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Review Article: Modern Trends in Imaging XI

Impedance measurements in the biomedical sciences

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Abstract. Biological organisms and their component organs, tissues and cells have unique electrical impedance properties. Impedance properties often change with changes in structure, composition, and metabolism, and can be indicative of the onset and progression of disease states. Over the past 100 years, instruments and analytical methods have been developed to measure the impedance properties of biological specimens and to utilize these measurements in both clinical and basic science settings. This chapter will review the applications of impedance measurements in the biomedical sciences, from whole body analysis to impedance measurements of single cells and cell monolayers, and how cellular impedance measuring instruments can now be used in high throughput screening applications.

Keywords: Bioimpedance, body mass index, tumor detection, cell-based assays, apoptosis, high throughput screening

1. Introduction

Electrical impedance is defined as the opposition to an electrical current within a circuit. In systems utilizing direct current, the impedance is simply the resistance, but in systems utilizing alternating currents, the changing electric and magnetic fields create additional and varying opposition to the applied current. Since the initial measurements of electrical impedance by Ohm in the early 19th century, the conductive properties of nearly every conceivable substance has been tested. The ability to collect impedance data from electrode arrays and calculate three-dimensional conductivity maps from that data has led to the development of extremely useful applications in many different branches of science and industry. Impedance values can be measured through layers of soil and rock, and the resulting maps can track plumes of underground contaminants, detect leaks in underground storage tanks, monitor the effectiveness of subsurface barriers, and track moisture movement in rock layers. In industrial processes, impedance measurements are used to visualize multi-component flows within pipes and mixing chambers, and can monitor mixing processes that involve combining two different phases (liquid/gas or solid/liquid), or that combine two liquids of differing viscosity, or that cause precipitation, or the extraction of a compound from one phase to another, or any multi-component process where at least two components differ significantly in electrical conductance [1].

The impedance properties of biological tissues have been studied for over 100 years, and papers describing impedance studies of different disease states have been published since 1901 [2, 3]. Currently, at least one organization, the International Society for Electrical Bio-Impedance (ISEBI), and one journal, the Journal of Electrical Bioimpedance, were created specifically to promote the biological applications of impedance measurements. Different biological tissues and biomaterials have a wide range of impedance properties, and impedance measurements of these tissues, both *in vitro* and *in vivo*, have lead to the development of numerous useful biomedical applications. Impedance measurements are routinely used to determine body composition and body mass index (BMI), monitor cardiac output and lung function, check the placement and effectiveness of probes and catheters, and scan

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organs and tissues for the presence of tumors (this latter approach is also used to determine the fat content of commercially sold meat and thus assist in the grading process). More recently impedance measurements have been adapted to formats amenable to the analysis of individual cells or small cell populations, specifically as add-ons to flow cytometry or Coulter counter instruments, and in multi-well formats to examine impedance characteristics of cell monolayers. These instruments are currently being utilized in high throughput screening approaches for drugs and bioactive agents in both academic and industrial settings, as well as for real time data collection in assays monitoring cellular activities such as death, activation, differentiation, migration and invasiveness. In this chapter we will review the various biological applications of impedance studies both *in vivo* and *in vitro*, and discuss the potential utility of *in vitro* studies as probes of the functional as well as molecular properties of pathologic cells and tissues.

2. Basic principles

In simple systems utilizing direct current and a homogeneous conductor, the opposition to the current supplied by the circuit is the resistance, which is constant and due to the structure and composition of the circuit material interfering with electron flow. In such systems the relationship between the current, potential and resistance is expressed by Ohm's Law:

$$V = IR \tag{1}$$

Where:
V is the potential difference
I is the current
R is the resistance

In systems utilizing alternating current, which is a sinusoidal signal, the opposition to a current is the impedance, which takes into account both the magnitude of the opposition to the applied current and the phase shifts between the current and voltage caused by the components of the circuit. Mathematically, impedance is composed of two components, the resistance, which is a real component, and the reactance, which is an imaginary component. The reactance may be operationally defined as opposition to current due to the contributions of both capacitance and inductance, which are not constant but shift according to electromagnetic frequency and the properties of the circuit. Capacitance is the ability of a material (in this case a cell, cell component or tissue) to store charge, and inductance is the creation of local potential differences by changes in the current passing through. The complex and variable nature of capacitance and inductance in biological systems contribute significantly to the unique aspects of impedance profiles for each cell and tissue, and thus to the utility of impedance as a tool for probing cell and tissue properties.

Impedance may be expressed as a complex number Z:

$$Z = R + iX \tag{2}$$

Where:
Z is the impedance
R is the resistance
i is the square root of -1
X is the reactance

The magnitude of the impedance, |Z|, is the ratio of the magnitudes of the voltage and current (V/I). The phase difference between the voltage and current due to reactance is θ (in radians), and the following equations may be derived which tie together impedance amplitudes and phase differences [4]:

$$\begin{aligned} &|Z| = (R^2 + X^2) \\ &R = |Z| \cos\theta \text{ and } X = |Z| \sin\theta \end{aligned} \tag{3}$$

The reciprocal of impedance is admittance, which is the ease of which a circuit allows current to flow:

$$Y = G + j\omega C \tag{4}$$

Where:
Y is the admittance
G is the conductance
i is the square root of -1
ω is the angular frequency
C is the capacitance

