto et al. (1990) used a coaxial needle electrode inserted into the breast to estimate the bioimpedance of breast tumors based on the three-electrodes method in the range of 0–200 kHz. The impedance (Z), the extracellular (R_e) and intracellular (R_i) resistances, and the membrane capacitance (C_m) were obtained. R_e and R_i of malignant tumors were considerably higher than benign tumors, while C_m was considerably lower. Regarding C_m, these results are opposite to those obtained by Singh et al. (1979), where higher electrical permittivity values were obtained in breast tumors in the range of 0.1 Hz – 1 kHz. The discrepancy between these two studies can be explained by the fact that in the study of Singh et al. (1979), the measurements were made with ECG electrodes attached to the skin of the breast, so the effects of polarization at the skin interface could affect the values of C_m. In addition, the measurements were not made directly on the breast tumor.

Bioimpedance has also been used to detect skin cancer based on the estimation of four different indexes, which have been used as indicators of the electrical impedance in the complex space (Emtestam et al., 1998). These indexes not only can be used to detect basal cell carcinoma (BCC), but also allergic reactions, irritation, and hydration of the skin (Beetner et al., 2003). Such indexes are described by Equations 2.4–2.7:

$$\text{MIX} = \frac{abs(Z_{20\text{ kHz}})}{abs(Z_{500\text{ kHz}})} \quad \text{Magnitude Index} \tag{2.4}$$

$$\text{PIX} = \arg(Z_{20\text{ kHz}}) - \arg(Z_{500\text{ kHz}}) \quad \text{Phase Index} \tag{2.5}$$

$$\text{RIX} = \frac{\text{Re}(Z_{20\text{ kHz}})}{abs(Z_{500\text{ kHz}})} \quad \text{Real part Index} \tag{2.6}$$

$$\text{IMIX} = \frac{\text{Im}(Z_{20\text{ kHz}})}{abs(Z_{500\text{ kHz}})} \quad \text{Imaginary part Index} \tag{2.7}$$

Intact skin yields a straight-line plot spectrum between 10 kHz and 1 MHz; however, 20 kHz and 500 kHz were considered sufficient to characterize most of the information in this frequency range (Nicander et al., 1996). The most significant difference found between BCC and normal skin is manifested in the MIX and IMIX values (Beetner et al., 2003; Emtestam et al., 1998), where both indexes were lower for BCC. Distinction between nonmelanoma skin

cancer and benign nevi by electrical bioimpedance is also feasible. Aberg et al. (2004) demonstrated the possibility of differentiating skin cancer from benign nevi with 75% of specificity in the range of 1 kHz – 1 MHz. However, the reasons why the impedance values are different have not been investigated, although it is believed to be due to the histopathological difference between both tissues.

In the case of prostate tissue, significant differences in permittivity have been observed between prostate cancer (CaP) and benign prostatic hyperplasia (BPH). Halter et al. (2009) observed a significantly higher permittivity in CaP at 100 kHz, which is attributed to the variability of the glandular and cellular content. The mean conductivity of CaP tissue was slightly higher than that of BPH between 0.1 kHz and 100 kHz, and significantly lower than that of non-hyperplastic glandular tissue and Stroma, specifically at 0.1 kHz. This is attributed to the fact that the extracellular space has been reduced by the proliferation of cancer cells causing neoplastic cells to infiltrate the Stroma (Halter et al., 2008).

Bioimpedance measurements not only detect the differences in electrical properties between malignant and benign tissues but have also shown their usefulness in estimating the stage of cancer. Qiao et al. (2010) have shown that the relaxation frequency increases as the stage of cancer advances. A possible hypothesis that can explain this, is based on the fact that the capacitance in cancer cells is lower than in normal cells, and this value decreases as cancer progresses (Han et al., 2007). Therefore, this makes the tissue less polarizable, causing the relaxation frequency to increase (Gregory et al., 2012).

2.1.3 Electrodes and probe

In biological tissues, ions are the charge carriers, giving rise to ionic currents. In wires and electronic circuits, the charge carriers are electrons, giving rise to electronic currents. In that sense, there must be an interface that allows the interaction between the biological tissues and the electronic instrumentation. The electrodes allow the exchange of charge carriers because they transform ionic currents into electronic currents and vice versa. It is a transduction function, and it can be carried out with or without galvanic contact. Although it is not the intention of this chapter to discuss the electrode theory in depth, it is important to understand the fundamentals of the electrode transduction mechanism.

When a metal comes into contact with an electrolyte, double layers are formed near the electrode surface where electric charges of opposite polarity are distributed across the interface, acting as a capacitor (Rigaud et al., 1996). Depending on the rates of diffusion of ions into and out of the metal, an equilibrium is reached that gives rise to a half-cell potential. The value of

this potential depends on the material of the electrode, the ionic concentration in the electrolyte, the temperature, and other second-order factors (Webster, 2009), and it is produced under conditions of no current flowing through the interface. When a current crosses the interface, the half-cell potential is altered. If such alteration is considerable, the electrode is considered polarizable. If such alteration is negligible, then the electrode is considered non-polarizable (Grimnes and Martinsen, 2015). The former acts as a capacitor and does not allow charge exchange at the metal-electrolyte interface. In this kind of electrode, no DC currents are available, unless a high dc voltage is applied. The latter acts as a resistor and it does allow an unhindered transfer of charges between the metal and the electrolyte. For applications that involve the injection of AC signals, platinum electrodes are often used as they have a lower polarization impedance than electrodes made of silver and stainless steel (Rigaud et al., 1996). According to Ivorra (2003), electrodes made of noble metals (platinum, gold) or stainless steel are used in bioimpedance measurements to avoid tissue damage or electrode degradation. In Electrical Impedance Spectroscopy (EIS), when using polarizable electrodes, the effect of the double layer can be notorious at low frequencies (< 10 kHz). To compensate for this effect, different approaches have been proposed (Ishai et al., 2013).

