Effect of magnetic and electric fields on plasma membrane of single cells: A computational approach

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Funding information
Departamento Administrativo de Ciencia, Tecnología e Innovación, 712-2015 No. 50457

Abstract
Cell membrane is a lipid bilayer that allows the flow of ions through their ionic pumping proteins. The ionic flow can be stimulated with external stimuli to activate specific signaling pathways intracellularly. Although studies have applied electric and magnetic stimuli to modify the cell function, the parameters to stimulate the cell membrane are unknown. Accordingly, a computational model to simulate the effect of electric and magnetic fields on the cell membrane was developed. Cells were stimulated with electric fields from $45 \times 10^3$ V/m to $12.6 \times 10^5$ V/m and magnetic fields of 2 mT, at frequencies of 60 kHz, 10 MHz, and 1 GHz. Results showed that the electric fields applied to the cell membrane tend to increase according to the frequency used, while magnetic fields do not have any effect on it. It was observed that electric fields generate a high voltage concentrator in the cell membrane of ellipsoidal cells when a frequency window from 1 kHz to 1 GHz was applied. These findings demonstrate that depending on the intensity of the field and frequency, it was possible to stimulate different cell membrane zones. This model is a promising tool to establish the adequate parameters to stimulate cells, and accurately predict if the stimulation modifies the cell membrane potential.

KEYWORDS
cell membrane, computational model, electric field, magnetic field

1 | INTRODUCTION

Electric and magnetic potentials are crucial in the functioning of living organisms because these stimuli promote and control cellular functions such as proliferation, migration, differentiation, morphology, and molecular synthesis.¹ For instance, electric fields (EFs) are responsible, either in the communication of nerve cells or in the opening of signaling channels, for the exchange of molecules from the extracellular matrix to the cytoplasm.² Regarding the magnetic fields (MFs), they are produced around the human body by the activation of muscles,³ due to the electrical activation cause by an associated MF. The EFs and MFs are being extensively investigated to find efficient therapeutic methods, for instance, to treat diseases without performing surgical interventions, since these stimuli increase cell metabolism fostering a faster...
healing of injured tissues. The EFs and MFs have demonstrated to have a positive effect on cell dynamics such as increasing the proliferative rate and stimulating the protein synthesis such as collagen, aggrecan, and glycosaminoglycans.\(^4\)\(^-\)\(^6\) This positive effect on cellular dynamics is due to an application of external stimuli over the cell membrane, which generates a longer activation of membrane proteins and a flow of ions such as \(\text{Ca}^{2+}, \text{Na}^+, \text{and K}^+\).\(^7\) The value of the potential differential across cell membrane usually varies between \(-20\) and \(-200\) mV, depending on the cell type and the organism.\(^8\) This transmembrane potential is established by ion channels and proteins that are integrated into the cell membrane.\(^7\)\(^-\)\(^9\) Therefore, if the external stimulus generates transmembrane potentials equal or superior to those generated by the cell, it would be possible to modify the gradient concentration of ions through the cell membrane pumps.\(^1\) Accordingly, during the stimulation of cell membranes with external biophysical fields, it is relevant to consider not only the magnitude of the field (electric or magnetic), but also the frequency, because it can modify the behavior of the cells.\(^\)\(^10\)

On the one hand, Foster et al., mentioned that the excitation of the cell membrane occurs with values of 1 KHz or higher.\(^11\) Moreover, they suggest that 50.000 V/m are required to produce an effect in the plasma membrane in round cells. There is experimental evidence proving that EFs applied at frequencies between 20 Hz and 448 kHz promote cell proliferation and protein synthesis.\(^4\)\(^,\)\(^11\)\(^-\)\(^14\) However, the most used frequencies are from 10 to 100 Hz because these are considered natural frequencies, and they have been reported as the most effective parameters for bone formation when tissues are stimulated.\(^1\) On the other hand, there are other reports in which cellular stimuli are performed in vitro with MFs between 0.4 and 2.3 mT.\(^5\)\(^,\)\(^15\)\(^,\)\(^16\) Some studies made by Liu et al.\(^17\) and De Mattei et al.\(^18\) concluded that MFs preserve the content of sulfated proteoglycans, which are mainly responsible for maintaining the hydration and compressibility of the cartilaginous tissue. Similarly, Stolf a et al., showed that static MFs of 0.6 T induced a significant increase in metabolic activity of chondrocytes after 72 hours of stimulation.\(^19\) These findings allow concluding that MFs are a potential therapeutic method for cartilage recovery.

