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Dielectric Characterization of Human Dermal Fibroblast Monolayers Using a Capacitive Sensor Array

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Abstract– ***This study presents a non-invasive electrical approach for the real-time characterization of human dermal fibroblast (HDFa) monolayers using a capacitive sensor array combined with phasor analysis. Voltage and current signals were acquired under low-intensity alternating excitation, enabling the estimation of the effective capacitance of the culture system across a controlled frequency sweep. The results revealed frequency-dependent capacitance variations that differentiate the electrical behavior of the biological culture from that of a passive dielectric substrate. A first-order phenomenological model was introduced to interpret these variations in terms of macroscopic dielectric properties. Although no direct biological assays were performed, the proposed metric should be understood as an electrical indicator sensitive to dielectric changes rather than as a direct measurement of cell viability. These findings highlight the potential of capacitance-based phasorial analysis as a complementary strategy for non-invasive electrical monitoring of in vitro cell culture systems.***

Keywords-- Bioelectrical Sensing; Human Dermal Fibroblast (HDFa); Phasor; Real-time cell analysis (RTCA); Dielectric Characterization.

I. Introduction

The assessment of cell viability and proliferation is crucial for determining the impact of various treatments on monolayer cell cultures. Conventional techniques, such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assays, are commonly employed for this purpose. However, these methods are time-consuming, costly, and require stringent experimental conditions to minimize the risk of culture contamination and reduce data variability. Consequently, there is an increasing need for innovative approaches that facilitate the monitoring of cellular dynamics through less invasive, more efficient, and cost-effective techniques. Cell electrostimulation using electric fields is a promising technique for identifying the behavior of cell cultures and the progression of specific processes in tissues. Electrical stimulation triggers various cellular responses, including the intracellular modulation of calcium ions, ATP production, and other events such as the clustering or reorganization of cell surface receptors and cytoskeletal remodeling, among others. Characterizing the electrical properties of cell culture media under applied electric fields is crucial for determining cell dynamics as a function of the applied intensities and frequencies, as well as for assessing whether the input electrical parameters are appropriate to influence such behavior.

Moreover, in cell culture monitoring systems, capacitance is a key factor that can be associated with various cellular processes, including viability, migration, and apoptosis, among others.

In the work developed by Isabella Surowiec, the relationship between capacitance measurement in bioreactors and frequency-dependent polarization in a volume of living cells is described, considering that its value may be related to the number of viable cells, cell density, and viable cell concentration, all critical parameters in bioprocess monitoring. Another important aspect of biocapacitance is its ability to be measured in real time, unlike other methods, making it a valuable tool for continuous and non-invasive assessment of cellular behavior in biotechnological applications [1].

Moreover, in protein production based on Chinese hamster ovary (CHO) cells, single-frequency capacitance measurement has enabled reliable determination of cell concentrations for real-time process monitoring across different scales [2].

In the electrical behavior of cell cultures, several derived parameters stand out, such as delta capacitance, the system's critical frequency (fc), and the Cole-Cole alpha parameter, which provide additional information on cellular processes, including cell size and intracellular states. This data can further enhance the monitoring system.

Among the most recent applications of biocapacitance spectroscopy measurement are real-time monitoring of specific cell growth rates, cellular responses to nutrient additions, cell size, cellular conductivity, membrane capacitance, early apoptosis detection, and prediction of apoptotic cell percentages. Consequently, this approach provides crucial insights into cell viability and the onset of the death phase in culture media [3]-[6].

In the process of measuring cell viability across different

treatments, the integrity of culture media can be compromised if protocols and procedures are not carried out by trained personnel.

In the study conducted by Goeun Park et al., an electric drug monitoring system based on capacitance was introduced as a complementary approach to existing cell viability assays; this study assessed viability by analyzing capacitance changes at the electrode surface due to adherent cells, including both cancerous and normal cells. One of the most significant conclusions of this research highlights how capacitance-based cell viability determination is a promising alternative, offering a low error rate compared to traditional methods [7].

