Electrical bioimpedance spectroscopy as biosensor technique to identify cells lineages and cell differentiation process

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Abstract— **The identification and characterization of diverse cells types and cell differentiation process requires complex techniques as flow cytometry, immunocytochemistry and the exploration of molecular markers; such techniques require infrastructure and qualified personnel. The objective of this study was to analyze the use of Electrical Bioimpedance Spectroscopy (EBIS) measurements as a non-complex alternative technique to identify populations of undifferentiated mouse Pluripotent Stem Cells (mPSCs), Mouse Embryonic Fibroblasts (MEFs) and the differentiation process from preadipocytes (3T3-L1) to mature adipocytes. EBIS measurements were compared in populations of cells which were characterized previously using microscopy. The results indicate that EBIS technique has a potential sensitivity at certain frequency range to discriminate between both evaluated cell populations and some differentiation process. Additional studies with different concentrations to evaluate quantitatively the sensitivity and specificity of the proposed technique are recommended.**

I. Introduction

Cell culture techniques and cell differentiation process have become relevant due to their capability to originate different cell lineages under certain culture conditions [1]. This cell expansion and differentiation ability is the trend in medical engineering for its use in tissue regeneration therapy [2, 3]. To identify undifferentiated and differentiated cells, specialized techniques are required, such as flow cytometry, immunocytochemistry and genes exploration [4, 5, 6]. Characterization of mouse Embryonic Stem Cells (mPSCs) has been developed by the exploration of pluripotent markers such as Nanog, Oct4 and SOX2 and specific genes according to obtained cell lineage [7, 8, 9]. Other cell differentiation processes such as adipogenic differentiation to evaluate the effects of different compounds or drugs have been characterized by invasive and laborious techniques (Noriega-col). The mentioned techniques are very efficient but they required specialized personnel and infrastructure, so it is appropriate to propose emerging techniques that could help to facilitate the identification of diverse cell lines.

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Bioimpedance measurements have been proposed since 1957 as an emerging tool for the study of the passive electrical properties of cells in suspension with potential value in biomedical applications [11]. The main advantages of the use of Electrical Bioimpedance Spectroscopy (EBIS) in biomedical research are: it requires low-cost instrumentation, is easily applicable in practice and enable on-line monitoring. Important EBIS biomedical applications have been focused on cell characterization. Coulter counter is a typical application of impedance methods in the cellular field; it is used to count the amount of cells in a suspension and eventually to extract information about the cell sizes. Another important EBIS application was proposed in 2007 by Arum Han et al. Han reported the feasibility to identify different tumor cell lines through the characterization of their electrical properties, measured by EBIS [12]. Similarly, the study by Seidel and coauthors, in which the means of electrical bioimpedance that follows a process of differentiation and neuronal maturation, demonstrating that changes in cellular impedance correlated with as changes in cellular structure, being a non-invasive sensitive monitoring system [13]. Cell growth characterization during culturing is also an important issue in a variety of biomedical applications. In 2013, Yi-Yu Lu et al. proposed the use of electrical bioimpedance spectroscopy-based on multi-electrode as a culture monitoring system to characterize PC12 cell growth, their findings showed important correlation coefficients between impedance and cell growth characterization [14].

In this study, the potential use of EBIS to identify populations of undifferentiated and differentiated cells as well as cell differentiation process was evaluated, thus as a first approach EBIS measurements in mPSCs, MEFs, 3T3-L1 and mature adipocytes were analyzed.

II. Materials and Methods

A. Experimental design

The biophysical concept of EBIS measurements in suspended cells is shown in Figure 1A. 3 cell lines were evaluated: mPSCs, MEFs and 3T3-L1. Approximately $2x10^5$ cells of each cell line were suspended in 200 µl of phosphate buffer solution (PBS) and placed over a quartz crystal surface with embedded gold electrodes in such a way that capillarity kept a consolidated volume. The electrodes represent the electric-ionic Interphase in which our biosensor proposal of the 3 studied cell lines is based (using EBIS measurements). Bioimpedance spectra of multiple frequencies were measured for each cell type by triplicate assays.