Cancer detection by electrical impedance can be carried out by *in vivo* measurements, which can be invasive, minimally invasive, or non-invasive. *In vitro* measurements can also be performed, which are carried out on a piece of excised tissue, on a single cell, or on cells in suspension. When measuring in tissues (*in vivo* or *ex vivo*), it is common to use arrangements made up of multiple electrodes to cover a larger measurement area of the tissue and, in some cases, to be able to reconstruct images from impedance values of the tissues involved. By performing multiplexing between the different injection electrodes and the voltage detection electrodes it is possible to perform tetrapolar measurements. Bioimpedance measurements through the surface of the skin involve several factors that can mask impedance measurements, e.g., skin conditions and movement artifacts. To avoid this, invasive *in vivo* measurements are preferable, where needle electrodes that penetrate the tissue are used.

For *ex vivo* measurements, stainless steel needles with an exposed pair of electrodes have been proposed. In this way, it is possible to measure the electrical properties of tissues simultaneously with the extraction of the tissue (Mishra et al., 2013). For this, an 18-gauge stainless steel needle has been used. It consists of two concentric elements separated by 54 μm of air. Both elements are insulated by a polyimide coating adhered to the inner element, allowing only two electrodes to be exposed for tissue contact. With this probe, measurements are made using a bipolar configuration.

For the detection of skin cancer by EIS, many studies have used the spectrometer manufactured by SciBase AB (Hudinge, Sweden). This system initially used a probe made up of four concentric circular gold electrodes deposited on a ceramic. The inner electrode is a sink electrode, and the next electrode is a guard, helping to reduce the effects of surface currents. The two outermost electrodes are the source electrodes (Beetner et al., 2003). Between these two electrodes, a virtual electrode is created which can be "moved" by changing the distribution of current between the source electrodes (Aberg et al., 2004). This probe allows measurement at five different depths by varying the position of the virtual electrode. A recent model changed the probe configuration to a square array made up of five disposable electrodes covered with gold microinvasive pins. Similarly, four levels of depth can be achieved by alternating between the different electrode bars (Braun et al., 2017).

Cancer detection by electrical bioimpedance can also be performed at cellular level. One way to do this is by using microelectrodes to measure the impedance of a single cell. Another way is by measuring impedance on cells in suspension, which minimizes errors such as variability in the size and shape of each cell, polarization effects of the electrodes, and heating effects from high current density (Guofeng Qiao et al., 2012). To measure cells in suspension, chambers have been proposed that allow controlling factors such as temperature, volume, and cell distribution within the chamber.

2.1.4 Instrumentation

To extract the electrical properties of tissues and/or cells, the response to a certain stimulus containing a specific range of frequencies must be measured, hence the term EIS. The stimulus can be a step function, electronic noise, a sinusoidal signal, or a chirp signal, among others (Bertemes-Filho, 2018; Xu and Hong, 2020). There are different techniques based on the number of electrodes used to apply the stimulus and measure the response (Figure 2.1a). The bipolar (2 electrodes), the tripolar (3 electrodes), and the tetrapolar techniques (4 electrodes) have been used. Each of these techniques has its advantages and disadvantages, which have been well described in the literature (Grimnes and Martinsen, 2015; Grossi and Riccò, 2017; Kassanos, 2021; Morimoto et al., 1990).

In EIS, the most common technique is tetrapolar since it reduces the effects of the contact impedance of the electrodes and the effects of polarization. In this technique there are four electrodes connected to the tissue/cell (Figure 2.1b): two electrodes (Z_{HC}, Z_{LC}) inject current (or voltage) and the other two electrodes (Z_{HP}, Z_{LP}) measure the response to that current (or voltage). Each block of the injection and the detection systems must have

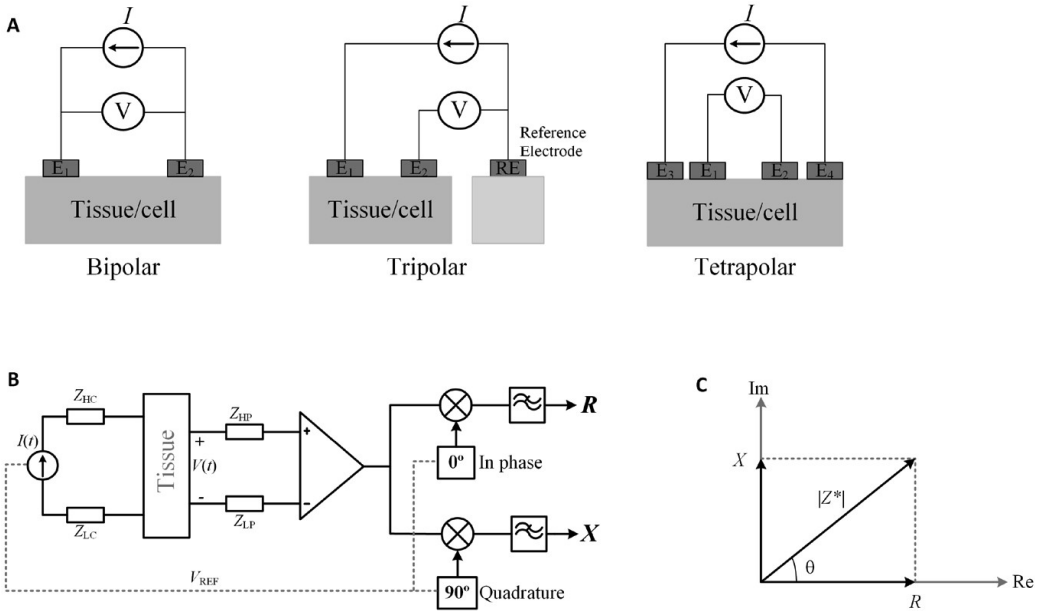

Figure 2.1. (A) Different techniques based on the number of electrodes used in Bioimpedance measurements, (B) Block diagram of the instrumentation involved in Bioimpedance measurements, (C) Phasor diagram where the real and imaginary parts of Z^* are depicted.

certain characteristics to reduce the errors that could cause misinterpretations of the impedance measurement. Briefly:

Oscillator: It is responsible for generating the waveform. In EIS applied to cancer detection, it is necessary to be able to generate a wave (usually sinusoidal) in a frequency range between 100 Hz and 1 MHz. For this, the oscillator must have high linearity (low THD[1]) over a wide frequency range, which affects the precision of the EIS system. There are oscillators implemented with off-the-shelf components and embedded on a chip.