Phasor analysis is a well-known technique used for the analysis of AC electronic circuits, particularly for linear circuits operating in a sinusoidal regime. This approach simplifies the use of differential equations by converting them into more manageable algebraic equations. A phasor is a complex representation of a sinusoidal signal, which, instead of being expressed as a function of time, incorporates key characteristics of the sine wave such as magnitude, phase, and frequency. In the phasor domain, voltage and current signals are typically represented as complex numbers. The fundamental laws governing electronic circuits can be applied in alternating current using phasor algebra. In direct current circuits, opposition to current flow is termed resistance; however, in alternating current circuits, this opposition is described as complex impedance in the case of capacitors and inductors. In this research, the capacitance of the cell culture is considered a key electrical parameter. A methodology is proposed to monitor the behavior (frequency-dependent electrical response) of a fibroblast cell culture through the characterization, identification, and temporal tracking of electrical parameters using phasor algebra. This approach involves the application of a low-intensity electric field to the culture, followed by the acquisition of voltage and current signals using a National Instruments data acquisition system. The recorded data are subsequently processed in MATLAB to estimate the effective capacitance of the culture medium. The article is structured into the following sections: (I) Introduction, (II) Materials and Methods, (III) Results, (IV) Discussion, and (V) Conclusions.

II. Materials and Methods

2.1 ACEF exposure system

To develop the methodology for characterizing an HDFa fibroblast culture medium, architecture illustrated in Figure 1 was proposed. This setup highlights the power amplifier, electrodes arranged parallel to the culture dish, and the culture medium.

The proposed electrostimulator features a bandwidth ranging from 10 Hz to 500 kHz, enabling a variety of tests based on frequency. Additionally, the circuit delivers an effective power output of 100 W for a 4 Ω resistive load, allowing for experiments focused on the intensity of the applied electric field. In this study, a voltage of 6.3 VRMS was applied between the parallel electrodes, with a frequency sweep conducted in the range of 1 kHz to 10 kHz.

The defined frequency range (1 kHz – 10 kHz) was based on the β-dispersion region, which is associated with cellular membrane polarization phenomena, where interfacial polarization effects dominate the dielectric response of biological tissues. In this range, changes in effective capacitance depend on membrane integrity and cellular structure.

Numerical simulations were conducted using specialized software to gain insight into the voltage distribution experienced by the fibroblast cells. For this purpose, theoretical values of the electrical constants relevant to the experimental conditions were adopted as initial input parameters. Table 1 summarizes the electrical constants used in the finite element simulations.

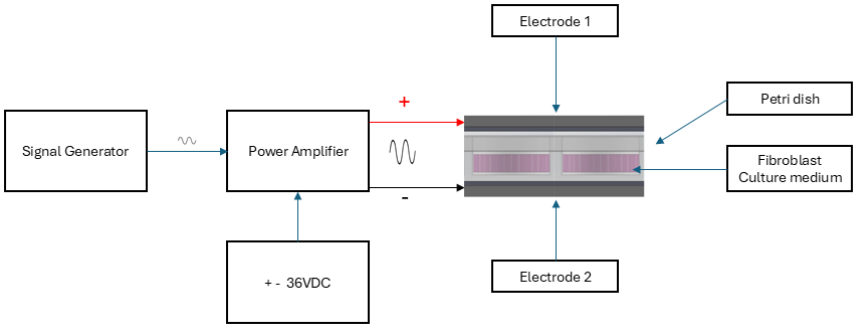

Fig. 1 Proposed device for performing electrostimulation with the aim of conducting the electrical characterization of the HDFa fibroblast culture medium.

TABLE I
ELECTRICAL PROPERTIES OF THE MODELLED GEOMETRY [8].

Domain	Material	Relative Permittivity
1	Culture Medium	80
2	Polystyrene	2.6
3	Stainless Steel	1.1×10^6
4	Air	1.0006

2.2 Numerical Simulation

To quantify the voltage applied to the cell culture medium, a 96-well culture plate was designed in SolidWorks, incorporating parallel electrodes and the culture medium. The CAD model was subsequently exported in IGES format for analysis using ANSYS software. The distance between the electrodes was set at 15.8 mm. A frequency sweep was conducted from 1 kHz to 10 kHz. As evidenced by the simulation results, frequency does not exert a direct influence on the voltage indicating its independence from frequency variations.

2.3 Data Acquisition and Processing

For data acquisition and processing, a National Instruments data acquisition card (model NI-DAQ6009) and a laptop running LabVIEW software were used to record the data. The output of the power amplifier was connected to a resistor in series with the cell capacitive array, as shown in Figure 2.