Biological systems are highly heterogeneous and contain numerous components that are not good conductors but can hold a charge and thus contribute to reactance. Humans and other higher multicellular organisms are composed of many different organs and tissues. The organs and tissues are composed of cell layers surrounded by aqueous fluids and supporting

tissues. The cells themselves contain aqueous fluids, proteins, nucleic acids and membranes, and are surrounded by a phospholipid bilayer. The aqueous fluids both within and outside of cells contain ions and have intrinsic resistance but are otherwise conductive. The cell membranes are phospholipid bilayers that also contain transmembrane proteins, sphingolipids and cholesterol, and at lower frequencies are very poor electrical conductors, however their composition gives them significant capacitance as well as significant resistance. The electrical properties of these heterogeneous biological tissues vary with the frequency of the applied field, thus different frequencies produce different impedance values from the same biological sample. The electric dispersions in biological tissues in response to applied electric fields have been separated into three regions defined by frequency: alpha dispersion is seen at low frequencies (10 Hz–10 kHz) and is believed to be caused by the ionic environment immediately surrounding cells; beta dispersion occurs in midrange frequencies (10 kHz–10 MHz) and is due to capacitive charging of cell membranes; gamma dispersion occurs at higher frequencies (10 MHz–10 GHz) and is due to dielectric relaxation of water molecules [5, 6]. At the highest frequencies the current can pass through cell membranes and the impedance values are reflective of molecules both inside and outside of cells, while at lower frequencies the current cannot penetrate the relatively nonconductive phospholipid bilayers and the impedance values reflect molecules and structures outside of the cell membranes. The instruments that collect impedance data from human subjects often collect over a wide range of frequencies due to the complex composition of tissue samples and the physical area needed to be scanned, while the instruments which collect impedance data from cell monolayers usually collect data at a few predetermined optimal midrange frequencies (the xCELLigence instrument collects data at 10, 25, and 50 kHz).

While collecting and displaying impedance values as a function of time or frequency is relatively straightforward, the process of recovering impedance information and then reconstructing a three dimensional image from that information is mathematically complex. The initial approach to defining the problem of image reconstruction was formulated by Calderon [7], and several numerical algorithms for this problem have been devised since [8, 9]. One of the first combinations of equipment and algorithms that produced images suitable for clinical interpretation was a 64 electrode array that collected data which was processed using a matrix inversion algorithm [10].

3. *In vivo* measurements

3.1. Overview

The term bioimpedance describes the response of a living organism to an externally applied electric current, and is a measure of the opposition to the flow of that electric current through the various tissues. Biological organisms are composed of many different organs and tissue types, and different tissues have different impedance properties. Graphs displaying both permittivity and conductivity data from multiple published studies have been assembled for blood, bone, fat, grey matter, white matter, kidney, spleen, heart, liver, lung, muscle and skin, over a very large range of frequencies (from 10–10^{10}) [11]. Changes in these parameters do not change in a linear fashion with frequency; for example, the conductivity of blood changes very little over the low and midrange frequency ranges of 1,000–1,000,000,000 Hz, but increases over 100-fold within the high frequency range as the frequency increases from 1,000,000,000 to 100,000,000,000 Hz. At any one given frequency, the conductivity and permittivity of one tissue type can be very similar to that of another tissue type, or up to several orders of magnitude different. Within a single biological tissue, the electrical impedance also varies depending on temperature and other physiological factors; for example, lungs are measurably less conductive when the alveoli are filled with air, the conductivity of bone samples is different if the bone samples are dry or wet, and the conductance properties of many tissues change somewhat with age [12].

Bioimpedance measurements are obtained using both invasive and noninvasive approaches. Invasive approaches involve inserting electrodes (needles) into tissues, applying a current, and measuring the impedance between pairs of electrodes. Noninvasive approaches involve attaching a series of surface electrodes to the skin, and then impedance is measured either between successive pairs of electrodes as with the invasive approaches, or current is applied and measured simultaneously at all electrodes and whole body patterns are measured. The success of noninvasive methods depends upon having constant, good contact between all electrodes and the skin. The use of a high

conductivity gel can ensure optimal electrical contact between the electrodes and the skin, but risks potential external field coupling. Electrode contact with dry skin avoids this potential problem, but electrode contact with dry skin is dependent upon the pressure applied to the electrodes, thus a good adhesive is necessary.

Electrical impedance tomography (EIT) is a noninvasive technique that re-creates images of the internal impedance distribution from surface electrical measurements [1]. Electrodes are attached to the skin of the subject in predetermined patterns and small alternating currents, usually 1–10 mA at a frequency of 10–100 kHz, are applied across two or more electrodes. The resulting electrical potentials are measured at the other electrodes, and the process is then repeated multiple times, with different combinations of electrodes supplying the current with the resulting different patterns of electrodes reading the potential differences. The currents used are small enough so they will not cause local neural stimulation, Ohmic heating or other biologic effects that could affect the experimental outcome. Many EIT systems are capable of working at several frequencies and can measure both the magnitude and phase of the voltage. The current may be from a single source and switched between electrodes, or from multiple sources, one for each electrode. Likewise, the measurements may be taken either by a single voltage measurement circuit covering all electrodes or a separate circuit for each electrode. The voltage values are then utilized to reconstruct and display an image [13–17].

Bioimpedance measurements have been used to monitor a number of physiological properties and processes. It is a commonly used method to measure body composition and hydration, and thus can be used as part of routine heath evaluations as well as following patient nutritional status and hydration status in chronic or progressive disease states. Impedance measurements are used to monitor cardiac and lung function, gastroesophageal reflux, gastric emptying and pharyngeal transit time [18], and have been used to examine brain tissues for evidence of strokes or epileptic foci [19]. As tumor cells generally have lower impedance values than surrounding normal tissues, impedance measurements of several different tissue types have been used as an adjunct to other scanning procedures to localize tumors. As impedance-based tumor detection depends upon the size of the tumor, the depth of the tumor from electrodes, and the relative difference in conductivity between the tumor and its surroundings, the primary applications of impedance in cancer detection have been in detecting cancers of the skin and breast, where tumors are localized within relatively small areas that are close to the skin and amenable to proximal electrode placement [19].