As mentioned above, there is evidence that demonstrate the biological effect generated for both EFs and MFs. However, there are no studies that show how the parameters of the external biophysical stimuli are selected and how the EFs and MFs influence and stimulate the cell membrane. Accordingly, computational models have been implemented to assess the effect of external EFs and frequencies on the cell membrane. In a study developed by Krassowska et al., a single cell was modeled in order to observe how external direct EFs affect the polarization of the cell and the physiological state of the cell membrane.\(^20\) Similarly, a study carried out by Valič et al., simulated prolate and oblate spheroidal cells under direct EFs.\(^21\) Even though the results from these studies evidenced that the cell responds to EFs, and the permeabilization of cell membrane is not only a function of EF and cell size, but also of the cell shape and orientation, one of the models considered the round cell as a dimensional domain,\(^20\) which is not similar to the actual cell morphology. Moreover, in these works the frequency was not considered as an important factor to stimulate the cell membrane in different zones. For this reason, Taghian et al., assessed the effect of external EFs on a single cell cultured in monolayer using different frequencies.\(^22\) Results evidenced that EFs penetration in the cell membrane depend on the frequency applied; nevertheless, in this study only one-dimensional round cell morphology was used to simulate the effect of EF over the cell membrane. Regarding the computational models to assess how MFs affect cells, Zablotskii et al., implemented a theoretical model to evaluate the effect of MFs on intercellular processes.\(^23\) Results indicated that MFs (1 T) may significantly change cell membrane potential and have a significant impact on the properties and biological functionality of cells. Although this finding opens new routes to apply the MFs, it is known that MFs greater than 0.5 mT cause DNA damage.\(^24\) In this context, we consider that some factors need to be taken into account: (a) different cell topologies to assess the cell membrane behavior after a stimulation with EFs and MFs, since a cell varies its morphology according to the stage and environment, (b) intensity of the electric or MFs to be applied to enhance the cell dynamics and avoid cell damage, and (c) the correct frequency to activate specific cell membrane zones according to the aim of the study. Accordingly, we hypothesize that well-established EFs or MFs may exert a positive effect on the cell membrane of different cell morphologies. The effect induced by the field generates a stimulation in specific points of the cell membrane where the ionic pumping proteins are located.\(^1\)\(^,\)\(^9\) To prove this hypothesis, a finite element model was implemented to consider the cell membrane as nonconductive layer that separates two conductive regions: the culture medium and the cytoplasm. Although a single cell was modeled in this study, it was simulated as a cell cultured in vitro which acquires different morphologies. The cell shape was varied in three different geometries: one spherical and two different ellipsoidal topologies. Results showed that there is a load concentration when the shape of the cell is ellipsoidal. It means that the EFs were accumulated when the area of the cell was smaller in the border. Regarding the MFs, there was not a perceptible effect on the cell membrane varying both the geometry and the frequency magnitude. This model is a computational tool that may be used to find the appropriate parameters to stimulate cells and tissues. Moreover, this computational simulation allows to predict the
influence that an external stimulation has on the cell membrane potential. Because the computational model allows controlling the variables used to stimulate cells such as field strength and frequency, this parameter characterization could be essential for non-invasive therapeutic methodologies focused on tissue recovery.