Current source: The signal coming from the oscillator is an alternating voltage. To transform that voltage into a current that is injected into the target tissue/cell, a circuit known as a V-I converter is required, specifically a Voltage Controlled Current Source (VCCS). This circuit must have a high input impedance (Z_i), to reduce the loading effects when connecting it to the oscillator. It must also have a high output impedance (Z_S) to reduce drifts in the injected current when the biological material is connected to the VCCS output. However, the values of Z_i and Z_S are frequency dependent, so it is a challenge to keep the values of these impedances high in multifrequency applications.

[1] Total Harmonic Distortion: is a measurement of the harmonic content of a signal that indicates the level of waveform distortion.

Front-end: This stage is responsible for measuring the voltage response due to the current injected into the tissue. $V(t)$ is a signal modulated in amplitude by the impedance (Z_X) of the tissue. The amplitude level of this signal is between 100 μV and 100 mV (Bertemes-Filho, 2018), so it is susceptible to errors from common mode signals, electrode contact impedance noise, and movement artifacts.

Demodulator: The measurement system must be able to extract the real part and the imaginary part of Z^*. The electrical impedance of the tissue not only modulates the amplitude (AM) of the injected current but also causes a phase shift (PM). Synchronous demodulation is a technique that has proven to be robust in bioimpedance measurements. It offers a high dynamic range and rejects non-synchronous signals. For this, the AM and PM signal is multiplied by a reference wave that is synchronous with the injected current, but can be in-phase (0º) or in quadrature (90º) (Figure 2.1b) with respect to the reference wave. The measurements in-phase (I) allow to extract the real part of Z^*, and the measurements in quadrature (Q) allow to extract the imaginary part of Z^*. From these components, it is possible to estimate the magnitude and phase of Z^* (Figure 2.1c). However, any mismatch between the IQ channels must be avoided to reduce phase errors (Yang et al., 2006).

2.2 Bioimpedance applications

In 1959, the Nobel laureate Richard Feynman, pronounce an emblematic speech… "There's Plenty of Room at the Bottom"… in that time, Feynman wanted to encourage his colleagues to think about the possibility of developing very small devices at the micro and nano scales, for instance, he mentioned the possibility of storing the British encyclopedia on the head of a pin, even more, he mentioned that there are no physical reasons that make it impossible to store information at the molecular level. It seems that Feynman's message was not fully understood by the scientific community till the last two decades. Now, several researchers worldwide work on the design and implementation of micro-devices at micro and nanoscale dimensions, and micro and nano biosensors are the current challenges in lab-on-a-chip development.

2.2.1 Circulating tumoral cells detection (CTC)

Breast cancer (BC) is an important health problem worldwide. In general, BC is the cause of high levels of mortality due to the absence of early identification, lack of response to therapy, and metastasis. Through several investigations in which the process of metastasis has been studied, the possibility to detect the presence of Circulating Tumoral Cells (CTC) exist, which could be a relevant signal of dissemination of the disease, prognosis, and response to treatment

(Samia et al., 2012) (Morán et al., 2014) (Giuliano et al., 2014). Metastasis causes high morbidity/mortality associated with BC, and 30% of the patients with BC develop micro-metastases in connective tissue. Approximately 50% of women with localized BC, treated with surgery show high recurrence and poor outcomes by oncological treatment, such as radiotherapy and chemotherapy, often ineffective because the status of CTCs in the body is not well known (Bray et al., 2018) and (Sua et al., 2011).

Currently, several techniques for the detection of CTC based on biological and physical properties have been proposed, since their detection might represent a non-invasive technique to monitor the evolution of cancer patients. However, the amount of CTC in peripheral blood is very low compared to the total blood cells (Markou et al., 2011). So, improvements in these technologies are continually being sought, which require expensive equipment and specialized processes, which require emerging technological proposals that may represent CTC detection alternatives that can be used in low-income healthcare levels. An ideal solution should be a method with very high sensitivity, good reproducibility, intuitive, easy to use, as well as practical implementation in the clinical field. Research focused on new techniques to deter these drawbacks to achieve low-cost and easily accessible technology is still necessary (Sua et al., 2011) (Adams et al., 2015).

In a study reported by Xu et al. (2011), the feasibility of a technique to perform the separation of CTC from whole blood by immunomagnetic techniques is shown. Han et al. (2007) showed the feasibility of differentiating tumor cells from normal ones through the characterization of their electrical properties in a study (Han et al. 2007). CTC detection through EIS measurements assisted with magnetic nanoparticles (MnP) bound to an antibody (Ab) to recognize proteins in the cell surface has been proposed (Silva et al., 2014). The main idea is to add nanoprobes "Magnetic Nanoparticle-Antibody" (MnP-Ab) on the CTC membrane and then, through a microfluidic system, separate immunomagnetically by the effect of a permanent magnet on the surface of a microelectrode array, thus perform EIS measurements for detection.

