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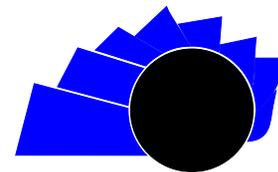
VISIÓN ELECTRÓNICA

A RESEARCH VISION

The effect of frequency in the electrical stimulation of chondrocytes
El efecto de la frecuencia en la estimulación eléctrica de condrocitos

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ABSTRACT

Electrical stimulation is a non-invasive therapy used to stimulate chondrocyte dynamics: proliferation, migration, morphology and molecular synthesis. Some studies have evidenced the role of frequency in the generation of electric fields; however, the electrical stimulation sensed by chondrocytes when the frequency varies is not well-documented. Accordingly, a computational model was implemented to assess the frequency dependence of electric fields that stimulate chondrocytes. Cells were modelled in three different scenarios: monolayer cultures, cartilage explants and scaffolds. Chondrocytes were stimulated with 100 Vp-p at frequencies of 0.001, 1, 10, 50, 100 and 1000 kHz. Results showed that frequency is a relevant factor when considering the stimulation of biological samples, since electric fields increased as frequencies were higher. Moreover, chondrocytes experienced different electric fields in both cytoplasm and extracellular environment. This model provides relevant information about the electrical parameters to stimulate cells; in fact, it could enhance experimental procedures, predicting the stimulation that improves chondrocyte dynamics. Electric fields are a promising tool to maintain either well-structured chondrocytes or biomimetic materials used in regenerative therapies such as autologous implantation to treat hyaline cartilage injuries.

RESUMEN

La estimulación eléctrica es una terapia no invasiva utilizada para estimular la dinámica de los condrocitos: proliferación, migración, morfología y síntesis molecular. Algunos estudios han evidenciado el rol de la frecuencia en la generación de campos eléctricos; sin embargo, la estimulación eléctrica percibida por el condrocito cuando la frecuencia varia no está bien documentada. Por esto, se implementó un modelo computacional para evaluar la dependencia de la frecuencia de los campos eléctricos que estimulan los condrocitos. Las células fueron modeladas en tres escenarios diferentes: cultivos monocapa, explantes de cartílago y andamios. Los condrocitos fueron estimulados con 100 Vp-p a frecuencias de 0.001, 1, 10, 50, 100 y 1000 kHz. Los resultados mostraron que la frecuencia es un factor relevante para estimular muestras biológicas, ya que los campos eléctricos aumentaron a medida que la frecuencia se incrementó. Además, los condrocitos experimentaron diferentes campos eléctricos tanto en el citoplasma como en el ambiente extracelular. Este modelo provee información relevante acerca de los parámetros eléctricos para estimular células; de hecho, este puede mejorar los procedimientos experimentales, prediciendo la estimulación que mejora la dinámica de los condrocitos. Los campos eléctricos son una herramienta prometedora para mantener ya sea condrocitos bien caracterizados o materiales biomiméticos usados en terapias regenerativas tales como la implantación autóloga para tratar lesiones del cartílago hialino.

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1.   Introduction

Hyaline cartilage is an avascular tissue composed by a single cell type, the chondrocyte [1]. This cell is responsible for synthetizing the main macromolecules located in the cartilaginous tissue: collagen and proteoglycans [2]. Hyaline cartilage is located in two specialized structures of long bones: articular cartilage and growth plate [3–5]. The former acts as a smooth, lubricated and low friction surface that supports mechanical loads and it facilitates the movement between joints [1, 6], while the latter structure is responsible for the longitudinal growth and the shape of long bones [6]. Considering that hyaline cartilage is exposed to a combination of mechanical loads, its avascularity and low proliferative capacity are counterproductive factors that limit the self-healing process of the tissue [7, 8].

Considering that hyaline cartilage responds to external biophysical stimuli, it has been demonstrated that tissue dynamics are modified when electric fields (EFs) are applied. For instance, in vitro assays have assessed the effect of EFs on chondrocyte behavior within the hyaline cartilage using different culture systems such as monolayer cultures, cartilage explants and tridimensional constructs [9–23]. These studies focused on the assessment of viability, proliferation, synthesis of extracellular matrix components and morphological changes of chondrocytes. However, there are discrepancies in the obtained results, since there are limitations in the adequate frequency to apply the EFs, the voltage required to generate the EFs, the stimulation times and the amount of days that the cultures need to be under stimulation. Moreover, the methodology to calculate, generate and homogeneously distribute the EFs remains a largely unexplored field. Accordingly, different computational approaches have been implemented to assess the effect generated by the application of EFs to cells. For instance, mathematical models were developed to observe the membrane potential in a spherical cell suspended in an electrolyte medium. Results evidenced that induced potential decreases due to surface conductance in small cells, while for bigger cells this potential decreases due to membrane conductance [24]. On the other hand, an electrolyte medium with low conductivity increases the charging time of the cell membrane; therefore, cells immersed in this kind of medium need to be stimulated with higher EFs to induce electroporation in the cell membrane [25]. In a study carried out by Krassowska et al., the polarization and physiological state of a single cell electrically stimulated was modeled [26]. A similar study analyzed the variation of the transmembrane potential on prolate and oblate spheroidal cells stimulated with EFs. Results evidenced that the transmembrane potential strongly depends on the cell orientation when a cell is being stimulated [27]. Similarly, computational models to simulate the effect of alternating current EFs on spheroidal cells were implemented. Results indicated that time-dependent geometry changes influence the induced membrane potential of the cell; additionally, the time-dependent charging and discharging of the membrane can be modeled when the frequency dependence is transformed into a time dependence [28, 29].