2 MATERIALS AND METHODS

2.1 Geometrical model of a single cell

A bidimensional domain to represent a single cell in a monolayer culture was performed in this study (Figure 1). The geometrical configuration to simulate the cell at three specific morphologies was considered: one spherical and two ellipsoidal. On the one hand, the spherical morphology was used to model a cell suspended into the culture medium (Figure 1A). On the other hand, the ellipsoidal morphology was modeled to simulate a cell that is starting the adhesion process to the well plate (Figure 1B). Finally, a second ellipsoidal morphology was used to simulate a cell completely attached at the bottom of the well plate (Figure 1C). These ellipsoidal shapes mimic a leading edge with pseudopods and extensions containing focal contacts where integrins and other adhesion molecules reside.25 Regarding the ellipsoidal shape, the minor axis (Y coordinate) was reduced, while the major axis (X coordinate) was increased in order to maintain a similar internal area in the three geometries used. The dimensions implemented to model the cell are shown in Table 1.

2.2 CELL CULTURE CONFIGURATION

Regarding the cell cultures configuration for electrical stimulation, two stainless steel electrodes were simulated to ensure the homogeneous and isotropic distribution of the EFs (Figure 2A). On the other hand, a coil was simulated in vertical and horizontal orientations to apply the MFs (Figure 2B). In the middle of the electrodes and the coil, a well plate was simulated to reproduce the surface in which the cells are attached. The air, culture media, and the single cell in their three morphologies were considered within the cell culture configuration. Both domains were considered as axisymmetric

![Figure 1](image1.png)

**Figure 1** Cell morphologies used in the computational model. A, Spherical morphology to simulate a cell in suspension in an in vitro monolayer environment; B, Ellipsoidal morphology of a cell which is starting the adhesion process at the bottom of a well plate; C, Ellipsoidal morphology of a cell completely attached to the bottom of the well plate

| Component       | Parameter     | Dimension (μm) | References |
|-----------------|---------------|----------------|------------|
| Cell membrane   | Thickness     | 0.005          | 26         |
| Major axis (μm) | Minor axis (μm) | 5 5           | 22,27      |
| Cytoplasm       | 8 2           | 9 1           | 22,27      |

**Table 1** Cell dimensions used in the computational model
models because these rotational configurations reduce computational analyses and the results can be extrapolated to a three-dimensional environment performing a revolution in the central axis.

2.3 Geometric discretization and boundary conditions

The domains and cell geometries were meshed using a triangle mesh with elements of 1.5 μm, and with a mesh refinement of 0.005 μm around the cell membrane reaching a minimum size of 0.003 μm. In Figure 3 it is possible to observe the axisymmetric domain used for the electrical simulations. The domain is composed by the electrodes, the well plate, the culture media, the cell, and air (Figure 3A). The domain for electrical simulations was meshed with 495.925 triangle elements (Figure 3B), while for magnetic simulations the domain was meshed with 291.019 triangle elements (See Appendix A). A mesh refinement near to the cell membrane of the cell was performed in order to better visualize the results when the electric and MFs reach the plasma membrane (Figure 3C). Figure 3D shows the cell membrane in detail, which is divided into two regions in order to measure the electric potential in the inner and outer regions of the cell. The boundary conditions for both electric and magnetic simulations consisted of a temperature of 310 K and a standard atmospheric pressure of 1 atm. The domain implemented for magnetic simulations and mesh refinement can be observed in Appendix A. The same domain and mesh refinement of the cell membrane was used for the ellipsoidal cell morphologies that were electrically and magnetically stimulated (Data does not show).

2.4 Electric and magnetic properties

The computational model was solved in the frequency domain solving the Maxwell equations, which describe the electromagnetic phenomena through the time (See Equations (1) to (4)).28

\[
\nabla \cdot E = \frac{\rho}{\varepsilon_0}, \quad (1)
\]

\[
\nabla \cdot B = 0, \quad (2)
\]

\[
\nabla \times E = -\frac{\partial B}{\partial t}, \quad (3)
\]

\[
\nabla \times B = \mu_0 j + \frac{1}{c^2} \frac{\partial E}{\partial t}, \quad (4)
\]
where $E$ is the EF, $B$ is the MF, $\rho$ is a charge function, and $j$ is an electric current. The quantities $\varepsilon_0$ and $\mu_0$ are physical constants called the permittivity and permeability of vacuum, respectively. The speed of light $c$ results from the relation $c^2 = 1/\varepsilon_0\mu_0$. When boundary conditions for the fields are specified, these equations completely and uniquely determine the fields. The electric and magnetic properties and dimensions of the domains used in simulations are shown in Tables 2 and 3.