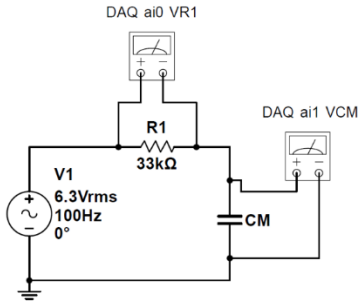

Fig. 2 Capacitive arrangement circuit to determine the input constants for phasor analysis

As illustrated in Figure 2, the power amplifier has an output of $Vo = 6.3\,VRMS$. The resistor R_1 in the circuit of Figure 2 serves the purpose of measuring the current in the series circuit. The voltage across the resistor was measured using analog input 0 of the DAQ6009, while the voltage across the capacitive array was acquired through analog input 1 of the DAQ6009. The electrical characterization of the culture medium was determined using the following equations, based on the data obtained from the DAQ. The primary variables of the capacitive array within the main cell include its impedance, capacitance, and conductivity:

$$I_{R1} = I_{CM} = \frac{V_{R1}}{R_1}$$ Current determination (1)

$$X_C = \frac{V_C}{I_{CM}}$$ Impedance determination (2)

$$Z = R + X_C i$$ normally the total impedance of a circuit is given by a real part and an imaginary part, which in this case corresponds to the capacitive reactance. (3)

$$X_C = \frac{1}{2\pi f C}$$ Capacitive impedance as a function of applied frequency (4)

$$C = \frac{1}{2\pi f X_C}$$ Capacitance determination (5)

$$G = \frac{1}{X_C}$$ Conductivity (6)

The LabVIEW data logging program includes two modules for analog-to-digital conversion and a logger for exporting data to Excel upon completion of the measurement. It is important to note that one of the main limitations of the DAQ6009 is its maximum sampling rate of 50 kS/s, which restricts the handling of signals with frequencies up to 25 kHz to prevent aliasing, in accordance with the Nyquist theorem.

2.4 Cell culture

For the present study, a human dermal fibroblast cell line, HDFa (human dermal fibroblasts, adult), was used, and the culture conditions required for implementation in a 2D monolayer configuration were established. The cells were maintained in an incubator at 37°C with 5% CO_2 and 95% humidity. The culture medium consisted of DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% glutamine. The medium was replaced every two days. The DMEM culture medium was selected due to its well-established compatibility with fibroblast cell cultures, providing a balanced composition of amino acids, glucose, and vitamins. This supports key cellular processes such as migration and proliferation and ensures proper metabolic activity under *in vitro* experimental conditions.

To apply ACEF using the electrostimulation system developed in this study, two electrodes were positioned parallel to the culture plate. The distance between the electrodes was 15.8 mm, the applied voltage was 6.3 VRMS. The applied voltage corresponded to a value of 6.3 VAC, which falls within the range of low-intensity ACEF and enables the assessment of cellular behavior through non-thermal mechanisms. The stimulation duration was set to 10 minutes for each frequency to ensure sufficient exposure time for inducing detectable bioelectrical responses, while avoiding potential damage to structurally intact cells due to immediate thermal effects associated with energy dissipation,

as described by Joule's law.

2.5 Dielectric Interpretation of Capacitance Variations in Fibroblast Cultures

This section introduces a phenomenological framework aimed at interpreting how variations in electrical capacitance reflect modifications in the dielectric properties of the culture system. Although several biological techniques exist for assessing cell viability, they are often invasive, time-consuming, and experimentally demanding. Therefore, the following formulation is presented as an electrical approximation intended to characterize the macroscopic dielectric behavior of the culture medium rather than to provide a direct biological quantification.

The effective capacitance of the system is governed by the polarization properties of the medium and by the dielectric contribution of suspended cells. Due to the insulating nature of the plasma membrane, structurally intact cells can be electrically modeled as polarizable particles within a heterogeneous dielectric environment. The term N_V/N_0 denotes the normalized fraction of structurally intact cells within the measurement volume.

$$C_e(t) = \varepsilon \frac{A}{d}\left(\frac{N_v(t)}{N_0}\right) \quad (7)$$

Where:

$C_e(t)$: Effective capacitance at time t or frequency f

ε = Dielectric permittivity of the medium
$\varepsilon = \varepsilon_r \varepsilon_0 = 7.0832 \times 10^{-10}$ F/m

A = Electrode Area = $0.010795\ m^2$

d = Electrode separation = 0.0158 m

$N_v(t)$ = Number of structurally intact cells at time t

N_0 = Initial number of structurally intact cells

For interpretative purposes, Equation (7) can be rearranged to obtain a normalized dielectric indicator expressed as a percentage.

$$\text{Dielectric Indicator (\%)} = 100\left(\frac{C_e(t)}{\varepsilon\frac{A}{d}}\right)$$

$$\varepsilon\frac{A}{d} = 4.8394 \times 10^{-10}\text{F}$$

$$\text{Dielectric Indicator (\%)} = 100\left(\frac{C_e(t)}{4.8394 \times 10^{-10}\ F}\right) \quad (8)$$

This model is supported by previously reported studies [5], [4], [9].