This subsection presents the advances in the development of a new CTC biosensor based on EIS measurements. Different types of cancer cells commonly used in the study of BC (MCF-7, MDA-MB-231 and SK-BR-3) were employed as potential targets for the use of an MnP-Ab nanoprobe. The three cell lines were quantified and incubated with the corresponding doses of bioconjugate Mnp-Ab according to their predominant surface protein (Huerta-Nuñez et al., 2019). Immunomagnetic isolation of the cells and subsequent measurement by multifrequency electrical bioimpedance was developed by the use of a ScioSpec-ISX3 impedance measurement system. The biosensor designed for isolation/detection of BC cells by the use of a microfluidic system and magnetic nanoparticle-assisted with EIS measurements is technically

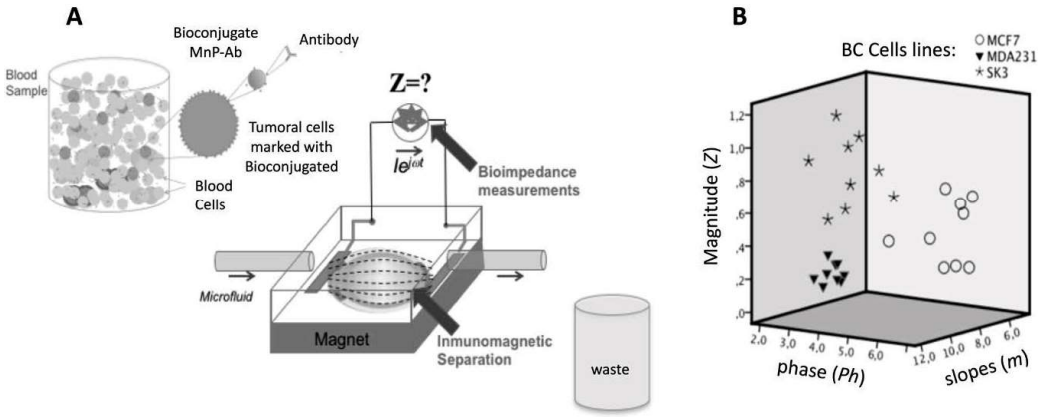

Figure 2.2. (A) Schematic representation of CTC detection by EIS measurements assisted with bioconjugated MnP-Ab. A microfluid system allows immunomagnetic anchoring by the use of a permanent magnet. (B) Three-dimensional representation of principal components for detection and classification of different types of BC cell lines.

viable to detect low concentrations of cancer cells and magnetic nanoparticles (Huerta-Nuñez et al., 2019).

The EIS magnitude parameter at low frequencies seems to represent the best option to get relevant sensitivity. A main factors analysis allows for identifying sensitive components as representative elements of the EIS spectra; thus, components for Magnitude (Z), phase (Ph) and slopes (m) were selected as a three-dimensional representation for cell lines characterization. The results indicate that the detection and classification of CTC through the proposed technique is feasible and allows supporting the operation principle of a biosensor for detection of CTC based on EIS measurements assisted with nanotechnology (González-Díaz and Golberg, 2020). Figure 2.2 shows the schematic representation of CTCs detection by EIS measurements assisted with MnP-Ab, as well as a three-dimensional representation of the main factor for classification of BC cell lines.

2.2.2 Breast cancer detection by EIS

BC is an uncontrolled malignant proliferation. In the search for diagnostic methods for this disease, it has been found that bioimpedance measurements of tumor tissues reveal important data. The commonly accepted concept of electrical bioimpedance is the opposition that a body presents to the passage of an alternating current through it, where the body is a biological tissue.

The structure of the tissues has to be considered since it affects the bioimpedance and the electrical properties of biological tissue change according to the frequency of the current; at high frequencies, the conductivity of the tissue increases until it becomes constant at frequencies from

10 to 100 MHz. The opposite is observed with the permittivity, which decreases at higher frequencies (Schwan, 1957).

Currently, the use of nanomaterials for the diagnosis of numerous diseases is somewhat feasible. These materials also work as drug carriers for the treatment of some pathologies and have also been used coupled with antibodies to have greater specificity when releasing the drug. Magnetic nanoparticles coupled to an antibody that recognize an overexpressed antigen as a receptor in breast cancer cells could help in the diagnosis/treatment of the disease, modifying the cellular structure and increasing its conductivity through nanomaterials.

The infusion of "magnetic nanoparticle - anti Her2" (MnP-Ab) inside of a biological organism and its physiological effect in tumor tissue through electrical bioimpedance measurements at different frequencies has been evaluated to show changes in tissue electrical conductivity. BC was induced in rats of the Sprague Dawley strain with 1-Methyl 1-Nistrosurea (MNU) intraperitoneally (30 mg/kg), according to the model reported by Thompson et al. (1983). Five rats with tumor development were anesthetized with acepromazine (300 mg/100 ml), butorphanol (10 mg/50 ml), and ketamine (1000 mg/10 ml), and a central venous catheter was placed by jugular venous dissection for systemic MnP-Ab infusion. The "magnetic nanoparticle–anti Her-2" bioconjugate was made according to a previous report (Silva et al., 2015). An impedance analyzer (Agilent: mod. 4294 A) was configured to perform impedance measurements in a broad range of 100 Hz to 100 MHz at 181 logarithmically spaced steps in three conditions: Basal (before MnP-Ab infusion), $t = 0$ hrs (immediately after MnP-Ab infusion), and $t = 24$ hrs (24 hours post- MnP-Ab infusion). The placement of a magnet in the tumor region was used in order to attract the magnetic nanoparticles to the region of interest. In all cases, bioimpedance measurements were normalized concerning their contralateral value in healthy tissue. The systemic injection of MNP-Ab that recognizes an antigen in cancer cells promotes a lower bioimpedance of cancer tissue at low frequencies and 24 hours post-infusion; such a condition allowed higher tissue electrical conductivity values. Specific descriptions of the findings could be found in a study reported by Silva et al. (2015).

2.2.3 Breast cancer detection by MIS

In general, limitations in the treatment of BC are associated with a late diagnosis. The scarcity or non availability of modern imaging systems at the primary level of medical attention promotes the risk of BC in women candidates since early detection opportunities in the early stages of the disease by screening studies were lost and it became impossible to continuously monitor the therapeutic response. Currently, no portable, non-invasive and

low-cost technology has the potential to assist in early diagnosis, as well as give timely alarms in remote places that do not have modern imaging systems.