Even though the computational models evidenced that transmembrane potential vary according to cell shape, size and orientation, the models did not consider different frequencies and dielectric properties of the in vivo extracellular environment where the cell is located such as living tissues and three-dimensional structures. The computational model that has evaluated the effect of external EFs on a single cell cultured in monolayer was developed by Taghian *et al.*, [30]. The results showed that the cell membrane behavior of the cell strongly depends on the frequency applied; however, the computational analysis was performed using a one-dimensional round cell morphology in one of the possible scenarios where a cell may grow. In this context, the role of the frequency and extracellular matrix in terms of distribution of EFs around and within the cell has not been well-documented. For this reason, we hypothesize that a computational model may estimate the EFs sensed by the cells according to the environment where they are located. To prove this hypothesis, a finite element model was implemented to simulate chondrocytes cultured in three different scenarios: 1) a cell cultured in monolayer an surrounded by culture media [22], 2) a chondrocyte within a cartilage explant of two day-old bone rat [21], and 3) a cell inside of a chondrogenic scaffold composed by two biopolymers such as hyaluronic acid and gelatin [31]. The computational model was simulated to mimic the stimulation of a biological sample using two external stainless-steel electrodes located at the bottom and upper surface of a culture well plate. Result evidenced that the EFs sensed by the cells are frequency-dependent, since at higher frequencies the EFs are higher. Moreover, it was possible to determine that the EFs in cells cultured in monolayer is different intra and extracellularly, since the EFs near to the cell membrane are higher than the EFs in the cytoplasm of the cell. Additionally, the EFs sensed by the chondrocytes located at different positions within the explant were different. The EFs that stimulated cells in the superficial part of the explant were higher compared with the EFs perceived by the chondrocytes located close

to the ossification of the explant. The findings derived from this computational model could be used to estimate the EFs desired to trigger intracellular responses from the cell. In fact, it has been reported that external EFs modifies the ionic fluid flow of the cell membrane; for this reason, this computational model may be a useful tool to improve experimental procedures. For instance, the model could predict the field strength and frequency that stimulate the cell membrane receptors such as ionic transmembrane channels responsible for activating different signaling pathways within the cell cytoplasm.

2. Materials and Methods

2.1. Geometrical models and boundary conditions

A bi-dimensional axisymmetric domain was implemented to represent the capacitive coupled system. The capacitive coupled system is composed of two parallel stainless-steel electrodes, which are located at the top and bottom of a culture well plate. Both culture media and biological samples were placed within the culture well plate, respectively (Figure 1). In this study, biological samples were modelled using two different well plate dimensions according to previous experimental procedures performed in our laboratory [21, 22, 32]. The single cell and the cartilage explant were simulated in a 6 well plate, while the chondrogenic scaffolds was modelled in a 48 well plate. The dimensions of the different materials that compose the capacitive system are listed in Table 1, while their dielectric properties, i.e. relative permittivity (ϵ) and electric conductivity (σ), are shown in Table 2. The boundary conditions for electric simulations consisted on a temperature of 310 K and a standard atmospheric pressure of 1 atm. The EFs were generated by applying 100 Vp-p at frequencies of 0.001, 1, 10, 50, 100 and 1000 kHz sine-wave form. The computational simulation was solved by a finite element analysis using an electromagnetic simulations software (COMSOL Multiphysics, Comsol Inc, Los Angeles CA, USA).

Figure 1: Geometries used for computational simulations. A) Culture well plate used for electrical stimulations. B) Three-dimensional representative scheme of the three culture systems simulated. C) Representative scheme of the bi-dimensional axisymmetric domain.
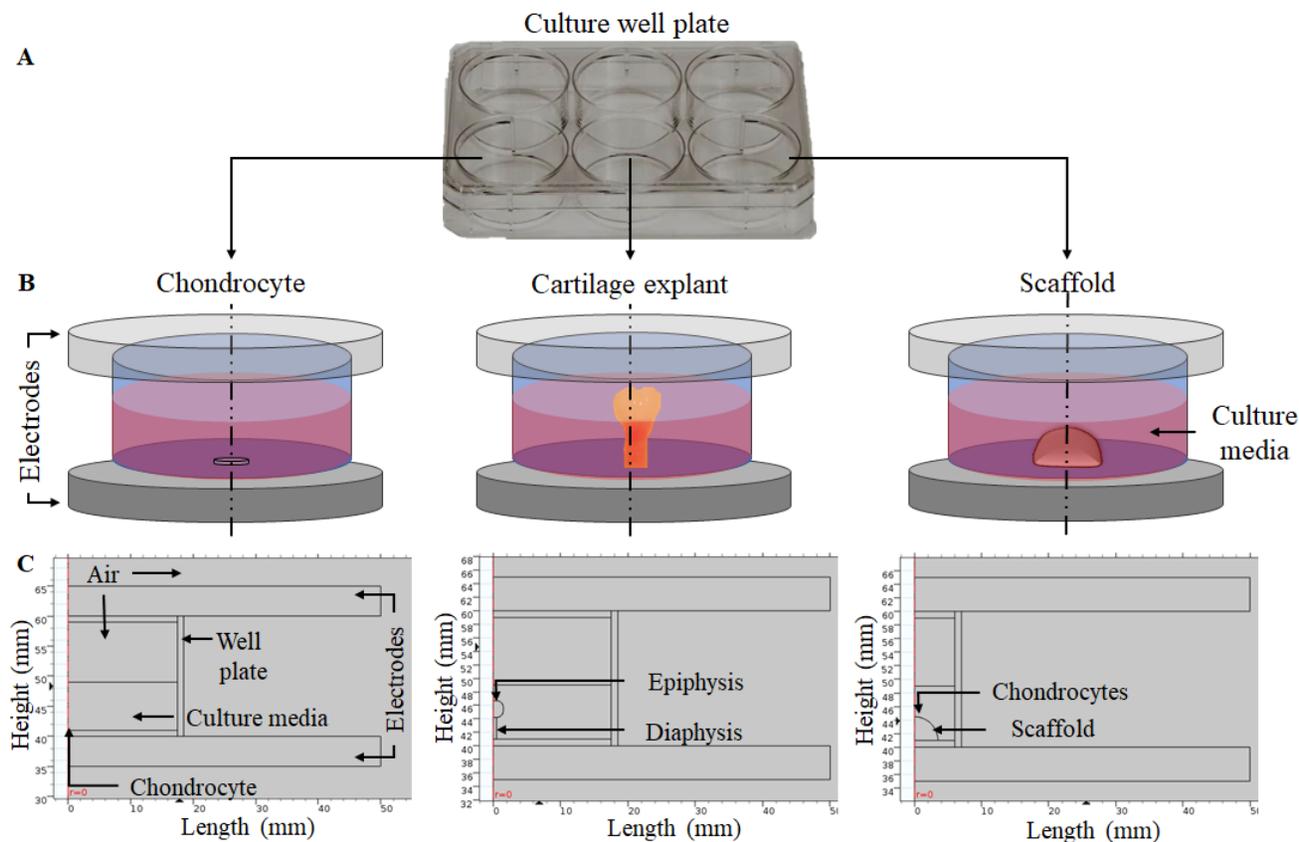
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Table 1: Dielectric properties and measurements of the capacitive coupled system.