Future work will focus on correlating this dielectric indicator with standardized biological assays.

III. Results

3.1 Numerical Simulation of ACEF

In Figure 3, the distribution of the applied electric potential (6.3 V) in the capacitive arrangement is shown. In this case, the electric field is distributed perpendicularly between both electrodes. A region of high field density is observed in the central area, where the spacing of the equipotential lines is narrower. In this region, HDFa cells are expected to experience more significant effects of electrical stimulation. The potential gradient directly influences the force exerted by the electric field as follows in Equation 9:

$$E = -\nabla V = -\frac{dV}{dl} \quad (9)$$

dl = distance differential along the direction in which the electric field is evaluated.

dV = voltage differential along cell capacitive array.

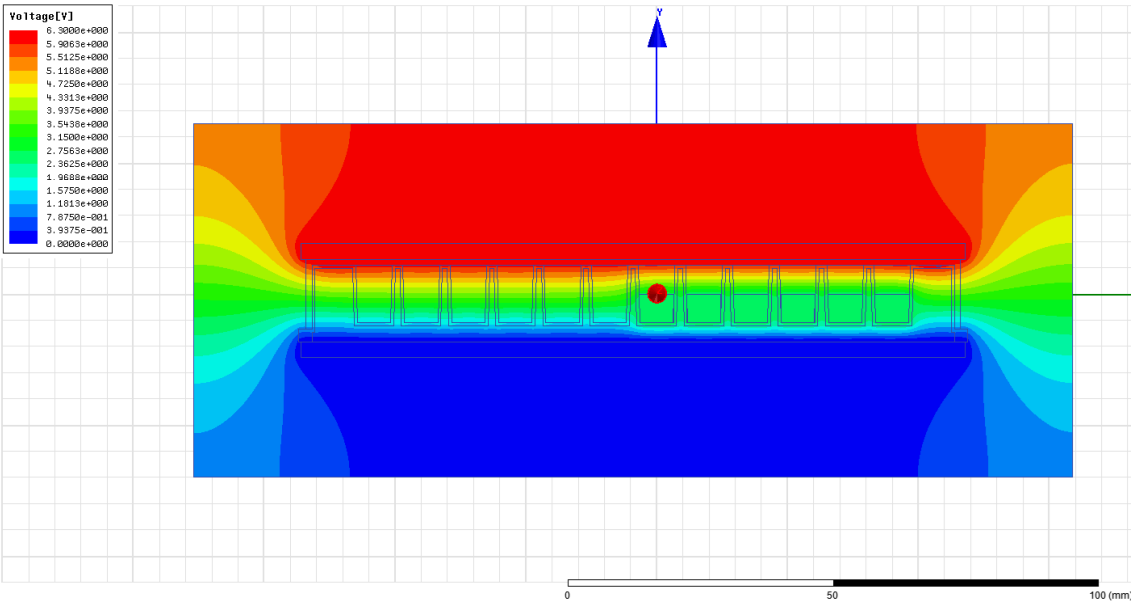

Fig. 3 Voltage along the electrodes

3.2 Electrical characterization of the culture box in the absence of culture medium using phasor techniques

The identification of the electrical parameters of the culture box in the absence of the culture medium was conducted to compare data variability with respect to the chemical and biological components influenced by the cells. This serves as a baseline for determining how cellular dynamics change in response to variations in electrical parameters, representing a non-invasive approach for monitoring cellular states (real-time cellular analysis) using simple phasor techniques. In this test, the polystyrene dielectric of the culture box maintained its physicochemical properties, thereby functioning as a pure capacitor. The primary electrical variable determined for the polystyrene culture chamber was capacitance, as referenced in Equations 1–6. Figure 4 presents the capacitance as a function of frequency. The culture chamber acts as a dielectric for the capacitive array, with the constants listed in Table 1. Purely capacitive behavior is observed, where capacitance remains constant, impedance decreases, and conductivity increases as frequency rises.