Hyper-vascularization commonly found in malignance represents important changes in the tissue's electrical properties. Such a condition suggests the use of the bioimpedance technique in order to detect cancer. Hutten et al. (1998) reported the use of EIS to produce relevant bioimpedance parameters associated with abnormal conditions. Newell et al. (1996) and Holder et al. (1999) proposed the Tomography through Electrical Impedance (EIT) for monitoring neoplasms in different organs/tissues. EIT depends on an adequate electrode-skin galvanic coupling, and this is frequently affected by sweating or skin hydration levels, thus it represents the main disadvantage. On the other hand, Griffiths et al. (1999) and Griffiths (2001) have shown the technical feasibility of using mono-frequency magnetic fields through non-contact coils for monitoring the status of organs and/or tissues. Al-Zeiback (1993) and Korzhenevskii (1997 and 1999), proposed the use of Magnetic Induction Tomography (MIT) to produce contactless bioimpedance imaging. The potential use of EIT and MIT to assist the diagnosis of BC is a feasible technique because the conductivity in a malignant tissue is different with respect to a healthy condition. Burdette (1982), reported that normal and BC tumors have different conductivities, for instance, 4 $mS \cdot cm^{-1}$ and 8 $mS \cdot cm^{-1}$, respectively.

Bioelectrical measurements through magnetic induction at multiple frequencies have been proposed as a relevant technique for non-contact detection of neoplasms in breast tissues. An inductor-sensor system to produce low intensity magnetic fields at non ionizing frequencies, adapted in an ergonomic form to the human breast shape was designed; the prototype works based on the Magnetic Induction Spectroscopy (MIS) technique.

The induction of currents in conductive materials through oscillating magnetic fields (of a certain frequency) is explained by the Faraday's law of induction (alternating magnetic fields induce electric potentials in conductive mediums). This potential in turn induces a flow of electric currents (Eddy currents) inside the medium proportional to its conductivity and the electric potential induced, so the greater the conductivity of the material, the greater the energy that the material must absorb. It has been documented that the presence of neoplasms in breast tissue increases its volumetric conductivity (Kim et al., 2007), so the influence of magnetic fields of a certain frequency in a volume of normal breast tissue should induce a lower amount of Eddy currents than in those breast tissues with mammographic findings.

A volume of breast tissue placed between an inductor coil and a sensor coil was considered as a case study (see Figure 2.3). An alternating current

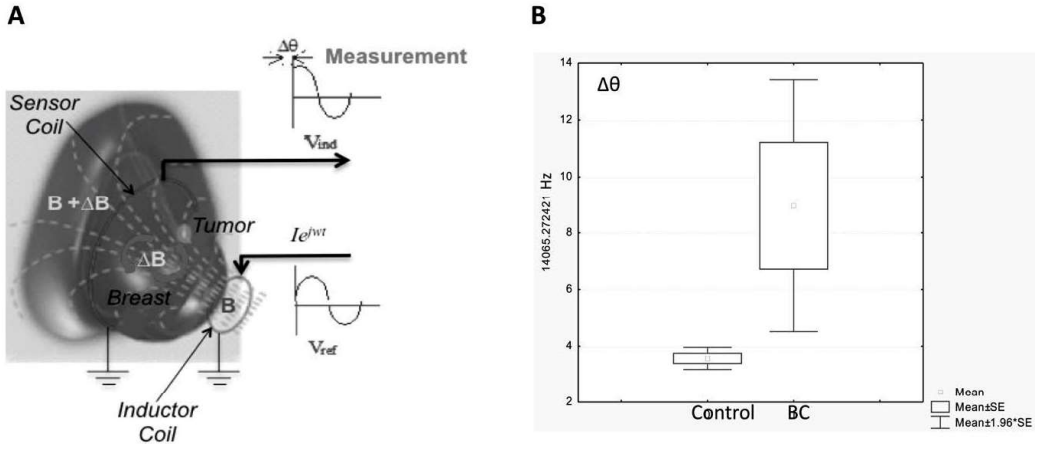

Figure 2.3. (A) Biophysical concept for non-invasive detection of malignant neoplasms in breast tissue by MIS measurements. (B) Inductive phase shift (Δθ) at a specific frequency for Control and BC groups (mean values and dispersion).

is injected into the inductor coil, and it induces a magnetic field **B** in the second coil (sensor). The phase of **B** is tied to the phase of the voltage (V_{ref}) in the field coil itself as a reference. The bulk of the tissue is considered non-magnetic with conductive properties. The induced current in the breast volume under study causes a modification Δ**B** in the initial **B** (Griffiths et al., 1999). The final result is a composite field **B** + Δ**B** and it is detected in the secondary coil out of phase with respect to **B** by an angle θ (González-Díaz et al., 2017). Thus, the phase shift ($\Delta\theta$) between V_{ref} and the voltage induced in the secondary coil (V_{ind}), can be calculated by Equation 2.8:

$$\Delta\theta = \theta(V_{ind}) - (V_{ref}) \tag{2.8}$$

The presence of neoplasia modifies the electrical properties of the breast volume, thus the conductivity increases (Martinsen and Grimnes, 2011) (González-Díaz et al., 2017). It means that conductivity increase promotes that the influence of a magnetic field is reflected as a selective induction of currents in the neoplastic region, which in turn causes a greater disturbance of **B** and consequent increase in Δ**B**, thus volumetric changes in $\Delta\theta$ are detected.

Women who were assisted with mastography screening were enrolled as volunteer patients separated into control and BC groups. All volunteers were explored by the MIS technique to determine multifrequency $\Delta\theta$ in breast tissue in the bandwidth of 0.001 to 100 MHz. In addition, patients were auscultated by experienced radiologists through conventional mastography. Statistical comparison of the two independent groups (Control vs BC) shows significant differences. Specific details regarding experimental design are described in (González-Díaz et al., 2017).