3.2. Body composition

One of the more common applications of bioimpedance measurements is to determine body composition and body mass index (BMI) [20, 21]. Commercial impedance instruments have been available to measure body composition since 1990. Impedance measurements of body composition are often referred to as bioelectrical impedance analysis (BIA). To date, BIA is one of the most widely used applications of biological impedance measurements, and is the basis of a number of commercially available body composition analysis instruments. These measurements have been utilized in a number of medical applications, including assessing the body composition of normal patients and monitoring the nutritional status in a variety of disease states, including a number of cancers and HIV [22–24]. BIA in combination with BIVA (bioelectrical impedance vector analysis) has been used to monitor COPD patients for nutritional analysis and water retention [25]. BIVA and B-type natriuretic peptide measurements have been tested as a way to rapidly and noninvasively assess fluid status in patients arriving at emergency rooms. As fluid retention increases with disease progression in cardiac failure and end stage renal disease, BIA has been used to monitor disease progression through monitoring fluid buildup in these heart and kidney patients [26].

3.3. Cardiovascular system

Bioimpedance measurements have been used as a non-invasive method for measuring cardiac output and circulating blood volume. Bioimpedance measurements can detect electrical changes occurring with altering fluid levels in the thorax [27]. Levels change as the left ventricular contracts and blood flows into the thoracic aorta. This causes a corresponding change in resistance within the thorax because the fluid level in the aorta increases. This change in impedance can be measured as a change in voltage passing between electrodes placed on a patient's chest. Measurements

across the chest cavity must deal with several sources of possible artifact, including the variances of electrical conductivity with breathing, however this technique is used in both routine clinical medicine and research. Some approaches have examined the bioreactance component of bioimpedance in analysis of cardiac function, following the changes in phase of the electrical current traversing the chest cavity, which become larger in proportion to larger cardiac stroke volume. Bioimpedance approaches are sufficiently established that insurance companies such as Aetna reimburse bioimpedance-based cardiac monitoring for a number of disease states and diagnoses, including differentiation of cardiogenic from pulmonary causes of acute dyspnea, evaluation for rejection in persons with a heart transplant (in place of a myocardial biopsy), monitoring response to medication changes in treatment of drug-resistant hypertension, optimization of atrio-ventricular interval for patients with pacemakers, optimization of fluid management in persons with congestive heart failure, and outpatient monitoring of continuous inotropic therapy for persons with terminal congestive heart failure.

3.4. Skin

Bioimpedance approaches have been used to analyze skin lesions, particularly to differentiate between skin cancers and benign lesions [28]. As electric conductance is affected by aspects of cells that are altered with carcinogenic transformation, including cell shape, cell membrane composition, cell structure, cell interactions with surrounding cells and matrix tissues, and the cell water content, impedance values of cancer cells differ from those of normal tissues and benign lesions. Typically, measurements of potentially cancerous skin lesions are taken at both the center of the lesion and at a normal reference skin site. Both skin loci are measured at multiple depths, approximately 0.1 to 2 mm into the tissue. Surface bioimpedance measurements have a high sensitivity for carcinoma *in situ* and thin melanomas, and using electrodes that penetrate a small distance into the skin increases diagnostic specificity. However, the electrical impedance properties of human skin vary significantly with anatomic location, patient age and gender, and even the season, thus more studies are needed to achieve a better level of standardization [29]. One relatively recent application of impedance spectroscopy is to monitor drug transport across an intact skin barrier using transdermal iontophoresis. This noninvasive technique facilitates drug transport through the skin by the use of an external electrical field, has expanded the scope of drugs that can be delivered transdermally and enables programmable drug delivery [30].

3.5. Lung

EIT is useful for monitoring patient lung function because the air has a large conductivity difference from the solid tissues of the lungs and chest [31]. Perhaps the most useful application of EIT is the monitoring patients during mechanical ventilation, as the technique can provide a continuous, noninvasive image of the lungs and thus the distribution of the ventilation [32]. As these patients are at risk for ventilator-associated lung injury, EIT can continuously monitor the distribution of air within all lung regions as the ventilation parameters are adjusted, thus giving the clinician instant feedback and allowing optimal ventilation for each patient while protecting the lung against damage [33]. Apart from the functional EIT studies focused on regional lung function, absolute EIT (a-EIT) approaches also have the potential to become a clinically useful, as this approach could allow the clinician to distinguish between lung conditions which result from regions with lower resistivity such as pleural effusion, hemothorax, and edema from those which result from regions with higher resistivity such as emphysema. The reconstruction of clinically useful a-EIT images from lung impedance data requires that the dimensions and shape of the area scanned as well as the precise location of the electrodes be taken into account, as simplified assumptions could lead to significant artifacts being introduced.

3.6. Breast

The composition and structure of the breast make it an ideal organ for impedance study. EIT is being investigated in the field of breast imaging as both a complementary and an alternative technique to mammography and magnetic resonance imaging (MRI) for breast cancer detection. The lower specificity of mammography [34] and of MRI [35] results in a significant number of false positives, resulting in temporary but great distress for the patient and the additional costs of followup procedures. The ionizing radiation used for mammography and the nephrotoxicity of the breast

MRI contrast agent gadolinium [36] make the development of alternative techniques highly desirable. Differences in the electrical conductive properties of normal breast tissues and breast tumors were first found in the 1920 s [37], and numerous studies since have firmly established impedance differences between normal and malignant breast tissues [5, 38–42], which has fueled efforts to develop impedance-based instruments for breast cancer monitoring and detection.