Component	Parameter	Value		Reference
Stainless-steel electrode	Separation	20 [mm]		[21]
	Radius	50 [mm]		
	Thickness	5 [mm]		
	ε	1		
	σ	1.73[MS/m]		
Well plate	Thickness	1 [mm]		[21], [31]
	Height	20 [mm]		
	Radius	48 well plate 6 [mm]	6 well plate 17.5 [mm]	
	ε	3.5		
	σ	6.6 x10⁻¹⁶ [S/m]		
Air	ε	1		[21]
	σ	0[S/m]		

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Table 2: Dielectric constants of the biological samples.

		Frequency (kHz)						Reference
		0.01	1	10	50	100	1000	
Cell	ε	74.3						[33]
	σ [S/m]	1.02						
Cartilage	ε	2.0E+07	3.2E+04	4.04E+03	2.7E+03	2.5E+03	1.3E+03	[34]
	σ [S/m]	1.6E-01	1.7E-01	1.76E-01	1.7E-01	1.9E-01	2.3E-01	
Bone	ε	1.0E+06	5.6E+03	6.76E+02	1.8E+02	1.1E+02	3.8E+01	
	σ [S/m]	9.7E-04	2.2E-03	2.73E-03	3.3E-03	3.2E-03	4.1E-03	
Scaffold	ε	3.3E+06	1.8E+05	4.81E+04	8.6E+03	3.2E+03	9.2E+02	[31]
	σ [S/m]	2.1E-03	1.1E-02	3.64E-02	7.0E-02	8.6E-02	1.1E-01	
Culture media	ε	1.6E+06	1.2E+05	5.77E+04	2.7E+04	1.1E+04	2.5E+03	
	σ [S/m]	1.6E-02	4.5E-03	1.78E-02	6.8E-02	1.7E-01	3.4E-01	

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2.2. Estimation of EFs in chondrocytes cultured in monolayer

An axisymmetric monolayer culture with a single chondrocyte was simulated (Figure **2**A). The chondrocyte, completely attached to the bottom of a 6 well plate, was modelled considering an ellipsoidal morphology. Cell dimensions were 9 μm of length and 1 μm of height [35], while dielectric constants used for the cell are described in table **2**. The domain was meshed using triangular elements obtaining 136,542 elements.

2.3. Estimation of EFs in hyaline cartilage explants

The cartilage explant, cultured in a 6 well plate, was simulated as an axisymmetric model (Figure **2**B). The explant was composed by two specialized tissues:

the bone (diaphysis) and the cartilage (epiphysis). The bone dimensions were 3.2 mm of length and 0.5 mm of thickness, while the cartilage dimensions were 2.5 mm and 1.5 mm for length and thickness, respectively [21]. Additionally, chondrocytes of 10 μm were located at the bottom, middle and upper surface of the cartilage to be simulated (Figure **2**B). The dielectric properties of the cell, bone and cartilage are described in table **2**. The domain was meshed using triangular elements obtaining 136,960 elements.

2.4. Estimation of EFs in chondrogenic scaffolds

The chondrogenic scaffold, cultured in a 48 well plate, was simulated as an axisymmetric model (Figure **2**C). The chondrogenic scaffold was composed by a mixture of two biocompatible polymers: hyaluronic acid

and gelatin. The scaffold was simulated as a drop with a radius of 3.5 mm with chondrocytes of 10 μm located at the bottom, middle and upper surface of the three-dimensional construct (Figure **2**C). The dielectric properties of the cell and scaffold are shown in table **2**. The domain was meshed using triangle elements obtaining 136,976 elements.