The EFs between the electrodes were generated using a differential potential of 100 Vp-p at frequencies of 60 kHz, 10 MHz, and 1 GHz. Additionally, a frequency window from 1 kHz to 1 GHz was applied in order to measure the EFs in five different parts of the cell plasma membrane. On the other hand, the current and number of turns of the coil to obtain MFs of 2 mT were 0.024 A and 15, respectively. Similar to the EF simulations, the frequencies tested were 60 kHz, 10 MHz, and 1 GHz. The coil was modeled as an extended area to avoid border effects.

### 2.5 MODEL IMPLEMENTATION

The domains and equations were discretized and numerically solved using the finite element method. The computational models were simulated and solved in COMSOL Multiphysics software (Comsol Inc. Los Angeles). In Figure 4 the procedure carried out to simulate the effect generated by the electric and MFs on a single cell is described. First, the
| Component        | Parameter              | Value               | References |
|------------------|------------------------|---------------------|------------|
| Cell membrane    | Relative permittivity  | 11.3                | 11         |
|                  | Electrical conductivity| 0                   |            |
|                  | Relative permeability* | 1                   |            |
| Cytoplasm        | Relative permittivity  | 80                  | 29         |
|                  | Electrical conductivity| 1.5 (S/m)           | 30         |
|                  | Relative permeability* | 1                   |            |
| Culture medium   | Relative permittivity  | 80                  | 29         |
|                  | Electrical conductivity| 1.5 (S/m)           | 30         |
|                  | Relative permeability* | 1                   |            |
| Copper           | Relative permittivity  | 1                   |            |
|                  | Electrical conductivity| 5.998E7 (S/m)       |            |
|                  | Relative permeability  | 1                   |            |
| Stainless steel  | Relative permittivity  | 1                   |            |
|                  | Electrical conductivity| 1.7391 (S/m)        |            |
| Well plate       | Relative permittivity  | 2.6                 | 22         |
|                  | Electrical conductivity| 10E-16 (S/m)        |            |
|                  | Relative permeability  | 1                   |            |
| Air              | Relative permittivity  | 1                   |            |
|                  | Electrical conductivity| 0 (S/m)             |            |
|                  | Relative permeability  | 1                   |            |

*Note:* The relative permeability for tissues is approximately 1.31

| Component        | Parameter       | Dimension (μm) | Reference |
|------------------|-----------------|----------------|-----------|
| Cell membrane    | Thickness       | 0.005          | 26        |
| Culture medium   | Height          | 25             |           |
|                  | Width           | 30             |           |
| Coil             | Width           | 2              |           |
|                  | Height          | 200            |           |
|                  | Axis distance   | 50             |           |
| Well plate       | Thickness       | 1              | 22        |
| Electrodes       | Separation      | 50             |           |
|                  | Thickness       | 5              |           |
|                  | Width           | 70             |           |
| Air              | Width           | 30             |           |
|                  | Height          | 23             |           |

axisymmetric configuration to represent both domains, electric and magnetic, was selected. Then, the module of alternating current (AC)/direct current (DC) was selected in order to simulate the EFs and MFs; additionally, the frequency domain study was selected since both fields were applied in AC. Thereafter, the domains composed by the well plate, cell culture media, air, cell, electrodes, and coil were built. The boundary conditions were assigned within the model to each domain. Once the domains were restricted, the material properties such as relative permittivity ($\varepsilon_r$), electrical conductivity ($\sigma$), and relative permeability ($\mu_r$) were defined for each component of the model. Then, the equations for electric...
and MFs were defined and inserted into the model. After, the domains were meshed using triangular elements; moreover, a mesh refinement in the cell membrane was performed. Finally, the model was solved in order to observe the field distribution in the whole domain. In the case that the model was not solved, the equations and their parameters were verified.