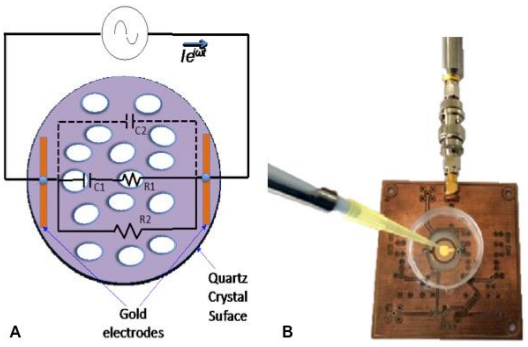

Figure 1. Biosensor. A. Schematic biophysical concept of biosensor based on EBIS measurements in suspended cells (not in scale). B. Biosensor based on EBIS measurements in suspended cells over a quartz crystal surface with gold electrodes embedded.

B. Cell cultures

Mouse embryonic stem cells (ATCC; SCRC-1011) were seeded at a density of 50,000 cells/cm^2 on monolayers of mitotically inactive MEFs feeder cells. We used mPSCs basal medium (ATCC; SCRR-2010), supplemented with 15% fetal bovine serum (FBS) (ATCC; 30-2020), 0.1 mM 2-mercaptoethanol (Invitrogen; 21985023) and 1,000 U/ml mouse leukemia inhibitory factor (Chemicon; ESG1107). Before EBIS measurements of mPSCs, these cells were separated from the monolayer of MEFs.

Mouse embryonic fibroblasts, STO cells (S, SIM; T, resistant to thioguanine; O, resistant to oubain), were cultured using Dulbecco's Modified Eagle's medium (DMEM) (ATCC; 30-2002), supplemented with 15% FBS and 1% penicillin–streptomycin (10,000 IU / ml–10,000 µg/ml) (Invitrogen; 15140).

Both cell types were incubated at 37 ºC in a humidified incubator (5% CO2, 95% air), and once the cultures reached 70% confluence, 3 samples of 2x10^5 cells were obtained and resuspended in +200 µl of PBS.

Adipogenic differentiation was made with murine preadipocyte 3T3-L1 cell line obtained from the American Type Culture Collection (ATCC). It was cultured according to the manufacturer's instructions, cells were seeded in 60 mm dishes (P60). In summary, preadipocytes 3T3-L1 were cultured in growth medium, consisting of Dulbecco's Modified Eagle's Medium (DMEM) (ATCC cat: 30-2002) supplemented with 10% newborn calf serum (NCS) (Equitech-Bio SNBU30-0500), 100UI/mL penicillin and 100 mg/ml streptomycin. Cell cultures were incubated at 37°C in a humidified 5% CO2 and 95% air incubator. The medium was replaced every 2-3 days. Adipocyte differentiation of 3T3- L1 cells was induced using a hormonal cocktail. When the cultures reached confluency (day 0), cells were treated with differentiation medium containing DMEM supplemented with 10% fetal bovine serum (FBS ATCC, cat: 30-2020), 0.25mM dexamethasone (Sigma-Aldrich - D4902), 5 mg/mL insulin (Sigma-Aldrich-I3536) and 0.5 mM isobutylmethylxanthine (IBMX, Sigma-Aldrich-I7018). At day 2, the medium was replaced with adipogenic medium containing DMEM supplemented with 10% FBS and 5 mg/ mL insulin, for an additional 3 days, at which time >90% of the cells were mature adipocytes with accumulated fat droplets.

C. Experimental Bioimpedance Measurements

An experimental bioimpedance measurement system was implemented on the basis of a precision impedance meter (Agilent; Model 4294A), which supplied a signal I cos (ωt) in the range of 100 MHz. The biosensor system was adapted by 2 electrodes positioned in such a way that the electric circuit is closed through a sample of PBS with suspended cells. Both electrodes are fabricated in gold embedded in a quartz crystal surface with radius r=9mm (QSX 301, Biolin Scientific Inc.) (Fig. 1B). Measurements were developed in the frequency range of 100 Hz to 100 MHz in 181 steps spaced logarithmically. Bioimpedance magnitude and phase values were normalized (Norm) in every spectrum in respect to its own maximum value. The concept behind the system is that the measurements are representative of the changes of volumetric electrical properties in each sample; it means the frequency response of the complex impedance Z changes as a function of the sample cell type.

III. RESULTS AND DISCUSSION

Microscopic observation of morphology in the cell cultures showed that mPSCs were rounded in shape with a tendency to grow in colonies (Fig. 2A). MEFs morphology was observed more complex, fusiform and did not make conglomerates (Fig. 2B). As for the pre-adipocytes, the elongated morphology (Fig. 2C) while the mature adipocytes have a polyhedral shape and numerous lipid vacuoles (Fig. 2D).