Figure 2: Meshes of the three different culture systems simulated in the study. A) Culture system used to stimulate a single chondrocyte cultured in monolayer. B) Culture system implemented to stimulate chondrocytes of a cartilage explant cultured in vitro. C) Culture system performed to stimulate chondrocytes embedded in a hyaluronic acid – gelatin scaffold.
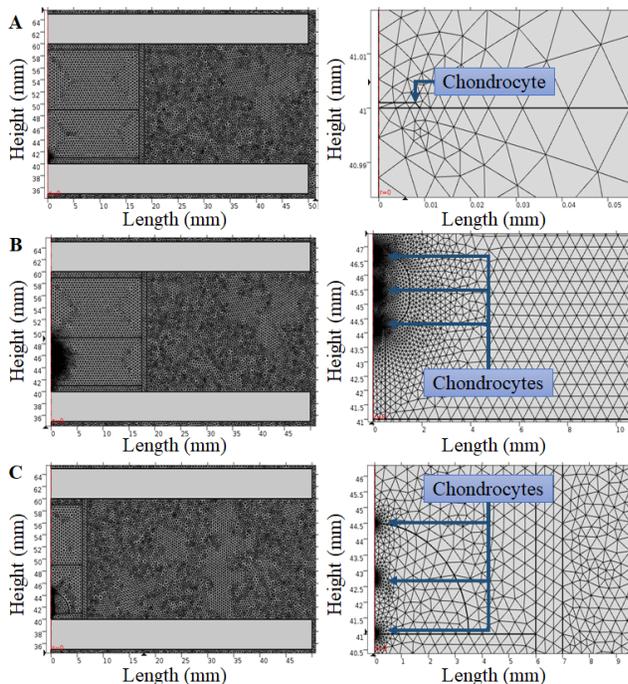
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temperature and pressure, were assigned within the model. Once the domains were restricted, the material properties ϵ and σ were defined for each component of the model. Then, the equation for EFs was defined and inserted into the model to be solved. After, each domain was meshed using triangular elements, followed by a mesh refinement where the cells were located. Finally, the model was solved to observe the field flow distribution in the whole domain.

Figure 3: Flowchart of the computational model implementation.
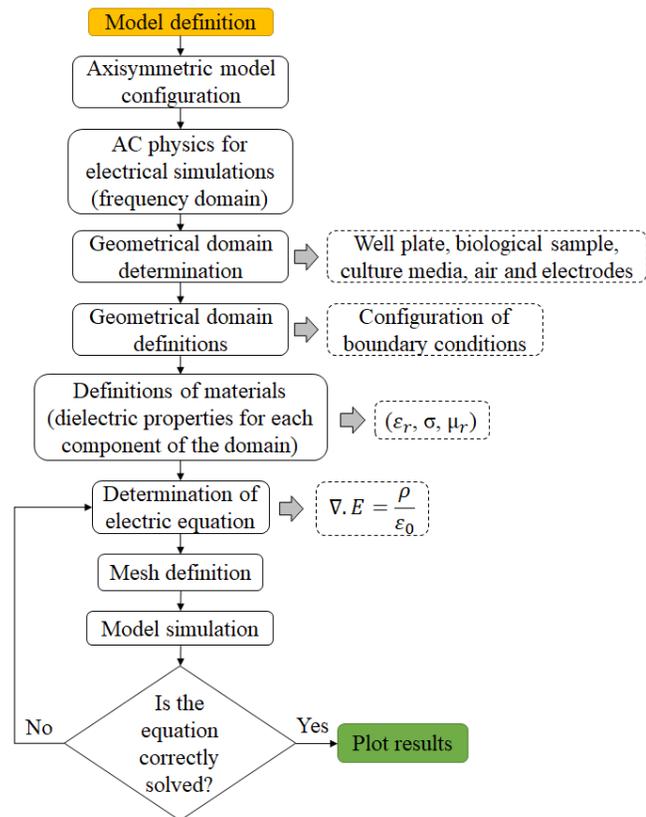
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2.5. Model implementation

The procedures to simulate the effect generated by EFs on a single cell, a cartilage explant and a scaffold are described in figure **3**. First, an axisymmetric model was selected to represent the domains of the EFs. Then, the physics to simulate the EFs was solved using the Electric Currents interface from the alternating current (AC) module. Next, the simulation of EFs was carried out in the Frequency Domain study. After that, the domain composed by the well plate, the biological sample, the cell culture media, the air and the electrodes were built. The boundary conditions for each domain, specifically

3. Results

3.1. EFs distribution in monolayer cultures

The flow of the EFs through the monolayer cell culture system is completely homogeneous, specially within the chondrocytes and the cell culture media where the cells were attached (Figure **4**A). Results evidenced that the EFs tend to increase as the frequency was higher. For instance, the EFs in the culture media at 1 kHz were around 1.8 mV/cm, while EFs at 100 kHz were around 9.2 mV/cm; moreover, a negligible decrease in the EFs was observed from the bottom to the upper surface of

the culture media (Figure **4**B). Regarding the EFs in the cytoplasm of the chondrocyte, EFs of 0.02 mV/cm for 1 kHz were observed, while EFs of 1.9 mV/cm for 100 kHz were perceived (Figure **4**C). Finally, the EFs near to the cell membrane of the chondrocyte were 2.4 mV/cm for 1 kHz and 12.1 mV/cm for 100 kHz (Figure **4**D). Monolayer cultures that were stimulated with 1 MGz evidenced similar EFs of 12.5 mV/cm in the cytoplasm of the chondrocyte and in the extracellular environment near to the cell membrane.