3 | RESULTS

3.1 Electrical stimulation

The first simulation consisted of finding the EFs that stimulate the cell membrane of spherical and ellipsoidal cells (Figure 5). A schematic representation to observe the EF distribution inside the cell membrane, the extracellular environment and the cytoplasm is shown in Figure 5A, as well as the flow of the EF around the cell membrane (yellow arrows in Figure 5A). Results showed that EFs on cell membrane of round morphology cells were of $45 \times 10^3$ V/m for a frequency of 60 kHz, while for frequencies of 10 MHz and 1 GHz the EFs were $69 \times 10^3$ and $72 \times 10^3$ V/m, respectively (Figure 5B). Results evidenced that the EFs tend to decrease at the edge of spherical cell membranes; however, this field reduction was uniform when the radius of the cell membrane was constant. The first ellipsoidal cell morphology stimulated with 60 kHz evidenced an EF of $8.1 \times 10^5$ V/m over the cell membrane (Figure 5C), while the second ellipsoidal morphology evidenced an EF of $1.31 \times 10^6$ V/m in the plasma membrane (Figure 5D). Both ellipsoidal cell morphologies stimulated with frequencies of 10 MHz and 1 GHz did not evidence the peak effect at the edge of the cell membrane. It was possible to observe that the effect of EFs on the cell membrane of ellipsoidal cells was higher when the frequencies increased. A physical phenomenon known as the peak effect occurred after stimulating the cell membrane of ellipsoidal morphologies. This phenomenon shows the accumulation of an electric charge on the surface of a material, which is stimulated by
The load value obtained by the peak effect is distributed equally over the entire surface; therefore, if the area is smaller, the load density will tend to be higher. Accordingly, the peak presented in Figure 5C, D represents a load density in a specific zone of the cell membrane when the cell was stimulated with a frequency of 60 kHz.

A comparison of the EF induced on the plasma membrane of cells that where stimulated with EFs at 60 kHz is shown in Figure 6A. It was possible to observe that cells with spherical shape had a lower EF compared with EFs at the edge of ellipsoidal morphology cells. In order to observe how the frequency influences the cell membrane in terms of EF distribution, a frequency window simulation from 1 kHz to 1 GHz was used to calculate the EFs in five different parts of the cell membrane of an ellipsoidal cell (Figure 6B). Results evidenced that in points A and B, near the center of the cell, the EFs were lower, at $0.43 \times 10^6$ and $0.51 \times 10^6$ V/m, respectively. At point C, near the edge of the cell, the EF concentration in the cell membrane when the cell was stimulated with lower frequencies was the highest ($1.25 \times 10^6$ V/m). A constant EF of $1.1 \times 10^6$ V/m was observed at point D when the cell was stimulated with frequencies from 1 kHz to 100 MHz. Finally, a constant EF of $0.77 \times 10^6$ V/m was obtained at the bottom of the well plate (point E) when the cell was stimulated with frequencies from 1 kHz to 1 GHz. Regardless of the zone in the cell membrane, the EFs tend to present an asymptotic behavior when the stimulation was applied at higher frequencies.

### 3.2 Magnetic Stimulation

The MF distribution across the ellipsoidal cell membrane and the culture system is shown in Figure 7. It was possible to observe that the MF was completely homogeneous between the extracellular environment, the cell membrane and the
FIGURE 6  Schematic representation of the peak effect in all cell morphologies and frequency window vs electric fields in different points of the cell membrane. A, Comparison of the peak effect generated at the edge of the cell membrane of spherical and ellipsoidal cells that were stimulated with a frequency of 60 kHz; B, Measurement of a frequency window from 1 kHz to 1 GHz performed to an ellipsoidal cell.

FIGURE 7  Distribution of the magnetic fields (MFs) through the cell membrane and coil with a frequency of 60 kHz. A, Distribution of MFs in the cell membrane of an elliptical cell, culture medium and cytoplasm at 60 kHz; B, Distribution of MF through the length of the coil.
cytoplasm (Figure 7A). The distribution of the MFs was uniform inside the coil with a value of 2 mT (Figure 7B). These results were the same varying the frequencies, the cell shape and the orientation of the coil (horizontal and vertical).