Gene expression studies (not showed in this work) confirmed the expression of characteristic molecular markers for every cell condition.

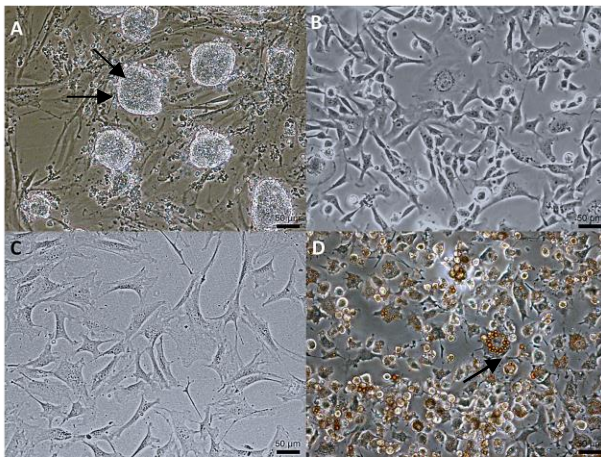

Figure 2. Morphology of cell cultures (100X, phase contrast). A. Pluripotent mouse embryonic stem cells forming colonies over mouse embryonic fibroblasts (arrows). B. Mouse embryonic fibroblasts. C. 3T3-L1 preadipocytes. D. Mature adipocytes with lipidic vacuoles stained with oil red (arrows) after differentiation process.

EBIS analysis showed significate magnitude differences between mPSCs and MEFs around $1x10^2$ (Fig. 3A), and in the range from $1x10^4$ to $1x10^5$ Hz for the process of adipogenic differentiation (Fig. 3B)

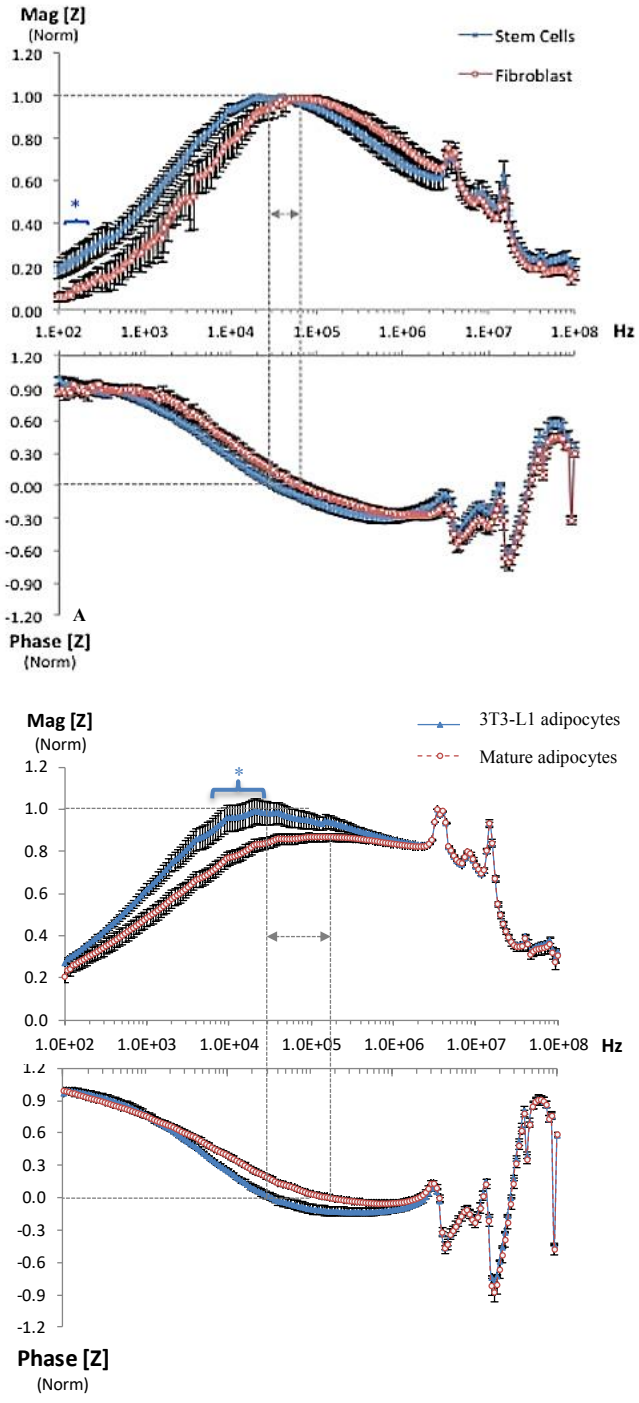

Figure 3. A) EBIS measurements in mouse Embryonic Stem Cell and Mouse Embryonic Fibroblasts. Magnitude Z and Phase Z. *p<0.05, U-Mann-Whitney test (Stem Cells vs Fibroblast). B) EBIS measurements in preadipocytes and adipocytes. Magnitude Z and Phase *p<0.05, U-Mann-Whitney test (preadipocytes vs adipocytes).