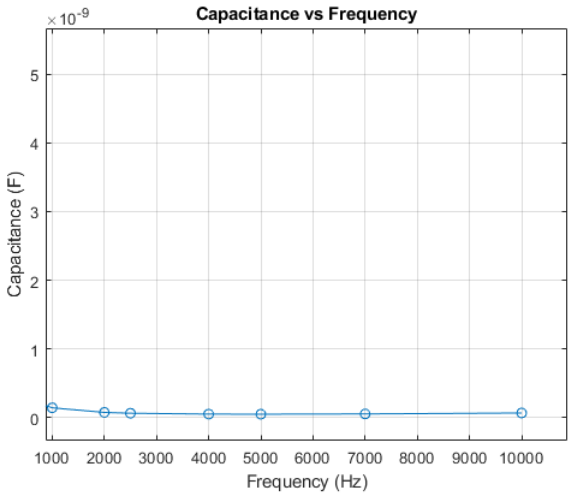

Fig. 4 Capacitance as a Function of Frequency (Capacitive Array in the Absence of Culture Medium). Capacitance remains approximately constant at 70.1 pF.

3.3 Electrical characterization of the cell culture medium using phasor techniques

For the electrical characterization of the culture medium, frequencies were varied within the range indicated (1-10 kHz), while applying a 6.3 VRMS sinusoidal potential between the electrodes. A total of 16,000 cells were cultured and evenly distributed across 48 wells of a 96-well culture plate, each suspended in 200 µL of medium, yielding a total volume of 9.6 mL. The experimental setup ensured uniform cell distribution and consistent medium conditions across all wells.

An AC capacitor acts as a short circuit at high frequencies, allowing most of the current to pass through while attenuating the voltage. Under normal conditions, the capacitance of a conventional capacitor remains constant, as the physical properties of its dielectric do not change. However, in this study, we worked with a biological capacitor composed of cells and culture media, whose physical properties vary in response to the applied electric field intensities and different frequency levels.

Figure 5 illustrates the capacitance as a function of frequency for capacitive array including culture medium and cells. This behavior aligns with that of a conventional capacitor, except for the capacitance variation as a function of frequency, a characteristic specific to biological capacitors.

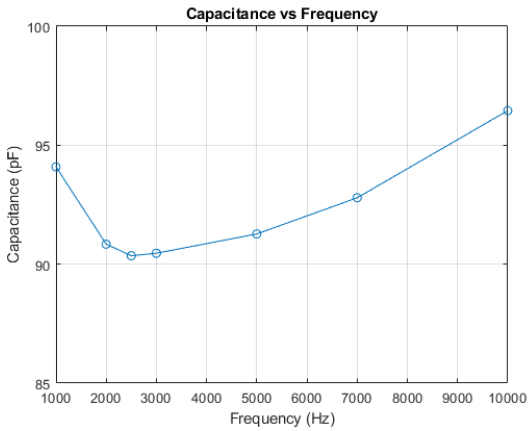

Fig. 5 Electrical parameters of the capacitive array in the presence of culture medium. (a) The capacitance varies as a function of frequency.

3.4 Model-Based Dielectric Interpretation of Capacitance Variations

Section 2.5 introduced the mathematical relationship used to associate variations in electrical capacitance with structurally intact cells. Figure 5 presents the capacitance values obtained from a frequency sweep ranging from 1 kHz to 10 kHz, which were used as input for the proposed phenomenological model. Based on these values, the corresponding structurally intact cells percentage was estimated for each frequency condition. Figure 6 shows the estimated relative structurally intact cells as a function of capacitance. Using Equation 8, the value of the Dielectric Indicator percentage was estimated as a function of the variation in the capacitance of the culture medium for each frequency. For this purpose, data from figure 5 were used, in which changes in the capacitance of the medium were detected for each frequency involved in the sweep from 1 kHz to 10 kHz.

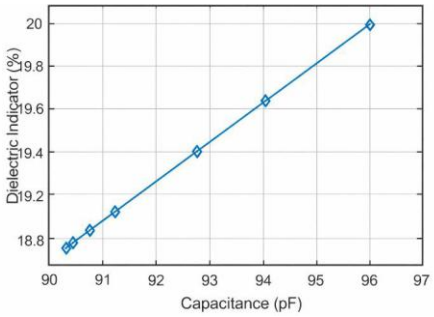

Fig. 6 Model-derived dielectric indicator obtained from capacitance normalization.

4. Discussion

The cellular response to the application of electric fields is a field that has been growing exponentially in recent years; the application of electric fields of different intensities and frequencies is proposed to generate biophysical treatments that contribute to solving problems, among which it is worth mentioning the reduction of tumor cell proliferation (TTFields), rehabilitation using tissue engineering, wound healing processes, and the generation of pores in the cell membrane to facilitate drug delivery, among others.