4 | DISCUSSION

This study presents a computational model to simulate and analyze the effect caused by an externally induced EF on the cell membrane of a single cell cultured in an in vitro configuration. The findings demonstrated that depending on the cell morphology, the EF intensities that stimulate the cell membrane can increase or decrease. Additionally, frequency is a relevant factor, since depending on the periodicity which the EF is applied, different cell membrane zones can be stimulated with a specific EF intensity. This computational result provides key information not only for understanding the mechanisms of cell responses to electrical stimulation, but also to predict which stimulation scheme may overcome the membrane potential to modify the gradient concentrations through the ionic pumps. Such ionic alteration could trigger the activation of intracellular proteins to increase the molecular activity of a cell.

Some studies that use EFs and MFs as external biophysical stimulus have observed an increased in proliferation, migration, cellular differentiation and synthesis of proteins. Although the effect over the cell membrane receptors and the cell dynamics such as proliferation and molecular synthesis were not simulated in this work, we have previously assessed the in vitro effects of EFs and MFs over monolayer chondrocytes. In a previous study, the findings indicated that EFs of 4 and 8 mV/cm applied at 60 kHz sine-wave form enhanced the proliferation rate and kept stable the synthesis of glycosaminoglycans. In our most recent work, it was evidenced that the best proliferation rate was obtained with MFs of 2 mT applied for 3 hours every 6 hours during 8 days. In addition, the increase in the synthesis of glycosaminoglycans was statistically significant when cells were stimulated with MFs of 2 mT applied for 5 hours every 6 hours during 8 days. The studies previously mentioned have evidenced that both EFs and MFs positively impact the cell dynamics of chondrocytes in terms of proliferation and molecular synthesis; nevertheless, the mechanism by which the external biophysical stimulations influence over the membrane receptors and their signaling pathways were not analyzed. In order to understand the effect that an electromagnetic stimulation has over the cell, some authors have suggested that electrical and magnetic stimuli enable the activation of signaling mechanisms through the voltage-dependent calcium channels (VDCC) and vanilloid transient potential receptors (TRPV4). These channels can be opened in response to changes in the membrane potential caused by an external stimulation. The activation of ionic channels allows the gradients of ionic inorganic diffusion to be greater across the cell membrane to activate different biochemical events such as gene and protein expression.

Whether VDCC or TRPV4 are electrically activated, an influx of ions such as intracellular calcium (Ca$^{2+}$) goes into the cytoplasm and activates different transcription factors. For instance, the EFs increase the influx of Ca$^{2+}$, which binds with calmodulin, an intracellular protein that regulates the signal transduction of calcium within the cell. This binding process induces the expression of SOX9, which promotes the production of proteins such as collagen type II and aggrecan. It has been demonstrated that the inductive MFs also influence Ca$^{2+}$ activation. Unlike the EFs, which directly affect the VDCC, the MFs increase the intracellular Ca$^{2+}$ levels stimulating organelles such as the mitochondria and the endoplasmic reticulum. The increase of Ca$^{2+}$ triggers the activation of cytoskeletal calmodulin, resulting in the activation of molecular synthesis. Multiple in vitro experiments have indicated that MFs promote cell proliferation and molecular synthesis of chondrocytes; therefore, this biophysical stimulus could be a potential therapeutic treatment for tissue regeneration.