The observations indicate that inductive $\Delta\theta$ increments in the β-dispersion region might correlate with differences in morphological patterns such as cell structure (Martinsen and Grimnes, 2011). Inductive $\Delta\theta$ increments could be used to detect tissue abnormalities such as BC (González-Díaz et al., 2017).

In conclusion, it seems necessary to search for new low-cost technologies and easy access molecular biomarker detection methods. Biosensors of different biological analytes designed based on bioimpedance measurements have been increased as an important emerging inexpensive technology; such developments require specialized knowledge of instrumentation for designing bioimpedance meters and constraints.

BC detection by EIS measurements assisted with nanotechnology as well as MIS exploration is an important approach for designing innovative diagnostic tools. Also, bioimpedance micro-devices integrated with different measuring techniques seem to be complementarily linked, thus, a combination of different techniques assisted with bioimpedance measurements seems to be the future key in biosensor designs.

Acronym/Abbreviation

AC	Alternating Current
AM	Amplitude Modulation
BC	Breast Cancer
BCC	Basal Cell Carcinoma
BPH	Benign Prostatic Hyperplasia
CaP	Prostate Cancer
CTC	Circulating Tumoral Cells
DC	Direct Current
ECG	Electrocardiography
EIS	Electrical Impedance Spectroscopy
IMIX	Imaginary part Index
MIS	Magnetic Induction Spectroscopy
MIX	Magnitude Index
PIX	Phase Index
PM	Phase Modulation
RIX	Real part Index

Reference list

Aberg, P., Nicander, I., Hansson, J., Geladi, P., Holmgren, U., Ollmar, S. et al. (2004). Skin cancer identification using multifrequency electrical impedance-a potential screening tool. *IEEE Transactions on Biomedical Engineering*, 51(12): 2097–2102. https://doi.org/10.1109/TBME.2004.836523.

Adams, D.L., Stefansson, S., Haudenschild, C., Martin, S.S., Charpentier, M. et al. (2015). Cytometric characterization of circulating tumor cells captured by microfiltration and their correlation to the cell search VR CTC test. *Cytometry Part A*, 87A: 137–144. https://doi.org/10.1002/cyto.a.22613.

Al-Zeiback. (1993). A feasibility study of *in vivo* electromagnetic imaging. *Physics in Medicine & Biology*, 38: 151–160. https://doi.org/.

Beetner, D.G., Kapoor, S., Manjunath, S., Zhou, X., Stoecker, W.V. et al. (2003). Differentiation among basal cell carcinoma, benign lesions, and normal skin using electric impedance. *IEEE Transactions on Biomedical Engineering*, 50(8): 1020–1025. https://doi.org/10.1109/TBME.2003.814534.

Bertemes-Filho, P. (2018). Electrical impedance spectroscopy. *Bioimpedance in Biomedical Applications and Research*, pp. 5–27. Springer.

Blad, B. and Baldetorp, B. (1996). Impedance spectra of tumour tissue in comparison with normal tissue; a possible clinical application for electrical impedance tomography. *Physiological measurement*, 17(4A): A105. https://doi.org/10.1088/0967-3334/17/4A/015.

Braun, R.P., Mangana, J., Goldinger, S., French, L., Dummer, R. et al. (2017). Electrical impedance spectroscopy in skin cancer diagnosis. *Dermatologic Clinics*, 35(4): 489–493. https://doi.org/10.1016/j.det.2017.06.009.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. et al. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68(6): 394–424. https://doi.org/10.3322/caac.21492.

Burdette, E.C. (1982), Electromagnetic and acoustic properties of tissues. pp. 105–150. *In*: Nussbaum, G.H. (ed.). *Physical Aspects of Hyperthermia*, *AAPM Medical Physics Monographs*, 8. https://doi.org/.

Emtestam, L., Nicander, I., Stenström, M. and Ollmar, S. (1998). Electrical impedance of nodular basal cell carcinoma: A pilot study. *Dermatology*, 197(4): 313–316. https://doi.org/10.1159/000018023.

Giuliano, M., Giordano, A., Jackson, S., De Giorgi, H. and Mego, M. (2014). Circulating tumor cells as early predictors of metastatic spread in breast cancer patients with limited metastatic dissemination. *Breast Cancer Research*, 16: 440. https://doi.org/10.1186/s13058-014-0440-8.

González-Díaz, C.A., Uscanga-Carmona, M.C., Lozano-Trenado, L.M., Ortíz, J.L., González, J.A. et al. (2017). Clinical evaluation of inductive spectrometer to detect breast cancer. *In*: Torres, I., Bustamante, J. and Sierra, D. (eds.). *VII Latin American Congress on Biomedical Engineering CLAIB 2016, Bucaramanga, Santander, Colombia, October 26th–28th, 2016. IFMBE Proceedings*, vol 60. Springer, Singapore. https://doi.org/10.1007/978-981-10-4086-3_170.

González-Díaz, C.A. and Golberg, A. (2020). Sensitivity analysis of electrical bioimpedance patterns of breast cancer cells labeled with magnetic nanoparticles: Forming the foundation for a biosensor of circulating tumor cells. *Physiological Measurement*, 41(6): 064001. https://doi.org/10.1088/1361-6579/ab9377.

Gregory, W., Marx, J., Gregory, C., Mikkelson, W., Tjoe, J. et al. (2012). The Cole relaxation frequency as a parameter to identify cancer in breast tissue. *Medical Physics*, 39(7Part1): 4167–4174. https://doi.org/10.1118/1.4725172.

Griffiths, H., Stewart, W.R. and Gough, W. (1999). Magnetic induction tomography - A measuring system for biological materials. *Annals of the New York Academy of Sciences*, 873: 335–345. https://doi.org/10.1111/j.1749-6632.1999.tb09481.x.

Griffiths, H. (2001). Magnetic induction tomography. *Measurement Science and Technology*, 12: 1126–31. https://doi.org/10.1088/0957-0233/12/8/319.