3.2.   EFs distribution in cartilage explants

A heterogeneous EF distribution was observed inside the explant (Figure **5**A). Results evidenced an increase of EFs in stimulated explants as the frequency was higher; in fact, the EFs inside the culture media were the same as reported in monolayer cultures with a negligible decrease of the EFs from the bottom to the upper surface (Figure **5**B). The EFs were measured in both bone and cartilage of the explant (Figure **5**C). On the one hand, different EF intensities along the bone were observed; for example, EFs of 8.3 mV/cm for 1 kHz and 573 mV/cm for 100 kHz

were evidenced at the base of the bone. The EFs in the middle of the bone were 3 mV/cm for1 kHz and 11.8 mV/cm for 100 kHz. The EFs near the ossification front were 3.9 mV/cm for 1 kHz and EFs 21 mV/cm for 100 kHz (Figure 5D). On the other hand, the EFs intensity through the cartilage tend to be the same; for example, EFs of 0.2 mV/cm for 1 kHz were observed, while EFs of 7.9 mV/cm for 100 kHz were perceived (Figure **5**E). The EFs were also measured in the cytoplasm of chondrocytes that were located in three different positions within the cartilage. The first measurement was performed in a chondrocyte located in the articular surface where the EFs were around 0.05 mV/cm for 1 kHz and 3.1 mV/cm for 100 kHz (Figure **5**F). The second measurement was conducted in a chondrocyte located in the middle of the cartilage where the EFs were 0.08 mV/cm for 1 kHz and 3.4 mV/cm for 100 kHz (Figure **5**G). Finally, the EFs were measured in a chondrocyte located near the ossification front. Here, the EFs were 0.03 mV/cm for 1 kHz and 0.3 mV/cm for 100 kHz; moreover, a negligible increase of the EFs inside the cytoplasm was observed (Figure **5**H).