Considering this evidence, this computational model is a useful tool from a biophysiological point of view, because it is possible to predict the intensity of the biophysical stimulus that is stimulating a specific zone of the cell membrane. Although this model can be extrapolated to stimulate different kind of cells to observe how membrane potentials can be altered, it could be improved simulating the outflow and inflow of inorganic ions and their effect on cellular dynamics, and taking into account the morphophysiological properties of each tissue and cells; for instance, to simulate how EFs and MFs affect the cell membrane proteins and cytosol organelles allowing the active (by ion channel opening and stimulation of pumps), and passive (by ionic diffusion) transport of ions through it. It has been well-documented that ion channels are relevant toward triggering different signaling pathways within the cell; for this reason, this computational model could be improved if the extra- and intracellular molecular regulation is modified while the cell is being stimulated with external biophysical stimuli. Although the signaling pathway derived from ion channels flux was not modeled in this study, this aspect should be the focus of future works to clarify how external biophysical stimuli act on the internal structures from an electrobiochemical point of view. Taking the foregoing into account, our results suggest that cellular mechanisms involved in the biological response to MFs are different compared to EFs. However, no similar models are available and further research is needed to confirm our observations.
The results derived from this study agree with those obtained by Taghian et al, since it was possible to evidence that frequency has an effect on the cell membranes when they are stimulated with EFs. However, there are notable variations compared with our results, as the EF in round cells stimulated with 10 MHz was $12 \times 10^5$ V/m for the above-mentioned study, while we obtained an EF of $72 \times 10^3$ V/m. This discrepancy in magnitude could be caused by the mesh refinement to the cellular domain and the dielectric properties used in the model. Additionally, in our study some changes were observed in the distribution of the EFs in the cell membrane as a function of cell morphology when the shape changes from spherical to ellipsoidal. Here, it is noteworthy to mention that the effect caused by external stimulation could vary if the actual cell topography is simulated, since there are significant height changes ($Z$ axis) over the range of the cell, being highest in the middle region with the contained nucleus. The most relevant differences seen were the load concentration area when the cell is adhered to the well plate. This may be relevant when interpreting in vitro results and their potential extrapolation to in vivo investigations. Based on this observation, some in vitro studies demonstrate that EF values induced cell membrane permeabilization. For example, Teissie et al applied EFs to Chinese hamster ovary cells and demonstrated that while the magnitude of the EF increases, the permeabilization of the cell rises up to a value of 1 kV/cm. Similar results were obtained by Saulis et al who increased the extracellular concentration of potassium ($K^+$) with a maximum value of 1.2 kV/cm. Comparing those results with our model, it is possible to say that the frequency of 60 kHz with a spherical shape has similar EF values over the cell membrane. Considering that some experimental studies have observed the effect generated by external biophysical stimuli over the cell membrane receptors, it is noteworthy to mention that this study presents certain limitations in order to validate the computational results obtained. For this reason, an experimental validation is necessary to observe how different frequencies modify the cell membrane response in terms of ion concentration flows and their incidence on the cell dynamics, such as viability, migration, proliferation, and molecular synthesis.

According to the results obtained by Caraglia et al, we can hypothesize that EFs could induce cell death, since in their study it was evidenced that EFs applied at 1.95 GHz decrease the viability of cancer cells. In this context, it should be concluded that stimulation at very high frequencies is not favorable for cell viability. Therefore, computational models could be an important tool to achieve a clear understanding of how external biophysical stimuli affect the integrity of the cell membrane and internal organelles. In this context, the model presented here has some limitations in terms of simulating the effect generated by the EFs and MFs on the cell membrane receptors and the internal organelle structures of the cell. Nevertheless, it has been evidenced that nano-pulsed EFs of 60 kV/cm at 60 ns applied to different type of cells induced damage to the cytoskeleton, nuclear membrane, and telomere sequences. These results contrast with the findings reported by other studies that have produced promising outcomes in terms of proliferation, migration and molecular synthesis. Considering that cell-recovery following external electrical or magnetic pulsing has been observed and is well documented, Pliquett et al, establish that destruction either in the nuclear membrane or other inner membranes could probably produce severe biological disruptions, leading to irreversibility and cell death.

It is relevant to mention that this study presents certain limitations, because a monolayer culture has a cell-cell contact population when abutting each other. This contact allows for cell-cell paracrine effects, affecting the electrical properties of the cell and the population in general. Furthermore, it is known that impacting a single cell can send waves of Ca$^{2+}$ signaling across the neighboring cells, and similar electrochemical effects. Due to this fact it is proposed that future studies should be directed to the realization of a simulation in three dimensions with several cells close to each other, so it can be analyzed if there is any change or interaction between the cells when they are exposed to EFs or MFs. It is important to find experimentally cell magnetic properties such as the relative permeability of the cell membrane and the cytoplasm in order to find an accurate explanation of the MF effect caused over the cell.