Apart from the purpose of treatment with electric fields, it is also worth highlighting the importance of electric fields as mechanisms for real-time monitoring of cellular biological behavior by correlating changes in electrical variables. There are various methods for real-time cellular analysis; however, as detailed in this document, many procedures involve complex and invasive techniques. Therefore, this study presents the use of phasor analysis to detect dielectric variations potentially associated with changes in the culture environment through a non-invasive approach and computationally efficient calculations.

In the experiments carried out in this research, it was demonstrated that for a fibroblast (HDFa) cell culture medium, as the frequency increases, electrical impedance decreases, allowing a greater flow of electric charge into the cells. This is consistent with the findings of Li et al., who, through their theoretical study, identified that this behavior causes an increase in the electric field in cytoplasm. Furthermore, in the same study, it is mentioned that when a cell is exposed to an external alternating electric field, the cell membrane acts as a capacitor, shielding the intracellular environment from the external electric field [3].

Among the main findings of this study, it is worth mentioning the difference between the capacitance values of the dielectric composed of the polystyrene plate and the dielectric involving the cell culture medium in the presence of cells. Normally, capacitance is a value that remains constant in electrical measurements; however, when the physicochemical properties of the dielectric change, capacitance also varies accordingly. Under the applied

excitation conditions, the electric field distribution was assumed to remain approximately stable, while frequency variations enabled the observation of measurable changes in the effective capacitance of the culture system.

In Figure 7, the circuit model for an individual cell composed of the cell membrane, cytoplasm, nucleus, and mitochondrion can be observed. It is important to note that the electrical parameters estimated in this study correspond to the effective response of the culture medium coupled with the entire cell population. Consequently, extracting the intrinsic electrical properties of an individual cell membrane would require further investigations under controlled single-cell conditions. A key limitation of this study is that the proposed dielectric indicator does not represent a direct measurement of cell viability, but rather an indirect electrical correlate sensitive to changes in the dielectric properties of the culture system. Consequently, variations in capacitance may be influenced by multiple factors, including cell morphology, medium conductivity, and electrode polarization effects. Additionally, this study focused exclusively on changes in electrical variables, without incorporating biological assays related to proliferation, differentiation, or migration. Therefore, further experimental work is required to establish a direct correlation between electrical measurements and underlying biological processes. Future studies should include validation against standard biological assays, such as MTT or live/dead staining, to confirm the biological relevance of the proposed metric

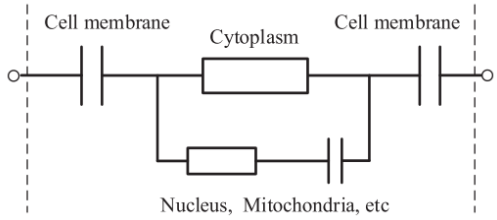

Fig. 7 Equivalent electrical circuit model of a single cell [3].

5. Conclusions

This study proposes a real-time electrical monitoring approach based on phasor analysis, employing a capacitive configuration with two parallel electrodes surrounding a dielectric culture environment composed of a polystyrene dish and an HDFa fibroblast cell culture. Following data acquisition, phasor-domain numerical analysis was performed using MATLAB, while the electric field distribution was evaluated through finite element simulations conducted in ANSYS Electronics Desktop. The results indicate that the electrical properties of the culture system vary as a function of the applied frequency, suggesting that the proposed method may enable the identification of dielectric variations potentially associated with changes in the culture environment.

The derived dielectric indicator exhibited a linear and monotonic dependence on effective capacitance, demonstrating the internal consistency of the proposed normalization approach and its sensitivity to dielectric variations within the culture system.

Although the present study evaluates the dielectric response of an HDFa fibroblast cell culture according to the electrical changes of the system, the work remains primarily within the methodological domain due to the absence of biological assays that allow correlating the dielectric changes. In this context, future work will be aimed at measuring different biological indicators such as cell viability, confluence, morphological changes, among others, with the purpose of identifying how they relate to the dielectric response. These biological analyses will be carried out through classical assays such as MTT/MTS and microscopy-based analyses. On the other hand, it will be important to perform tests with different cell types to determine the general applicability.

From an applied perspective, this work presents high potential for real-time and label-free monitoring of biological parameters in tissue engineering systems. From a biotechnological standpoint, the non-invasive estimation of the culture state will reduce the need for destructive sampling that alters cellular conditions.

Furthermore, from a clinical perspective, cellular responses to different therapies could be monitored, including mechanisms for drug evaluation. In the case of implantable systems, fibrotic processes that cause these devices to fail could be detected at an early stage.

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