Figure 4: Distribution of EFs in a chondrocyte cultured in monolayer. A) Electric flow diagram inside the culture well plate (measurement in mV/cm). B) Distribution of EFs inside the culture media. C) Distribution of EFs in the cytoplasm of the chondrocyte. D) Distribution of EFs around the cell membrane of the chondrocyte.
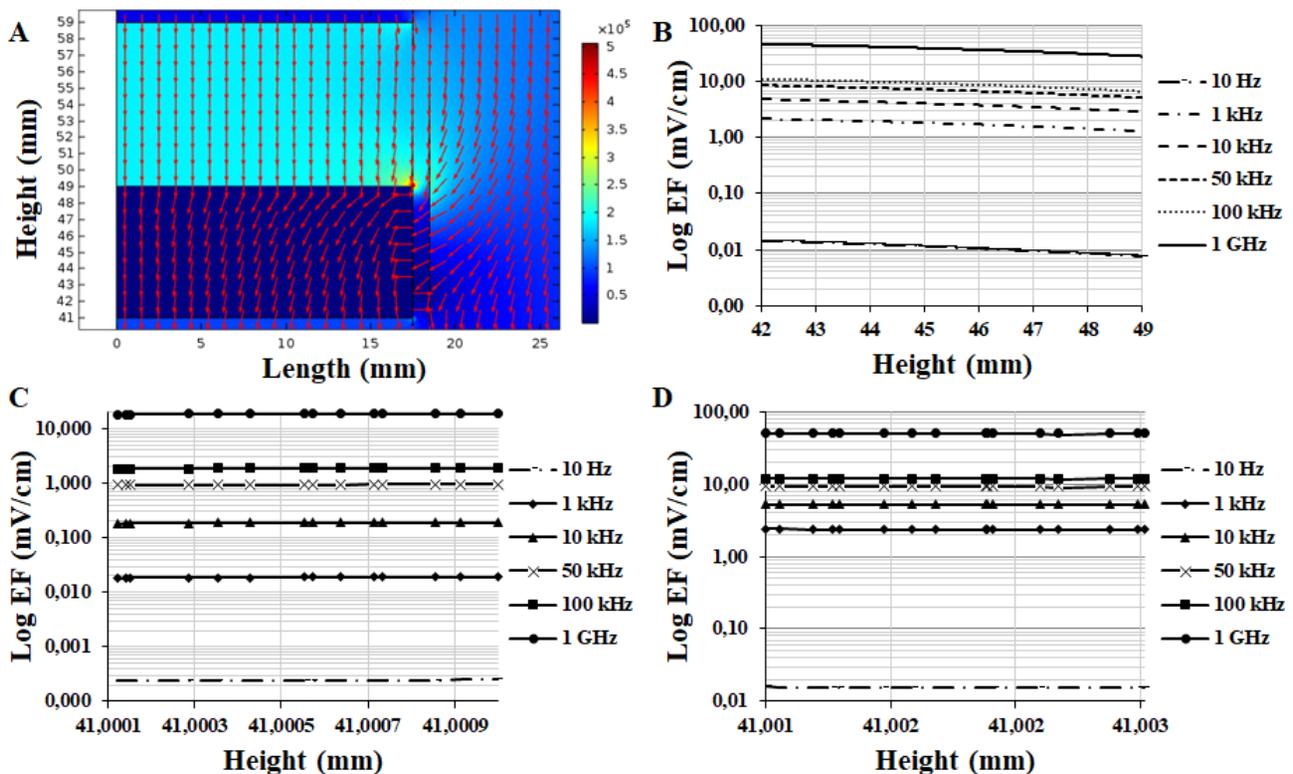
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Figure 5: Distribution of EFs in cartilage explant cultured in vitro. A) Electric flow diagram inside the culture well plate and around the explant (measurement in mV/cm). B) Distribution of EFs inside the culture media. C) Distribution of EFs in the entire explant. D) Distribution of EFs inside the bone. E) Distribution of EFs inside the cartilage. F) Distribution of EFs inside the chondrocyte located at the upper surface of the cartilage. G) Distribution of EFs inside the chondrocyte located in the middle of the cartilage. H) Distribution of EFs inside the chondrocyte located at the bottom of the cartilage.
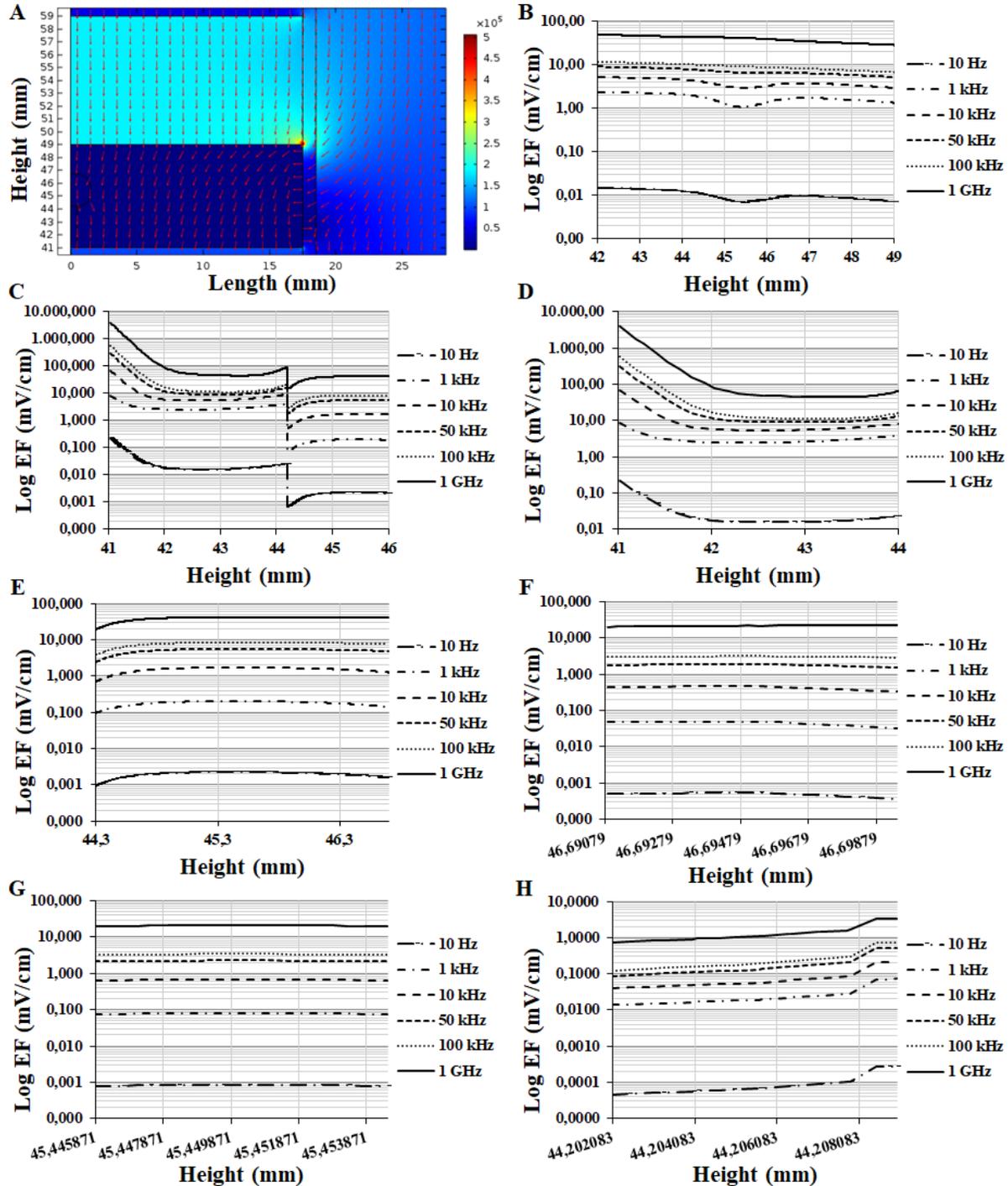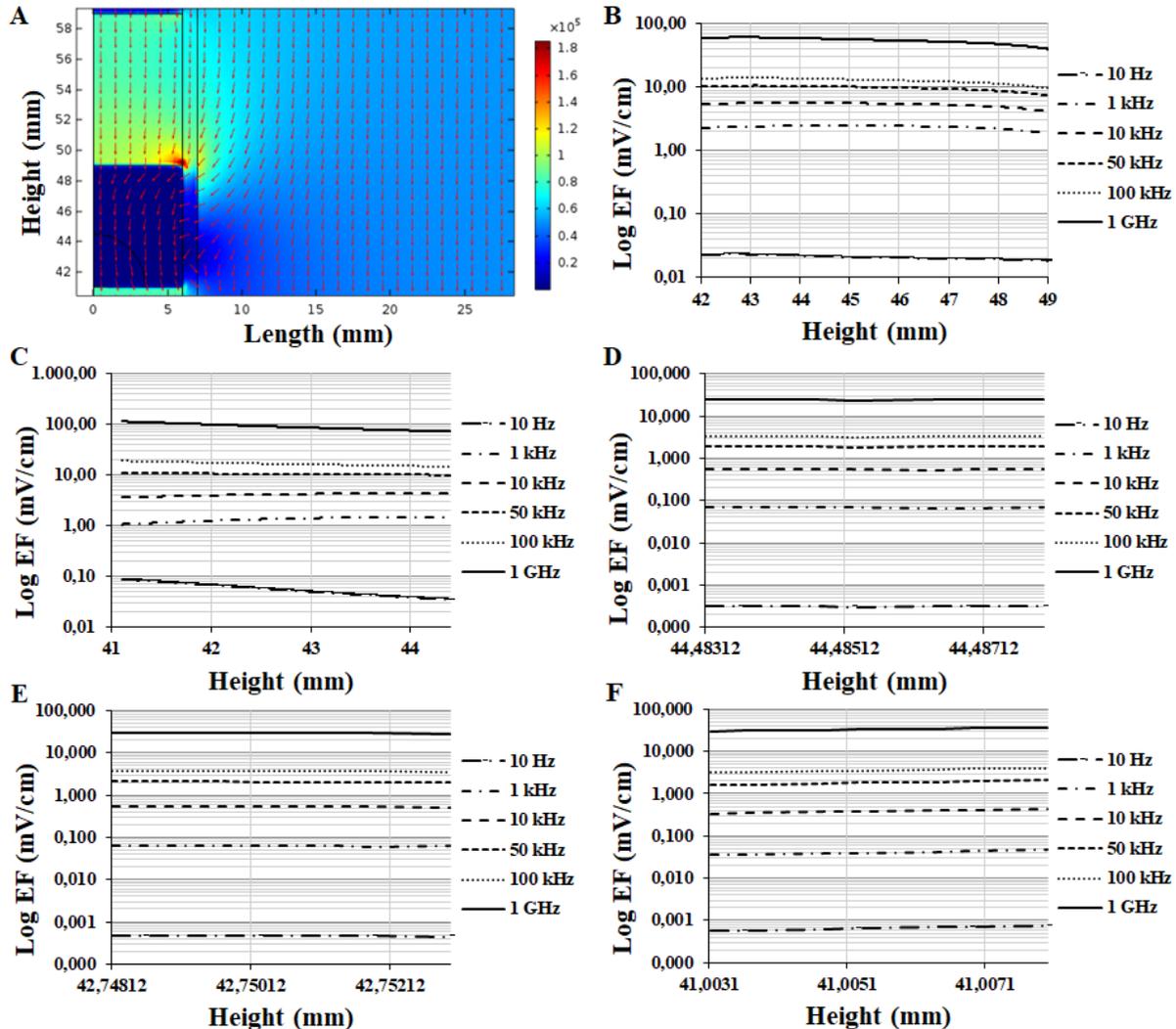