One successful commercial development of non-tomographic electrical impedance imaging is the T-Scan device (Assenheimer 2001), which has been demonstrated to improve sensitivity and specificity when used as an adjunct to screening mammography. The T-scan device consists of a hand-held probe that scans the breast for electrical conductivity, sending two-dimensional images of breast tissue to a computer screen. Because breast cancer cells usually have lower electrical impedance values than normal breast tissues, when an electric current is flowing through the area, the region of lower impedance would attract more current lines that the surrounding tissues and have an enhanced current density that causing them to appear different from normal cells (bright white on the screen). This is useful to evaluate potential tumors that have been previously detected by mammography. A report to the United States Food and Drug Administration (FDA) describes a study involving 504 subjects where the sensitivity of mammography alone was 82%, the sensitivity for the T-Scan alone was 62%, and the sensitivity for both methods combined was 88%. The specificity for breast tumors, however, favored the impedance approach: the specificity for mammography alone was 39%, the specificity for the T-Scan alone was 47%, and the specificity for both combined was 51%. According to the National Cancer Institute (NCI), electrical impedence imaging may reduce the number of biopsies needed to determine whether a breast mass is cancerous and also improve the identification of women for whom a biopsy is appropriate. The use of these kinds of impedance measurements to detect tumors is being expanded into other tissues; a recent study reports that cancers of the tongue have also been identified by impedance scanning techniques (Sun et al. 2010).

3.7. Brain

EIT has been utilized for brain imaging to enable the detection and monitoring of cerebral ischemia and hemorrhage, and epileptic foci localization, and progress has been made in impedance monitoring of normal brain function and neuronal activity [19], (Rosenblum 2007). The primary use of impedance brain scanning to date has been to monitor regions of abnormal fluid buildup that accompany edema and brain swelling, similar to applications in other anatomical regions. In more experimental brain imaging applications, EIT depends upon applying very low frequency currents above the skull that are less than 100 Hz. During neuronal rest, currents in this frequency range remain in the extracellular space and cannot pass through the cell membrane into the intracellular space within neurons. When a neuron depolarizes or creates an action potential, the open transmembrane channels allow some current to enter and reduce the membrane resistance by a factor of 80. When this occurs across large numbers of neurons, the change in total resistivity is about 1%, which is measureable and provides a signal for detecting coherent neuronal activity across large numbers of neurons and potentially the imaging of neural activity in the brain. Unfortunately while such changes are currently detectable, they are too small for current imaging technologies, and the use of this technique for imaging will depend upon improved signal recording and processing (Gilad 2009).

4. Impedance measurements of cells *in vitro*

4.1. Overview

The large scale extension of impedance scanning to individual cells and to cell monolayers has been a relatively recent event, dependent upon the development of commercially available instruments designed specifically for that purpose. Because parameters that affect cellular impedance change during a number of cellular processes, including activation, differentiation, and apoptosis, these instruments have been utilized in a large number of applications in basic and pharmaceutical research. Instruments examining cell suspensions share design principles with Coulter counters and flow cytometers, and measure impedance of individual cells as they flow in a line through a detection cell. Instruments examining cell monolayers utilize microwells with an electrode array on the bottom surface of the wells, and impedance data is collected in real time as the cell monolayers adhere, proliferate, and respond to any added biological effector.

4.2. Cell suspension instruments

Impedance measurements of individual cells are performed using flow type systems, in which individual cells passing single file through a capillary pass through an electric current and thus perturb an electric field. This technique has been available for over half a century (Cheung 2010; Mittag 2011), although generally it has been far overshadowed by fluorescence-based flow cytometry approaches. Using impedance cytometry approaches, different cell populations can be identified by their different bioelectrical properties, which are reflective of cell size, cell shape, and cell composition (Chapman 2011, Pierzchalski 2011). It has been used to discriminate lymphocytes from monocytes and neutrophils, distinguish normal from modified erythrocytes, follow monocyte and adipocyte differentiation, and monitor Jurkat cell apoptosis (Cheung 2010). The advantages of this label free approach is that cell populations can be tested rapidly without time-consuming labeling steps, and the cells are not affected by the labeling process, as can occur with some antibodies which can act as receptor agonists.

The disadvantage of the label-free approach is the inability to differentiate cell subsets as routinely occurs in fluorescence-based flow cytometry, due to the absence of impedance-modifying analogs of fluorescent labeled antibodies. Lymphocytes could be resolved from monocytes and neutrophils, but CD4+ and CD8+ T cells remained indistinguishable. A recent study utilized a novel approach towards solving this problem, and mixed latex beads coated with anti-CD4 antibodies with patient blood samples to bind CD4+ T cells. The bound latex beads shifted the size and dielectric properties of the T cell conjugates sufficiently to enable CD4+ T cells to be counted separately and as accurately as using traditional flow cytometry methods (Holmes 2010). The success of this approach has significant implications in two areas. First, this represents a potentially significant healthcare advance for HIV treatment in poor or rural areas. Fluorescence-based flow cytometry has not penetrated many of the poorer areas of the world affected by HIV, particularly sub-Saharan Africa. Impedance-based cytometry instruments are much less expensive than fluorescence-based instruments and utilize reagents that are less expensive and can be kept in storage in rural clinics far longer than fluorescent-labeled antibodies. Thus this approach represents a method by which physicians in rural clinics can monitor disease progress in their HIV patients. Second, it establishes a method of impedance labeling for cell subpopulations so that impedance cytometry could perform some of the same type of experiments now being performed on fluorescence-based flow cytometry. This impedance cytometry approach is currently limited to one label, where fluorescence based cytometry systems routinely follow 4-5 different labels simultaneously, and the more advanced systems can track over ten individual fluorescent labels in one sample.