Grimnes, S. and Martinsen, O.G. (2015). *Bioimpedance and Bioelectricity Basics* (3rd. ed.). Academic Press.

Grossi, M. and Riccò, B. (2017). Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: A review. *Journal of Sensors and Sensor Systems*, 6(2): 303–325. https://doi.org/10.5194/jsss-6-303-2017.

Haemmerich, D., Staelin, S.T., Tsai, J.-Z., Tungjitkusolmun, S., Mahvi, D.M. et al. (2003). *In vivo* electrical conductivity of hepatic tumours. *Physiological Measurement*, 24(2): 251. https://doi.org/10.1088/0967-3334/24/2/302.

Halter, R.J., Schned, A., Heaney, J., Hartov, A., Schutz, S. et al. (2008). Electrical impedance spectroscopy of benign and malignant prostatic tissues. *The Journal of Urology*, 179(4): 1580–1586. https://doi.org/10.1016/j.juro.2007.11.043.

Halter, R.J., Schned, A., Heaney, J., Hartov, A., Paulsen, K.D. et al. (2009). Electrical properties of prostatic tissues: I. Single frequency admittivity properties. *The Journal of Urology*, 182(4): 1600–1607. https://doi.org/10.1016/j.juro.2009.06.007.

Han, A., Yang, L. and Frazier, A.B. (2007). Quantification of the heterogeneity in breast cancer cell lines using whole-cell impedance spectroscopy. *Clinical Cancer Research*, 13(1): 139–143. https://doi.org/10.1158/1078-0432.CCR-06-1346.

Heinitz, J. and Minet, O. (1995). Dielectric Properties of Female Breast Tumors. *In Ninth International Conference on Electrical Bio-Impedance*, pp. 356–359. Heidelberg.

Holder, D.S., Gonzalez-Correa, C.A., Tidswell, T., Gibson, A., Gusick, G. et al. (1999). Assessment and calibration of a low-frequency system for Electrical Impedance Tomography (EIT), optimized for use in imaging brain function in ambulant human subjects. *Annals of the New York Academy of Sciences*, 873: 512–519. https://doi.org/10.1111/j.1749-6632.1999.tb09500.x.

Huerta-Nuñez, L.F.E., Gutierrez-Iglesias, G., Martinez-Cuazitl, A., Mata-Miranda, M.M., Alvarez-Jiménez, V.D. et al. (2019). A biosensor capable of identifying low quantities of breast cancer cells by electrical impedance spectroscopy. *Scientific Reports*, 9: 6419. https://doi.org/10.1038/s41598-019-42776-9.

Hutten, H., Scharfetter, H., Ninaus, W., Puswald, B., Petrova, G.I. et al. (1998). Inductively coupled wideband transceiver for bioimpedance spectroscopy (IBIS). *Proceedings of the 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 20(4): 1791–1794. https://doi.org/10.1109/IEMBS.1998.7469.

Ishai, P.B., Talary, M.S., Caduff, A., Levy, E., Feldman, Y. et al. (2013). Electrode polarization in dielectric measurements: A review. *Measurement Science and Technology*, 24(10): 102001. https://doi.org/10.1088/0957-0233/24/10/102001.

Ivorra, A. (2003). Bioimpedance monitoring for physicians: An overview. *Centre Nacional de Microelectrònica Biomedical Applications Group*, 11(17).

Jossinet, J. (1998). The impedivity of freshly excised human breast tissue. *Physiological Measurement*, 19(1): 61. https://doi.org/10.1088/0967-3334/19/1/006.

Kassanos, P. (2021). Bioimpedance sensors: A tutorial. *IEEE Sensors Journal*, 21(20): 22190–22219. https://doi.org/ 10.1109/JSEN.2021.3110283.

Kim, B.S., Isaacson, D., Xia, H., Kao, T.-J., Newell, J.C. et al. (2007). A method for analyzing electrical impedance spectroscopy data from breast cancer patients. *Physiological Measurements*, 28: S237. https://doi.org/10.1088/0967-3334/28/7/S17.

Korjenevsky, A.V. and Cherepenin, A. (1999). Progress in realization of magnetic induction tomography. *Annals of the New York Academy of Sciences*, 873: 346–352. https://doi.org/10.1111/j.1749-6632.1999.tb09482.x.

Korzhenevskii, A.V. and Cherepenin, A. (1997). Magnetic induction tomography. *Journal of Communications Technology and Electronics*, 42(4): 469–474.

López-Carrillo, L., Torres-Sánchez, L., Blanco-Muñoz, J., Hernández-Ramírez, R.U. and Knaul, F.M. et al. (2014). Utilización correcta de las técnicas de detección de cáncer de mama en mujeres mexicanas. *Salud Pública de México*, 56(5): 538–546.

Malich, A., Boehm, T., Facius, M., Freesmeyer, M., Azhari, T. et al. (2002). Electrical impedance scanning of lymph nodes: Initial clinical and technical findings. *Clinical Radiology*, 57(7): 579–586. https://doi.org/10.1053/crad.2001.0927.

Markou, A., Strati, A., Malamos, N., Georgoulias, V., Lianidou, E.S. et al. (2011). Molecular characterization of circulating tumor cells in breast cancer by a liquid bead array hybridization assay. *Clinical Chemistry*, 57(3): 421–430. DOI: https://doi.org/10.1373/clinchem.2010.154328.

Mishra, V., Schned, A., Hartov, A., Heaney, J., Seigne, J. et al. (2013). Electrical property sensing biopsy needle for prostate cancer detection. *The Prostate*, 73(15): 1603–1613. https://doi.org/10.1002/pros.22695.

Morán, M.E., Rodríguez, G.J., Lara, L.M., Piña, L.C., Thompson, B.M.R. et al. (2014). Células tumorales circulantes en cáncer de mama: un posible riesgo biológico. *Revista de Especialidades Médico-Quirúrgicas*, 19(1): 45–51.