5 CONCLUSION

It has been well described that EFs and MFs are used in physiotherapeutic trials to relieve the pain locally. However, recent studies have demonstrated that these biophysical stimuli not only have an effect on different injured parts of the human body, but also may trigger desired molecular responses at the cell and tissue level. For this reason, different researches have been working on assessing the effect generated by the EFs and MFs at a cellular level in order to understand how these biophysical stimuli modify the signaling pathways at an intra and extra cellular level. Considering that the standardization of protocols to find the best parameters to stimulate the cells is a challenge, and that the experimental procedures to assess the effect generated by the external stimulations are not well-documented, there is a need to find alternative methods that allow predicting the response of cells and tissues after a stimulation either with EFs
or MFs. Accordingly, numerical models are useful tools that can be implemented, not only to predict the influence of the biophysical stimuli over the intra- and extracellular environment of cells, but also to simulate the device configurations according to the needs of the experiment.

The computational modeling of electric and MFs has the potential of simulating different cellular processes such as viability, proliferation, migration, differentiation and molecular synthesis.\(^1\)\(^2\)\(^2\) In fact, computational models have the versatility to quantify, predict and understand the behavior of complex systems that show nonlinear behavior in space and time.\(^5\)\(^5\) The computational model developed in this study provides a predictive tool that quantifies the electric and MFs that stimulate different cell membrane zones. This finding not only allows understanding how the cell membrane potential can be stimulated, but it also serves as a tool to predict the EFs or MFs that could trigger different cellular responses. In fact, this computational model could be improved if the galvanotactic property of the cell is considered, since the direction, concentration, and isolation of cells could be simulated in order to predict the key role of the cell during tissue growing.\(^1\)

Overall, the computational model is a promising tool that, combined with experimental data, could be used to standardize the electric and magnetic intensities, the stimulation times, and the period that the biological samples need to be under stimulation in order to increase the proliferation rate and stimulate the molecular synthesis. This work shows a computational approach as a tool to understand the mechanism by which EFs and MFs impact the membrane potential. These results evidence that computational models are a useful mechanism to predict the effects caused by an external stimulus at micrometric scales and extrapolate these observations to a possible experimental scenario. In addition, the model presented in this study has the versatility to modify parameters such as electrodes and coil dimensions, well plate types, cell morphologies, frequencies and voltages in order to find the electric and magnetic mechanisms that better fit the required cellular responses. In this context, the electrical and magnetic stimulation devices that are used in therapeutic methods in clinical trials can be simulated before construction in order to improve patient comfort and treatment compliance.

ACKNOWLEDGEMENTS

The authors gratefully thank the research support from the Biotechnology Institute of the Universidad Nacional de Colombia for providing the lab space at the Biomimetics laboratory. This work was supported by the project “In vitro evaluation of the effect of biophysical stimuli on cell cultures, as a tool to stimulate the regeneration of joint tissues.” The project has received funding from the Departamento Administrativo de Ciencia, Tecnología e Innovación (COLCIENCIAS) through the grant 712-2015 No. 50457.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

J.F.E. was responsible for gathering data, building the computational model for magnetic simulations, analysis and interpretation of results and drafting the manuscript. J.J.V.-G. was responsible for gathering data, building the computational model for electric simulations, analysis and interpretation of results and drafting the manuscript. J.M.G. was responsible for the critical review of the manuscript for important intellectual content and drafting the manuscript. D.A.G.-A. was responsible for the critical review of the manuscript for important intellectual content and give final approval of the version to be published.

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**How to cite this article:** Escobar JF, Vaca-González JJ, Guevara JM, Garzón-Alvarado DA. Effect of magnetic and electric fields on plasma membrane of single cells: A computational approach. *Engineering Reports*. 2020;2:e12125. [https://doi.org/10.1002/eng2.12125](https://doi.org/10.1002/eng2.12125)