4.3. Cell monolayer instruments

Within the past decade, commercially available instrumentation has been developed to perform impedance analysis *in vitro* on cell monolayers, and how their impedance properties change in real time following the addition of bioactive agents. Among the more popular impedance instruments are the xCELLigence system (Roche), the ECIS system (Applied Biophysics), and the CellKey system (MDS Analytical Technologies). These instruments all have similar designs: specially prepared tissue culture wells of either the 96 well or 384 well format contain electrode arrays partially covering the well bottom, and following the addition of cells, impedance data is collected in real time by measuring the opposition to current flowing between the electrodes created by the cells. As data collection begins immediately following cell addition, the complete data set contains impedance profiles of the cells as they attach to the well bottom, assume their normal morphology, begin to proliferate, and react to whatever agent is being tested, which is usually added 24 hours after cell seeding to allow full recovery from cell passage (Irelan 2011).

A critically important aspect of these instruments is that they measure an integrative property of the cells. Measuring an integrative property means that one can observe a cell response without having to know (or correctly guess) the mechanism of response. An experimenter need not rely on changes in a single marker, of which a good candidate may not be known prior to the experiment. One can add an effector and see a change in real time, a change that has biological relevance, which creates a starting point for exploring the mechanism of the observed effect. For many types of assays, in which ligands are added that target a specific cell surface receptor, validation that the effect seen was due to the added ligand binding to

that particular receptor is relatively straightforward using specific agonists and antagonists. Effects that are not easily linked to one particular cellular component, or which appear to be mediated by multiple mechanisms, can be more difficult to understand, however the instrument does provide a platform in which multiple follow up experiments may be performed in a short period of time to pinpoint the major contributors to the observed impedance changes. The ability to follow complex mechanisms can be especially valuable, as in cases where impedance instruments were used to follow endothelial to mesenchymal transitions (Asphahani 2007). For some applications, elucidating the mechanism of impedance change is unimportant as long as there is a measurable change that is linked statistically to some cellular property, such as metastatic potential. These relationships need not be universal even within cell types – as with tumor markers, even the best tumor markers do not appear on 100% of a particular tumor cell type, but they are an important component of an overall diagnosis.

The types of events these instruments have been used to monitor have expanded significantly over the past several years (McGuiness 2007)(Ke 2011). A classic application is to measure cell death due to a variety of mechanisms, as impedance drops off as the dying cells loosen and dissociate from the electrode array on the well bottom. Not only have these instruments been used to measure cytotoxicity due to added drugs and cytokines (Li 2008; Hanusova 2010, Sharma 2011), but they have been used to measure cell death in diverse experimental systems such as antibody-dependent cell cytotoxicity (ADCC) (Yamashita-Kashima 2011), NK killing of astrocytes (Moodley 2011) and cell death in xenotransplantation models (Quereda 2010; Ramis 2011). Impedance values increase as cell number increases, so these instruments have been used to measure cell proliferation as well as cytostasis (Cai 2011; Li 2011). Perhaps the most valuable applications have been in monitoring complex cell activities such as activation, differentiation, atrophy, cell composition changes, cell migration, and changes in cell morphology and cell size that alter impedance readings (Abassi 2004; Abassi 2009; Dad'o 2009; Keogh 2010; Rakhilin 2011; Jiang 2011; Slania 2011; Hong 2011). This has been especially valuable in screening drugs and other small molecules (Ke 2010; Xi 2011; Stander 2011).

Impedance measurements have also been used to monitor endothelial cell junctions (Pannekoek 2011), measure cell migration and invasion of cell monolayers (Ungefroren 2011; Rahim 2011), track the expression of transfected genes (Raabe 2008), monitor cell responses to PAR2 activating peptides (Boitano 2011) and cell survival in response to bioactive materials released from acrylic (Ozturk 2011). Impedance measurements are not limited to human and animal cells, as one recent report described using this technique to screen helminthes for their response to panels of drugs (Smout 2010).

One great advantage to these instruments is that they collect data in real time. Since data is collected continuously, the experimenter does not have to estimate (or guess) when optimal times for sample collection will occur, as must happen in assays were one of a series of replicate samples is processed at a predetermined time point. In many assays, especially cytotoxicity assays, a single time point is used (usually a multiple of 24 hours), thus potentially missing important occurrences. In a single end point cytotoxicity assay, a cellular alteration that moves the onset of cell death beyond the assay end point will be interpreted as a change in sensitivity to the cytotoxic agent, when actually it is a shift in reaction kinetics. So long as the data is collected over a sufficient time period (which is very easy to do using these instruments), real time data collection will always collect the key time points, no matter when they occur. In addition, the same wells are measured throughout the experiment, as opposed to assays where replicate wells are harvested at predetermined time points. Since the data from each well is collected and stored separately from data from other wells throughout the experiment, any anomalies in one well remain with that well throughout the experiment (and serves as a kind of internal control). Data from any well that deviates in a statistically significant manner from the others throughout the time course can be removed.

In our own preliminary studies, we have monitored both intrinsic impedance time courses and impedance time courses following the addition of cytokines to neoplastic breast cell lines. The impedance time course of the breast cancer cell line MCF-7 with and without the addition of tumor necrosis factor alpha (Fig. 1) demonstrates the classic loss of impedance associated with apoptosis. The impedance time course of the normal breast stromal cell line MCF-12A with and without the addition of IL-10 (Fig. 2) demonstrates a cytokine-mediated increase in impedance that is due to growth enhancement, structural changes that diminish electrical conductivity, or a combination of both. The system could be used to explore the nature of the

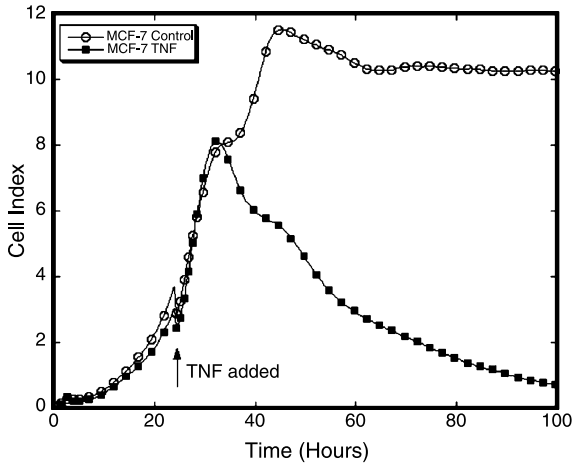

Fig. 1. MCF-7 human breast cancer cell line given 2.5 nM TNF-alpha 24 hours after seeding the cells in the E-plate. After 7–8 hours, the TNF-treated cells begin to undergo apoptosis, round up, and eventually detach from the plate, causing the loss in impedance.