The morphological characterization shows that the evaluated cell samples correspond to mPSCs and MEFs populations. mPSCs cultures grew in the colonies, showing an extensive capacity for replication, these colonies were composed of many round cells with diffuse cytoskeletons (Fig. 2A). In contrast, MEFs were adherent plasticware cells, that spread out to form a monolayer of actively dividing cells; under a light microscope these cells are tapered with thin extensions, closed oval nucleus and scant cytoplasm (Fig. 2B), such observations for mPSCs and MEFs agree with Ginis et al. and Acosta et al. respectively [9, 15]. In previous studies, our group as well as Chialin Chen et al. has reported the cell complexity characterization of mPSCs, we have shown that mPSCs retain a low cell complexity related to its undifferentiated state [6, 16]. Preadipocytes were characterized as adherent cells and elongated cells with cytoplasmic extensions and when they were differentiated, their morphology changed to a polyhedral, increasing its diameter, as well as acquiring accumulation of small lipid vacuoles surrounding the periphery of the adipocyte within the cytoplasm and per their maturity process such complexity tends to increase [10].

The bioimpedance measurements show sensitivity in the alpha dispersion bandwidth, this frequency range is associated with a low electrical conductivity of the cell membranes and most of the current must flow around the cells [17, 18], in such conditions the structural cell components and their capacitive effects are relevant. The mPSCs and MEFs populations evaluated show different morphological structural patterns (Fig. 2A and 2B); In the same way, the process of adipogenic differentiation, since there is a biochemical change as well as morphology and size (Fig. 2C and 2D); thus is assumed that the reactive components between both types of cell structures are evidently different. Observing the same behavior the spectrum of the process of adipogenic differentiation. The observation seems in agreement with previous report of changes in bioimpedance measurements as a function of cell morphology, maturity and functionality [19].

For analytical purpose, a classic, simplified electrical model for cells in suspension was adapted from previous reports [11, 17, 18] and was represented by solid lines in Figure 1A. The membrane and cytoplasm of the cells are represented by C1 and R1 respectively; the conductive extracellular medium is represented by R2. In addition, we have considered that the used macro-electrodes induce a significant parasite capacitive effect C2, and it was represented in dotted lines in Figure 1A, it might explain in part a not characteristically behavior for biological samples at frequencies below 2×10^4 Hz. Given the configuration of the whole involved passive elements, the integrated electrical model might correspond a pass band filter with a resonance effect approximately centered in the 10 to 100 KHz bandwidth, is evident that the specific resonance frequency could be defined as a function of C1 and R1, and such parameters are specifically associated to the morphological and biophysical characteristics of the evaluated cells. The morphology of mPSCs indicates less complexity than MEFs, while preadipocytes are shown to be less complex and smaller than mature, this condition is expected to reflect a different dielectric behavior in the integrated electrical model considered, in this sense, lower capacitive values of C2 correspond to increment of the resonance frequency of the system, this behavior is in agreement with the experimental

observation of the complex impedance frequency response between mPSCs-MEFs and preadipocytes-mature adipocytes, a shift of the resonance frequency is evidenced by coherent poles with maximum magnitude at zero phase values.

IV. Conclusion

The purpose of this experiment was to analyze the use of the EBIS as an alternative technique to identify populations of mPSCs and MEFs and monitoring a process of differentiation, for to determine the technical feasibility of using simple and inexpensive instrumentation to identify different cell lines. In this sense, this work is a basic study and the assumptions for interpretation of the findings represents a preliminary analysis, we have estimated than additional experiments and analytical studies regarding the effect of different cell concentrations and the passive electrical properties involved in the observed phenomena are warranted.

Acknowledgment

The authors thank CONACYT for the graduate scholarship to CIGR. The presentation of this work at IEEE-EMBS 2017 Conference is supported in part by "CONACYT CB-2012" under the grant no. 180536

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