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3.3. EFs distribution in scaffolds

A homogeneous EF flow distribution was evidenced inside the chondrogenic scaffold (Figure **6**A). Results evidenced that the EFs tend to increase as the frequency was higher. For instance, the EFs in the culture media at 1 kHz were around 2.4 mV/cm, while EFs at 100 kHz were around 12.8 mV/cm (Figure **6**B). Regarding the EFs within the hydrogel, it was observed that the EFs at 1 kHz were approximately 1.3 mV/cm, while the EFs at 100 kHz were around 16.7 mV/cm (Figure **6**C). Here, it was possible to observe a slight decrease in the EFs within the hydrogel when frequencies of 10 Hz and 1 GHz were applied. The EFs were also measured in the cytoplasm of chondrocytes located in three different positions of the scaffold. The EFs in chondrocytes located at the upper surface of the scaffold were 0.07 mV/cm for 1 kHz and 3.2 mV/cm for 100 kHz (Figure **6**D). The EFs in a chondrocyte located in the middle of the scaffold varied from 0.06 mV/cm for 1 kHz to 3.6 mV/cm for 100 kHz (Figure **6**E). Finally, the EFs in a chondrocyte located at the bottom of the scaffold were 0.04 mV/cm for 1 kHz and 3.6 mV/cm for 100 kHz (Figure **6**F).

Figure 6: Distribution of EFs in scaffold cultured in vitro. A) Electric flow diagram inside the culture well plate and around the scaffold (measurement in mV/cm). B) Distribution of EFs inside the culture media. C) Distribution of EFs inside the scaffold. D) Distribution of EFs inside the chondrocyte located at the upper surface of the scaffold. E) Distribution of EFs inside the chondrocyte located in the middle of the scaffold. F) Distribution of EFs inside the chondrocyte located at the bottom of the scaffold.

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4.   Discussion

This study presents a computational model that evidences the effects generated by EFs on chondrocytes cultured in three different scenarios: monolayer culture, cartilage explant and chondrogenic scaffold. The findings demonstrated that depending on the frequencies applied, the chondrocytes experienced several EF concentrations at intra and extracellular level in the three settings computed. For instance, the EFs perceived by the cytoplasm were smaller compared to the EFs sensed by the extracellular environment. This electrical behavior is controlled by both the dielectric properties of the biological materials and the insulating properties of the plasma membrane. Although literature reports evidence that the EFs increase as the σ is higher, the opposite effect was observed in this study, where a higher σ of the cytoplasm reduces the magnitude of the induced EF that flows through it. Similar results were observed by Taghian *et al.*, who evidenced that an increase in cytoplasm conductivity leads to lower EFs in the cytoplasm and higher EFs in the plasma membrane [30]. This particular behavior is also explained by the ϵ of the cytoplasm, since these dielectric constant decreases because the cytoplasm is polarized and overrides the internal EF. On the other hand, it has been described that the insulating effect of the cell membrane decreases as the frequency increases. For instance, cells are totally isolated by the plasma membrane when low frequencies are applied; therefore, the current is not able to penetrate the cell. Nevertheless, the insulating effect of the plasma membrane decreases as the frequency increases; thus, small current magnitudes are able to flow through the cell. Accordingly, when the plasma membrane is subjected to high frequencies, it behaves like a short-circuit and the current flows throughout the membrane by reaching the cytoplasm [36]. In this context, it is possible to mention that the plasma membrane acts as a capacitor, since the current that flows through a capacitor tends to increase as the frequency is higher.