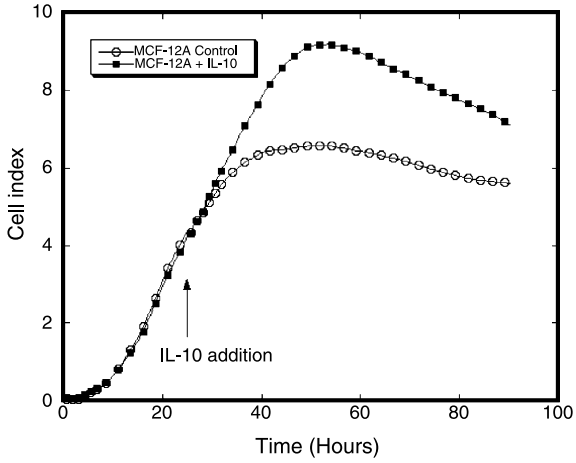

Fig. 2. MCF-12A human breast epithelial cell line given IL-10 24 hours after seeding in the E-Plate. The IL-10-treated cells show enhanced impedance compared to untreated cells beginning 6–8 hours after IL-10 addition and continuing for the duration of the experiment (65 hours).

impedance change, or if this was the result of a screening assay, the response alone might be sufficient for the purposes of the assay. The multiple well format allows high throughput screening, thus a large number of compounds can be tested in real time for their effects on specific cells and processes. Specialized systems have been constructed in which confluent cell monolayers are subject to tumor cell invasion and the loss of impedance as areas of the monolayer are lifted from the electrodes by the invading tumor cells. While these instruments are designed to measure impedance across adherent cell monolayers, impedance signals can also be obtained from nonadherent cell lines such as MOLT-4 T cell leukemia and U937 monocytic cell lines. However, the impedance values for nonadherent cells are over an order of magnitude smaller than those for adherent cells; fortunately because they contact a much smaller surface area than adherent cells, many more nonadherent cells can be used in a well than nonadherent cells.

One common use for these instruments in pharmaceutical research is screening for compounds that interact with specific G protein coupled receptors. Impedance measurements can not only detect changes that accompany binding, but can differentiate between ligands that bind to the same receptor but activate different signaling pathways (Kammermann 2011). The G protein-coupled receptor GPR109A is a receptor for niacin and is known to activate two pathways, the classic Gi pathway resulting in inhibition of adenylate cyclase and lowering of cellular cAMP, and the activation of ß-arrestin, which activates the ERK pathway. Both pathways alter cellular metabolism and induce rearrangements of the cytoskeleton, however ß-arrestin causes changes in cell morphology that are detectable by impedance profiling separate and distinct from the adenylate cyclase pathway. Thus the complexity of the impedance signal allowed, in this case, drugs to be screened not only for receptor binding, but by which signaling pathway they activated after binding.

A unique application has been developed to screen for potential cardiotoxic effects. A substantial number of developmental drugs are eventually withdrawn due to cardiac side effects. Unfortunately, these side effects are often not detected until late in the clinical development process, when tens of millions of dollars have been spent in clinical trials. Finding evidence of these side effects in the preclinical stage of drug development has been difficult, because the *in vitro* assays currently employed can only assess cellular properties such as cytotoxicity and proliferation, and cannot assess the effect of compounds on the coordinated electrically stimulated contractions that are necessary for cardiac function. The xCELLigence RTCA Cardio instrument utilizes murine cardiomyocytes that have been derived from murine embryonic stem cells and display a spontaneous beating phenotype nearly identical to cardiomyocytes *in vivo* (Abassi 2011, Xi 2011). Profiles of over 60 compounds were reported, including

modulators of ion channels and channel-linked receptors, hERG trafficking inhibitors, and drugs that had been withdrawn from the market due to cardiotoxicity. The results demonstrate that this system can identify compounds that can produce arrhythmias accurately and in some cases identify arrhythmia-inducing compounds missed by electrophysiological approaches.

While most studies using the instruments are performed using cell lines, results of impedance analysis of patient prostate biopsy samples showed promise as well. Prostate cancer cells are graded (Gleason score) in part by cellular structure and morphology. The electrical impedance properties of 546 prostate samples were taken and compared with clinical diagnosis. Of these biopsy samples, 71 were defined as cancerous (cancer cells comprising more than 50% of the area), and higher grade cancers had higher mean electrical properties (Halter 2011).