Morimoto, T., Kinouchi, Y., Iritani, T., Kimura, S., Konishi, Y. et al. (1990). Measurement of the electrical bio-impedance of breast tumors. *European Surgical Research*, 22(2): 86–92. https://doi.org/10.1159/000129087.

Newell, J.C., Edic, P.M., Ren, X., Larson-Wiseman, J., Danyleiko, M. et al. (1996). Assessment of acute pulmonary edema in dogs by electrical impedance imaging. *IEEE Transactions on Biomedical Engineering*, 43: 133–138. https://doi.org/10.1109/10.481982.

Nicander, I., Ollmar, S., Eek, A., Rozell, B.L., Emtestam, L. et al. (1996). Correlation of impedance response patterns to histological findings in irritant skin reactions induced by various surfactants. *British Journal of Dermatology*, 134(2): 221–228. https://doi.org/10.1111/j.1365-2133.1996.tb07605.x.

Pethig, R. (1984). Dielectric properties of biological materials: Biophysical and medical applications. *IEEE Transactions on Electrical Insulation*, (5): 453–474. https://doi.org/10.1109/TEI.1984.298769.

Qiao, G., Duan, W., Chatwin, C., Sinclair, A., Wang, W. et al. (2010). Electrical properties of breast cancer cells from impedance measurement of cell suspensions. *Journal of Physics: Conference Series*. https://doi.org/10.1088/1742-6596/224/1/01208.

Qiao, G., Wang, W., Duan, W., Zheng, F., Sinclair, A.J. et al. (2012). Bioimpedance analysis for the characterization of breast cancer cells in suspension. *IEEE Transactions on Biomedical Engineering*, 59(8): 2321–2329. https://doi.org/10.1109/TBME.2012.2202904.

Rigaud, B., Morucci, J.-P. and Chauveau, N. (1996). Bioelectrical impedance techniques in medicine part I: Bioimpedance measurement second section: impedance spectrometry. *Critical Reviews™ in Biomedical Engineering*, 24(4-6). https://doi.org/10.1615/CritRevBiomedEng.v24.i4-6.20.

Samia A. Ebeed, Nadia A. Abd El-Moneim, Ahmed Saad, Ebtsam R.E. Zaher et al. (2012). Diagnostic and prognostic value of circulating tumor cells in female breast cancer

patients. *Alexandria Journal of Medicine*, 48(3): 197–206. https://doi.org/10.1016/j.ajme.2012.02.005.

Sarode, G.S., Sarode, S.C., Kulkarni, M., Karmarkar, S., Patil, S. et al. (2016). Role of bioimpedance in cancer detection: A brief review. *International Journal of Dental Science and Research*, 3(1): 15–21. https://doi.org/10.1016/j.ijdsr.2015.11.003.

Scholz, B. and Anderson, R. (2000). On electrical impedance scanning-principles and simulations. *Electromedica*, 68(1): 35–44.

Schwan, H.P. (1957). Electrical properties of tissue and cell suspensions. *Advances in Biological and Medical Physics*, 5: 147–209. https://doi.org/10.1016/B978-1-4832-3111-2.50008-0.

Silva, J.G., Cárdenas, R.A., Quiróz, A.R., Sánchez, V., Lozano, L.M. et al. (2014). Impedance spectroscopy assisted by magnetic nanoparticles as a potential biosensor principle for breast cancer cells in suspension. *Physiological Measurement*, 35(6): 931–941. https://doi.org/10.1088/0967-3334/35/6/931.

Silva, J.G., López, J., Sánchez, V., Lozano, L.M., González, C.A. et al. (2015). Breast cancer tissue marked selectively by magnetic nanoparticles in an experimental animal model. *Journal of Nanoscience and Nanotechnology*, 15: 1–6. https://doi.org/10.1166/jnn.2015.10610.

Singh, B., Smith, C. and Hughes, R. (1979). *In vivo* dielectric spectrometer. *Medical and Biological Engineering and Computing*, 17(1): 45–60. https://doi.org/10.1007/BF02440953.

Sua, L.F., Silva, N.M., Vidaurreta, M., María, L., Maestro, M. et al. (2011). Detección inmunomagnética de células tumorales circulantes en cáncer de mama metastásico: nuevas tecnologías. *Revista Colombiana de Cancerología*; 15(2): 104–109. https://doi.org/10.1016/S0123-9015(11)70073-7.

Surowiec, A.J., Stuchly, S.S., Barr, J.R. and Swarup, A. (1988). Dielectric properties of breast carcinoma and the surrounding tissues. *IEEE Transactions on Biomedical Engineering*, 35(4): 257–263. https://doi.org/10.1109/10.1374.

Thompson, H.J. and Meeker, L.D. (1983). Induction of mammary gland carcinomas by the subcutaneous injection of 1-methyl-1-nitrosourea. *Cancer Research*, 43: 1628–1629. https://doi.org/10.1016/0304-3835(95)03761-K.

Webster, J.G. (1990). *Electrical Impedance Tomography*. CRC Press.

Webster, J.G. (2009). *Medical Instrumentation: Application and Design*. John Wiley & Sons.

Xu, J. and Hong, Z. (2020). Low power bio-impedance sensor interfaces: Review and electronics design methodology. *IEEE Reviews in Biomedical Engineering*, 15: 23–25. https://doi.org/10.1109/RBME.2020.3041053.

Xu, H., Aguilar, Z.P., Yang, L., Kuang, M., Duan, H. et al. (2011). Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood. *Biomaterials*, 32(36): 9758–9765. DOI: https://doi.org/10.1016/j.biomaterials.2011.08.076.

Yang, Y., Wang, J., Yu, G., Niu, F., He, P. et al. (2006). Design and preliminary evaluation of a portable device for the measurement of bioimpedance spectroscopy. *Physiological Measurement*, 27(12): 1293. https://doi.org/10.1088/0967-3334/27/12/004.