Considering the cell membrane as a capacitor, the EFs in this computational model were applied in alternating current (AC). Furthermore, the model considered a variation of the frequencies, because a capacitor submitted to an AC with different frequencies is being constantly charged and discharged. In this sense, the plasma membrane is alternately charged and discharged at a rate determined by the frequency of the supply. This phenomenon is related with the activation of the plasma membrane ionic channels, as the application of external alternating EFs modifies the opening and closing of the Na+, Ka+ and Ca+ channels [37–39].

Even though the activation of ionic channels of chondrocytes was not modelled, there are reports that have demonstrated that the voltage-dependent calcium channels (VDCC) are responsible to trigger different signaling pathways, which are involved in cell dynamics and molecular synthesis [40]. According to our results, this computational model is a useful tool that can predict both the EF intensity and frequency required to modify the influx and outflux of ions which may enhance the cell dynamics of chondrocytes. Although the EFs within the chondrocytes were similar in the monolayer culture, the cartilage explant and the scaffold, the extracellular EFs near the cell membrane varied considerably from each culture system to the others. This means that depending on the systems where the chondrocytes are cultured, specific stimulation schemes need to be applied to cells to trigger different cellular responses such as molecular gradient concentrations, proliferation, migration, among others [41]. For this reason, the computational model presented here is a promising tool that can be used to estimate the required EFs to overcome the chondrocyte membrane potential and modify the ionic gradient concentrations responsible for activating the signaling pathways of the chondrocytes.

Depending on the EFs applied and the stimulation time, the in vitro procedures of chondrocytes cultured in monolayer can be modified in order to increase cell proliferation and molecular synthesis. In fact, some experimental studies have evidenced that EFs in chondrocytes cultured in monolayer increase cell population and enhance the molecular synthesis of molecules such as glycosaminoglycans, collagen type II and aggrecan [14,15,23]. Accordingly, this computational model could be improved if the molecular events are modeled when external EFs are applied to the cells. Indeed, our most recent study evidences the role of EFs on the plasma membrane of single cells. This work demonstrated that depending on the intensity of the field and the frequency, it was possible to stimulate different cell membrane zones [42]. Combining these computational approaches, it is possible to establish the adequate parameters to stimulate cells, and accurately predict if the stimulation modifies the cell membrane potentials. A recent study simulated the EF distribution in a capacitive coupled system, in which a frequency of 60 kHz and an input voltage of 44.8 V were used to generate the EFs [43]. Although that computational model simulates a similar domain, the model did not consider the cell inside the culture well plate; for this reason, it was not possible to predict the EF around and inside the cell.

On the other hand, the EF intensities inside the cytoplasm of chondrocytes immersed in the native tissue were lower compared with the EFs in the extracellular matrix of the cartilage. In fact, the EFs tend to be similar within the chondrocytes regardless the location within the tissue. Experimental studies have demonstrated that electrical stimulation in cartilage explants stimulates the tissue growth and modifies the longitudinal position of the chondrocytes that are located near the ossification front [17]. In a previous study, we evidenced that the EFs influence the columnar organization of chondrocytes located in the middle of a chondroepiphysis explant. Moreover, it was possible to identify that the EFs accelerate the hypertrophic process of chondrocytes located in the columnar zone of the chondroepiphysis [21]. Although some studies have showed positive results in the application of EFs to cartilage explants, other works have evidenced that the electrical stimulation generates abnormal cartilage growth when the fields are not adequately applied [19]. In this context, the appropriate intensity of the EFs applied is a relevant factor to consider, since the variation of voltages and frequencies can alter cartilage growth and keep the tissue in a quiescent state [11].

Finally, the electrical stimulation on chondrogenic scaffolds evidenced that the distribution of EFs tends to be higher in an extracellular environment compared with the EFs perceived by the cytoplasm of chondrocytes. A study simulated the EF distribution in bioresorbable collagen type I scaffolds; however, the computational model considered just one frequency of 1 kHz and a voltage of 0.7 Vrms [44]. Moreover, that model did not consider the chondrocytes inside the scaffold. The computational model presented in this study provides a better understanding in how the EFs can distribute not only inside the three-dimensional construct but also inside the cytoplasm of chondrocytes. From an experimental point of view, the EFs have also been applied to tree-dimensional constructs. For instance, two studies evidenced opposite outcomes regarding cell proliferation and molecular synthesis in chondrocytes cultured in agarose systems and then electrically stimulated [20, 45]. Accordingly, this computational approach could be a promising tool that could estimate the EFs responsible to stimulate the chondrocytes and enhance their dynamics.

5.   Conclusions

Overall, electrical stimulation has proven to influence the cell dynamics in chondrocytes within a monolayer culture, a cartilage explant and a chondrogenic three-dimensional construct. This control is achieved by combining the appropriate input parameters, such as frequency and voltage. A suitable combination of these factors can provide the desired results, such as the increase in cell population, morphological changes of chondrocytes and stimulation of characteristic cartilage tissue molecules. Given the results obtained in relation to the frequency dependence of the effects of EFs in chondrocytes, a new field of research may be opened to study how a biophysical stimulus could improve the therapeutic outcomes of chondrocytes, cartilage explants and 3D constructs used to regenerate injured tissues. In addition, this computational approach is a novel and potential tool that can be used to create special bioreactors to generate distinct electrical intensities according to the necessity of a particular cartilage treatment. This study may contribute to tissue engineering because biophysical stimuli are the basis for the next generation of cartilage regeneration technology. Accordingly, those electrical stimuli help the development of biomimetic samples that recreate an environment where the functional, structural and biological features of cartilage remain stable at the time of cartilaginous replacement.

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