4.4. Practical considerations of in vitro impedance measurements

There are a number of practical considerations for those using instruments such as xCELLigence or ECIS that examine the impedance properties of cultured cells within small wells, typically the same size as those in a standard 96 well plate. First, one must choose an appropriate number of cells to be seeded into each well at the initiation of an experiment, and that number depends on the cell type, the cell size and surface contact area, the cell proliferation rate, and the design and length of the experiment. Some experiments are designed to begin with proliferating cells that are not in close contact, while others are designed to begin with a confluent monolayer. The initial number of cells must be sufficient to produce a reasonable initial impedance signal. If changes in cell proliferation are expected, the number of cells must be low enough so that at least one cell doubling can be achieved before a confluent monolayer is formed and contact inhibition halts cell proliferation (this is less of a problem in cancer cell lines that continue proliferation despite reaching confluence, but the change in impedance with increasing cell number when the cells grow on top of each other differs from the change in impedance when the cells proliferate under subconfluent conditions, and data interpretation under the latter conditions is not straightforward). Beginning an experiment with very low cell densities avoids this issue, but the impedance signals are very low. Additionally, cells that rely on autocrine growth factors will grow very slowly at low density because there will be too few cells to make sufficient quantities of the required growth factors; this condition will change once sufficient numbers of cells are produced and the required growth factors are made at the required levels. The loss of media nutrients, the lowering of the media pH, and the potential onset of autophagy become more significant factors the longer the experiment runs, and experiments are usually designed to conclude before these events occur. If cytotoxicity is an expected result, the initial cell number must be calibrated so that the impedance signal is significant when cell death commences, thus the signal loss due to cell detachment accompanying cytotoxicity will be optimal. In the authors experience using adherent mammalian cell lines from a variety of tissue sources, seeding numbers of 5,000–15,000 cells per well provide reasonable impedance signals and unique, reproducible time courses for adherent cells in most types of experiments. When setting up the experiment, it is critically important that the cells are in a single cell suspension (or as close to it as possible) when added to the wells, to insure uniformity of signal from well to well. This can be quite difficult when using particular adherent cell lines that form multicellular aggregates during normal passage, due to tight cell-cell interactions or the secretion of extracellular matrix-like components. In the authors experience, when harvesting some cell lines prior to seeding in xCELLigence plates, doubling the normal amount of trypsin, using significantly higher concentrations of EDTA (up to 30 mM), incubating the cells with trypsin/EDTA for longer periods of time at 37°C, and periodic mechanical disruption by repeated pipetting have been necessary to create optimal cell suspensions. When preparing such a cell line for an impedance time course experiment, it is critical to periodically monitor cell detachment and cell clumping using a microscope during the trypsinization process, in order to assess when optimal cell separation has occurred.

5. Summary and conclusions

Impedance measurements are currently used in both clinical and research applications. The main clinical applications have been in body composition analysis and monitoring lung and heart function, while

impedance instruments designed to discover or assess tumors have primarily been used to support primary finding by other scanning technologies such as MRI or mammography. Within the last 5 years, impedance instruments designed for use with cell monolayers have become available and have already become important tools for screening certain classes of pharmacologically active agents. These instruments have been used to study a variety of cellular events, including a system that examines cultures of cardiomyocytes that produce regular repeating electrical signals that can be used to identify potentially cardiotoxic activity in compounds being screened. The value of collecting data in real time and in utilizing an integrative signal that can reflect changes in many different cellular compartments is that the experimenter maximizes the opportunity to see the effects of added compounds, however they act and whenever they act. Although to elucidate the basis for the change in impedance requires follow up experiments, the instruments provide a platform upon which to perform them. In particular, impedance represents a biophysical parameter of the cell that may both provide insight into underlying biochemical events, and may provide information as to the impact of the changes in cell behavior. Ongoing studies by us and others are attempting to determine the utility of the approach for gaining prognostic information in cancer, in order to help determine optimal treatment regimes. Although said studies have just begun, they provide proof of concept for a biophysical approach to the study of pathological processes.

References

[1] M.S. Beck and R. Williams, *Process Tomography: Principles, Techniques and Applications*, Butterworth-Heinemann, Waltham Massachusetts, 1995.

[2] H.P. Schwan, The practical success of impedance techniques from a historical perspective, *Ann NY Acad Sci* **873** (1999), 1–12.

[3] S.B. Rutkove, Electrical impedance myography: Background, current state, and future directions, *Muscle Nerve* **40** (2009), 936–946.

[4] J. Jossinet, Elementary electrodynamics, *Technology and Health Care* **16** (2008), 465–474.

[5] Y. Zou and Z. Guo, A review of impedance techniques for breast cancer detection, *Medical Engineering and Physics* **25** (2003), 79–90.

[6] R. Pethig, Dielectric properties of body tissues. *Clin Phys Physiol Meas* **8**(Suppl A) (1987), 5–12.

[7] A.P. Calderón, On an inverse boundary value problem, in seminar on numerical analysis and its applications to continuum physics, Rio de Janeiro. Reprinted as Calderon, Alberto P. (2006). "On an inverse boundary value problem", *Mat Apl Comput* **25**(2-3) (1980), 133–138.

[8] G. Uhlmann, Developments in inverse problems since Calderón's foundational paper, Harmonic Analysis and Partial Differential Equations: Essays in Honor of Alberto P. Calderón, (editors M.E. Christ and C.E. Kenig), University of Chicago Press, 1999.

[9] D.C. Barber and B.H. Brown, Applied potential tomography, *J Phys E:Sci Instrum* **17**(9) (1984), 723–733.

[10] P. Metherall, D.C. Barber, R.H. Smallwood and B.H. Brown, Three dimensional electrical impedance tomography, *Natur* **380**, 509–512.

[11] C. Gabriel, S. Gabriel and E. Cortout, The dielectric properties of biological tissues: I. literature survey. *Phys Med Biol* (1996), 2231–2249.

[12] C. Gabriel, Dielectric properties of biological tissues: Variation with age. *Bioelectromagnetics* (Suppl 7) (2005), S12–S18.

[13] B.H. Brown, Electrical impedance tomography (EIT): A review, *J Med Eng & Tech* **27** (2003), 97–108.

[14] Adler, Andy; Lionheart, William, Uses and abuses of EIDORS: An extensible software base for EIT, *Physiol Meas* **27**(5) (2006), S25–S42.

[15] J.P. Morucci and B. Rigaud, Bioelectrical impedance techniques in medicine. Part III: Impedance imaging. Third section: Medical applications, *Critical Reviews in Biomedical Engineering* **24**(4-6) (1996), 655–677.

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

[17] M.E. Valentinuzzi, J.P. Morucci and C.J. Felice, Bioelectrical impedance techniques in medicine, Part II: Monitoring of physiological events by impedance. *Critical Reviews in Biomedical Engineering* **24**(4-6) (1996), 353–466.

[18] R. Fass, Sensory testing of the esophagus, *J Clin Gastroenterol* **38** (2004), 628–641.

[19] D.S. Holder, Electrical impedance tomography: Methods, *History and Applications, Institute of Physics*, 2004.

[20] M.C. Barbosa-Silva and A.J. Barros, Bioelectrical impedance analysis in clinical practice: A new perspective on its use beyond body composition equations, *Curr Op Clinical Nutrition & Metabolic Care* **8** (2005), 311–317.

[21] N. Sarhill, F.A. Mahmoud, R. Christie and R. Tahir, Assessment of nutritional status and fluid deficits in advanced cancer, *Am J Hospice and Palliative Medicine* **20** (2003), 465–473.

[22] A. Andreoli, A. De Lorenzo, F. Cadeddu, L. Iacopino and M. Grande, New trends in nutritional status assessment of cancer patients, *Eur J Medical Pharm Sci* **15** (2011), 469–480.

[23] R. Thibault, N. Cano and C. Pichard, Quantification of lean tissue losses during cancer and HIV infection/AIDS, *Current Opinion in Clinical Nutrition & Metabolic Care* **14**(3) (2011), 261–267.

[24] K. Norman, N. Stobaus, D. Zocher, A. Bosy-Westphal, A. Szramek, R. Scheufele, C. Smoliner and M. Pirlich, Cutoff percentiles of bioelectrical phase angle predict functionality, quality of life, and mortality in patients with cancer, *A, J Clin Nut* **92** (2010), 612–619.

[25] A. Walter-Kroker, A. Kroker, M. Mattiucci-Guehlke and T. Glaab, A practical guide to bioelectrical impedance analysis

using the example of chronic obstructive pulmonary disease. *Nutrition J* **10** (2011), 35.

[26] T. Tuy and F. Peacock, Noninvasive volume assessment in the emergency department: A look at B-type natriuretic peptide and bioimpedance vector analysis, *Contributions to Nephrology* **171** (2011), 187–193.

[27] R.P. Henderson and J.G. Webster, An impedance camera for spatially specific measurements of the thorax, *IEEE Trans Biomed Eng* **25**(3) (1978), 250–254.

[28] P. Aberg, I. Nicander, J. Hansson, P. Geladi, U. Holmgren and S. Olimar, Skin cancer identification using multifrequency electrical impedance – a potential screening tool, *Biomed Engin IEEE Tran* **51** (2004), 2097–2102.

[29] D.S. Rigel, J. Russak and R. Friedman, The evolution of melanoma diagnosis: 25 years beyond the ABCDs, *CA: Cancer J Clin* **60** (2010), 301–316.

[30] M.G. Kumar and S. Lin, Transdermal iontophoresis: Impact on skin integrity as evaluated by various methods, *Critical Reviews in Therapeutic Drug Carrier Systems* **25**(4) (2008), 381–401.

[31] M. Bodenstein, M. David and K. Markstaller, Principles of electrical impedance tomography and its clinical application, *Crit Care Med* **37** (2009), 713–724.

[32] O. Moerer, G. Hahn and M. Quintel, Lung impedance measurements to monitor alveolar ventilation, *Curr Op Crit Care* **17** (2011), 260–267.

[33] I. Frerichs, J. Scholz and N. Weiler, Electrical Impedance Tomography and its Perspectives in Intensive Care Medicine. Yearbook of Intensive Care and Emergency Medicine. Yearbook of Intensive Care and Emergency Medicine. Springer, Berlin, 2006, pp. 437–447.

[34] P.T. Huynh, A.M. Jarolimek and S. Daye, The false-negative mammogram, *RadioGraphics* **18**(5) (1998), 1137–1154.

[35] C.W. Piccoli, Contrast-enhanced breast MRI: Factors affecting sensitivity and specificity, *European Radiolog* **7** (1997), 281–288.

[36] P.H. Kuo, E. Kanal, A.K. Abu-Alfa and S.E. Cowper, Gadolinium-based MR contrast agents and nephrogenic systemic fibrosis, *Radiology* **242**(3) (2007), 647.

[37] H. Fricke and S. Morse, The electrical capacity of tumors of the breast, *J Cancer Res* **10** (1926), 340–376.

[38] J. Jossinet, The impedivity of freshly excised human breast tissue, *Physiological Measurement* **19**(1) (1998), 61–76.

[39] E.Y. Ng, S.V. Sree, K.H. Ng and G. Kaw, The use of tissue electrical characteristics for breast cancer detection: A perspective review, *Technology in Cancer Research & Treatment* **7**(4) (2008), 295–308.

[40] D. Gupta, C.A. Lammersfeld, P.G. Vashi, J. King, S.L. Dahlk, J.F. Grutsch and C.G. Lis, Bioelectrical impedance phase angle as a prognostic indicator in breast cancer, *BMC Cancer* **8** (2008), 249–256.

[41] R.J. Brenner and Y. Parisky, Alternative breast-imaging approaches, *Radiologic Clinics of North America* **45** (2007), 907–923.

[42] T.A. Hope and S.E. Iles, Technology review: The use of electrical impedance scanning in the detection of breast cancer, *Breast Cancer Res* **6** (2004), 69–74.